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OF WILLIAM L. PETERSGrowth and Life-cycle of Invertebrates
from Danish Springs

by

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(with 19 figs.)

The fauna of Danish springs has been the subject of earlier studies, the most notable of these being ANKER NIELSEN's study of springs in Himmerland (Jutland). In his work on trichoptera found in the springs of Himmerland (NIELSEN 1942) he also mentioned the life-cycle of the individual species. Apart from NIELSEN's studies, however, the growth-rate and life-cycle of fauna from Danish springs has not been the subject of further analyses, and even with regard to other countries the literature on this topic has been particularly sparse. The springs examined constitute a very uniform environment, with very small fluctuations in the magnitude of the individual ecological factors during the course of the year. An examination of the life-cycle and growth of the fauna would, therefore, appear to have special interest with regard to a study of the interdependence, if any, between these phenomena and the environmental conditions.

MATERIAL AND METHODS

The animals studied in the present work originate from material procured with a view to a comprehensive study of Danish spring fauna. The material was collected from two large springs in the Himmerland area of Jutland, Rold Kilde and Ravnkilde, which are both mentioned by NIELSEN (1942, 1950 a and 1950 b) and BERG (1951). *Asellus aquaticus*, however, was collected in a large helokren near the western part of Tystrup Lake in Zealand. In this material some species were so numerous that an examination of the growth-rate was possible even though not all the samples are of an ideal size, particularly in relation to a statistical analysis of the results.

In addition, the collecting technique employed permits an exam-

nation of the animals' rate of growth – as no selection of the animals was made at the time of collection. However, the fact cannot be overlooked that the smaller individuals are somewhat poorly represented in the material. The collecting technique depended on the particular type of substratum from which the animals originated. Those animals collected from a stony bottom were taken with MACAN's Bottom Sampler (MACAN 1958). Specimens from moss vegetation and from piles of dead foliage were taken by filling a 500 ml glass jar with the substratum in question. *Asellus* was collected by means of sieving a large amount of ochrous ooze. The material was later distributed in flat trays and the animals sorted from the substratum as soon as possible – preferably while still alive. The animals were thoroughly sorted out, in so far as they were visible to the naked eye, and preserved in 70 % alcohol.

Collecting took place in the period Aug. 1959—Oct. 1960, during which time 13 collections were made with approximately identical intervals between them. With each collection the temperature of the water was measured, and these measurements provide the basis for the temperature curves in the figs. 15—19.

With regard to some of the species there was a sufficiently large amount of material from two localities – giving the opportunity for some valuable comparisons.

The animals having been placed in a petri dish under the microscope, a silhouette was projected onto a piece of paper, with the aid of an Edinger Projector. The distances to be measured were then drawn on the paper and subsequently measured with a nonius or a curvometer depending on whether the lines were straight or curved.

The section of the animal measured was, as far as possible, a firm, chitinous part. When a good projection picture of this part could not be made, the animal's length, or part of it, was measured, as the end point of the measured line should be well defined, e.g. a tuft of bristles or a chitinous edge. Table I shows the measurements made of each animal and the magnification used in each case.

From these measurements the histograms were drawn, showing the individuals' distribution in groups according to size. Additionally, the average was calculated for each sample and from these figures the growth-curve fig. 1—7 and 10—13 were drawn. However, this type of growth-curve does not give a true picture of the growth size since, while it is composed on the basis of mean values, the growth may be assumed to stretch from a figure lying lower than the lowest mean value to a figure lying higher than the highest mean value, as there is often a considerable dispersal around the mean value (see further BRINCK 1949, p. 131 and MACAN 1957, p. 134). Finally, significance tests were made for the differences between two subsequent points in the

TABLE I

Measurement made of each animal and the magnification used in each case.

Species	Measurement taken	Magnification
<i>Baëtis rhodani</i>	Width of head capsule	40 : 1
<i>Brachyptera risi</i>	Width of head capsule	40 : 1
<i>N. (Nemurella) picteti</i>	Total length	7.3 : 1
<i>Leuctra hippopus</i>	Width of head capsule	40 : 1
<i>Agapetus fuscipes</i>	Front edge of pronotum - anal shield	40 : 1
<i>Crunoecia irrorata</i>	Width of head capsule	40 : 1
<i>Pericoma blandula?</i>	Front edge of 1. thoracal segment - back edge of the hindmost anal segment	40 : 1
<i>Simulium ornatum</i>	Width of head capsule	24.7 : 1
<i>Ancylus fluviatilis</i>	Length of shell	40 : 1
<i>Asellus aquaticus</i>	Width of telson	40 : 1

growth-curves. These were made as a t-test (HALD 1952) and a difference taken as being significant if the probability of a real difference lay over 99 %.

For the histograms as well as the diagrams there are two units for the measured size - one a relative unit, giving the length of the measured distance after the magnification and the basis for the calculations, and the other the actual length given in mm.

RESULTS

Baëtis rhodani PICTET:

Material: 1,440 nymphs taken in Rold Kilde (714 nymphs) and Ravnkilde (726 nymphs), both places from a stony bottom. The species may be said to be both characteristic of, and numerous in, this biotope.

The growth-rate of the species was earlier studied by ILLIES (1952), HARKER (1952), MACAN (1957) and HYNES (1961). ILLIES did not entirely succeed in clarifying the growth-conditions as he could not distinguish the smaller nymphs from *Baëtis pumilus* BURMEISTER. HARKER was equally unsuccessful in reaching a result but did, however, demonstrate that the nymphs could be distributed into several size-groups. On the other hand, by means of very thorough examinations involving the measurements of a large amount of material, MACAN was able to clarify the growth-rate of the species (including the smallest size-groups), as it appears in some brooks near Lake

Windermere. He showed that in this locality *B. rhodani* has two generations per year – a long-lasting winter generation with a flying time from April to July, and a short summer generation with a flying time from July to November. Moreover, HYNES also found two generations in his area of examination, although with a slightly deviating flying time.

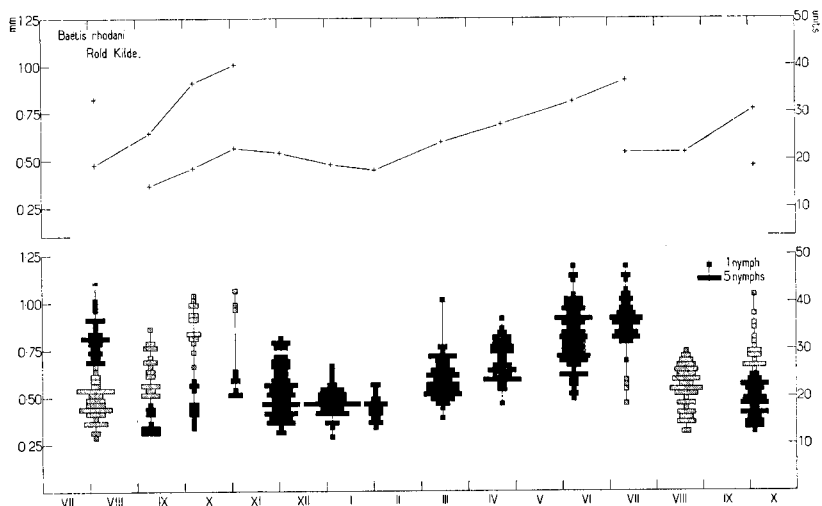


Fig. 1. Growth-curve and size distribution in each sample of *Baëtis rhodani* from Rold Kilde. Summer generation cross-hatched and winter generation black.

Not all the histograms fig. 1 and 2 indicate a normal distribution – certain of them may rather be considered as bi- or polymodal. This could be taken to show the simultaneous occurrence of generations of unequal age. The separation between these generations has been made rather haphazardly since they overlap each other in the histograms. However, the following rules have been followed throughout:

1. The separation between the generations should show that no, or only a few, animals were found in the size-group through which the separation line was drawn.
2. At the same time, the histograms should be divided in the same place for both of the springs examined and for the years 1959 and 1960 as well.

According to the division of the histograms it appears that the species has two generations per year, corresponding to those found by MACAN and HYNES. The flying times seem to be a little deviating in the present author's material (April to July (August) and October

to November), but nevertheless, here also there is a long winter generation with a flying time in spring and early summer, as well as a short summer generation in late summer and autumn.

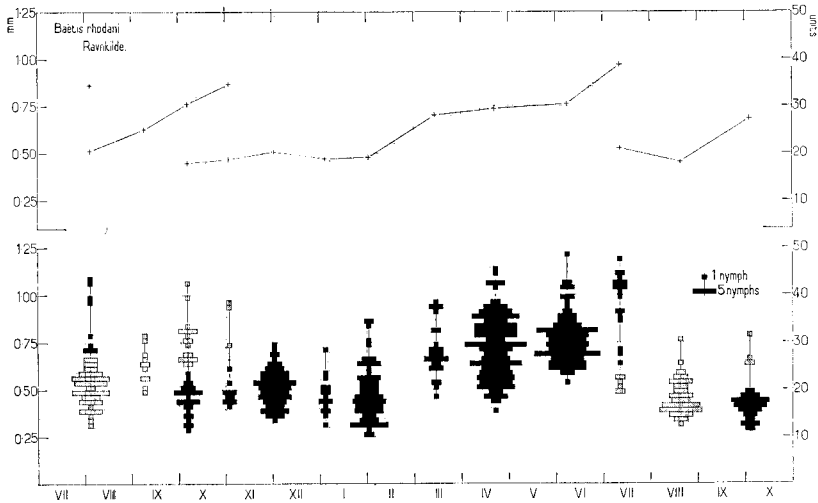


Fig. 2. Growth-curve and size distribution in each sample of *Baëtis rhodani* from Ravnkilde. Summer generation cross-hatched and winter generation black.

One question that MACAN does not answer completely is, how far these two generations ought again to be split up into two generations each. Certain features in his material on rather small nymphs seem to point in this direction and his histograms also give a similar indication. The author's own material does not include the smallest animals but the histograms suggest that in certain places there really does occur a further division of the two generations into another two generations each. With reference to the winter generation it is seen that the histograms for Ravnkilde in the period 1.2.1960—7.6.1960 and for Rold Kilde in the period 14.3.1960—7.6.1960, have a tendency to show two maxima. A similar pattern is seen for the summer generations in the histograms for Ravnkilde and Rold Kilde from 19.8.1960. That the histograms concerned have two maxima is further indicated by the method used by HARDING (1949) for analysis of polymodal distributions in the author's material from the months in question.

Further, it is seen as a characteristic of the growth-curves that a growth-stoppage takes place in winter. This is approximately the period from beginning of November to the beginning of February,

and to judge from the growth-curve even the average decreases during this period. However, it should be mentioned that there is no significant difference between consecutive mean figures in the period stated, with the exception of figures for 1.12.1959—4.1.1960 for Rold Kilde.

BERG (1938) found a similar pattern in measurements of *Caenis moesta* BENGSSON from Esrom Lake. Here the phenomenon is explained by the immigration of animals of the smaller size-groups together with the disappearance of animals of the larger size-groups, both resulting in a lowering of the average. In the instances examined here it appears that a withdrawal of individuals in the larger size-groups only takes place (see histograms fig. 1 and 2). This may be due to the fact that the large animals normally live on the surface of the stones, where they are more susceptible to the dangers of predatory animals and the stream's current than are the small individuals who establish themselves in more protected places, under and between the larger stones.

Brachyptera risi (MORTON):

Material: 63 nymphs taken from the stony bottom in Rold Kilde where the species may be considered a characteristic element in the fauna, even though it appears in rather small numbers and only during a very limited part of the year (Nov.—April). The species has been measured in spite of the paucity in numbers of animals in the present material. Since the result of the measurements show a distinct trend, and since the year-cycle appears to be so regular, it is considered worthwhile to make further mention of the growth-rate.

The growth-rate of the species has been studied earlier by BRINCK (1949) and HYNES (1961). Since in many places it appears in populations composed of many size-groups, BRINCK is of the opinion that in these cases it is usually very difficult to obtain a clear picture of the complete growth. At the same time he published a curve of the growth conditions found in a stream in the Skåne province of Sweden. The species is only found during the winter season. The smallest individuals appear in October and the largest in May (when the nymphs are hatched). A characteristic of BRINCK's curve is that it shows a retarded growth in January, and particularly, in February. This retardment was not mentioned by HYNES in connection with his material, although his histograms show that the growth is slow during the winter months. His observations correspond closely to those of BRINCK.

It has not been possible for the author to demonstrate any obvious growth-stoppage in the cold months, neither with the help of histograms nor growth-curves (fig. 3). However, while there is

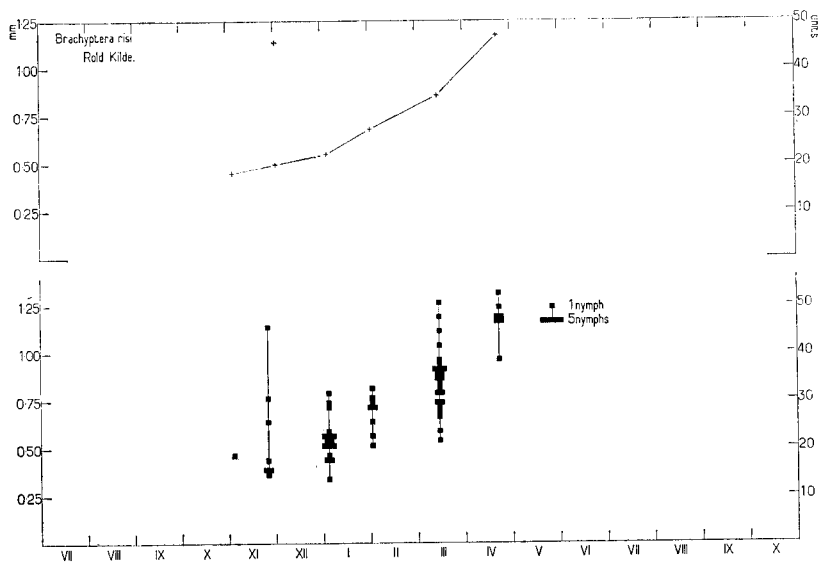


Fig. 3. Growth-curve and size distribution in each sample of *Brachyptera risi* from Rold Kilde.

no significant difference between the average for consecutive samples during the period 2.11.1959—4.1.1960, there is a significant difference between the remaining samples in question. The histogram for the month of December shows the presence of one specimen of the same size order as those individuals about to be hatched in the months of March and April. This may probably mean that a few individuals start their development early in autumn when they are able to take such good advantage of the favourable growth conditions prevailing at that time that within the course of a few months they are ready to complete their growth. However, it seems hardly feasible that the imagines would be ready for a flying time with mating and egg-laying in December, and since the author has not found large nymphs in the following months, it may be assumed that these large December nymphs perish whether they are hatched into imagines or not. It is possible that in this phenomenon we have an example of a less satisfactory adaptation to ecological conditions.

It seems then that the species is able to carry out all its development in a very short time. As it also seems that there is only one generation per year it may be assumed that the eggs have a very long period of incubation.

Nemoura (Nemurella) picteti KLAPÁLEK:

Material: 1698 nymphs taken in Rold Kilde (882 nymphs) and

Ravnkilde (816 nymphs), both places in moss-growth. Even though the species is found in all types of substratum it is the author's opinion that moss-growth, where it appears consistently and in great numbers, is the preferred substratum.

The growth-rate of the species has been studied earlier by BRINCK (1949), from a spring in Skåne, Sweden. He found that the growth is even throughout the entire growth period from September—May. In the southern part of Sweden it has a flying time from May—September; thus, no growth-stoppage takes place in winter.

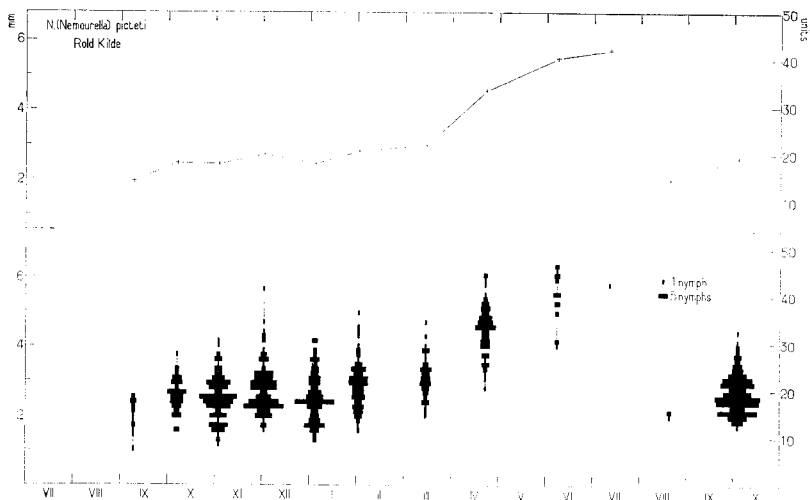


Fig. 4. Growth-curve and size distribution in each sample of *N. (Nemurella) picteti* from Rold Kilde.

The present studies deviate from this pattern, primarily in the growth-curve development (fig. 4 and 5). This shows a definite growth-stoppage in the months October—March/April, after which the growth takes place so quickly that the first nymphs are ready for hatching in April; the hatching continues until August. The first hatched nymphs obviously do not give rise to a short summer generation such as we saw with *Baëtis rhodani* (fig. 1 and 2), but the new generation probably starts in summer (in July?). In this way the nymphs in August/September have grown to such a size that they appear in the present samples. It must be assumed, therefore, that the first part of the growth is fairly rapid and that the complete growth-curve is S-shaped.

The significance tests show that the difference between the averages for consecutive months in the period March—August are significant for both localities.

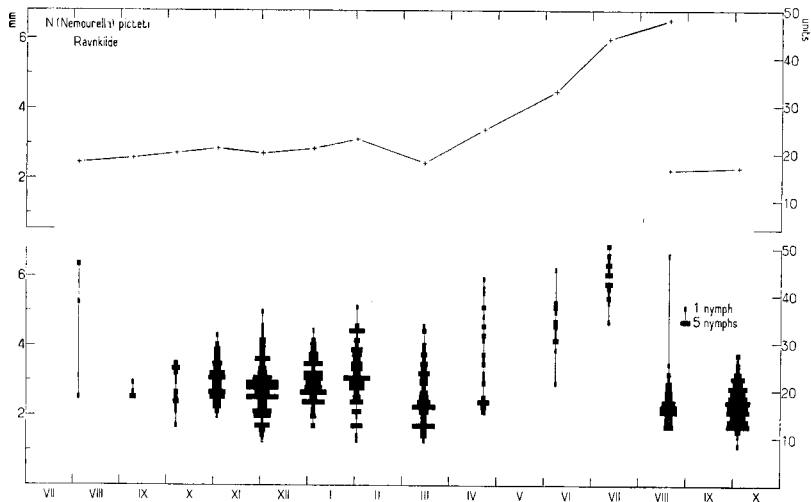


Fig. 5. Growth-curve and size distribution in each sample of *N. (Nemurella) picteti* from Ravnkilde.

Leuctra hippopus KEMPNY:

Material: 115 nymphs from stony bottom in Rold Kilde. The species often appears on a stony bottom but is not found exclusively on this substratum.

The growth-rate and life-cycle of the species were studied earlier by HYNES (1941 and 1961) and BRINCK (1949). Both authors found that the species has one yearly generation starting in the autumn, with hatching in early spring. They also found that the growth is more vigorous in autumn and decreases somewhat in winter, although without a complete growth-stoppage.

Despite the present material being rather small it has, nevertheless, been found worthwhile to publish the findings – even though the poor number of animals measured leads to some inaccuracy in compiling the growth-curve. However, the histograms (fig. 6) show a definite trend, and from these there can be no doubt that the species has at least one generation (winter generation) per year, exactly as was shown by HYNES and BRINCK. It is possible that there is also a summer generation, probably starting in March and ending in July. It is also possible, however, that the individuals from this generation belong to another species. But it has not been possible to distinguish with certainty between nymphs from the two generations. According to the histograms and the growth-curve the sizes appear to differ, and the tibiae of the summer generation nymphs seem to have more hairs than those of the winter generation; although there is no actual ridge

of hairs, as in *L. fusca* LINNÉ (HYNES 1941). Unfortunately, the present material does not contain imagines which can, with certainty, be referred to the summer generation; the last *Leuctra* imagines were taken in June and these all belong to *L. hippopus*.

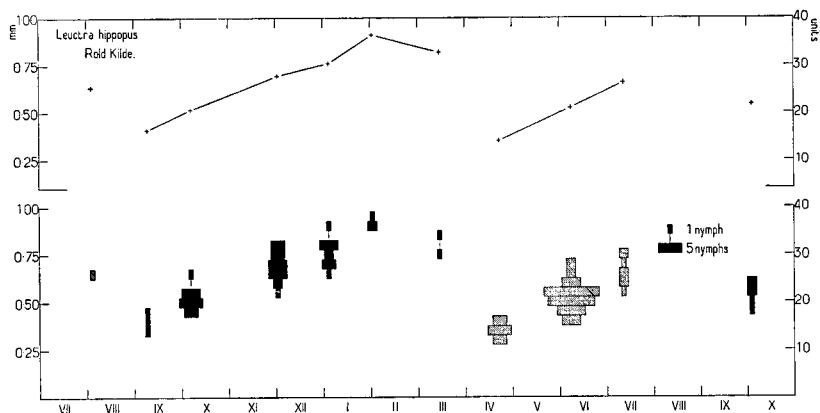


Fig. 6. Growth-curve and size distribution in each sample of *Leuctra hippopus* from Rold Kilde. Summer generation cross-hatched and winter generation black.

In this connection it is worth noting that HYNES (1941 and 1961) showed that *L. fusca* carries through its growth in the course of the summer and has a flying time in the latter part of August. This corresponds to the conditions for the summer generation in the present material, with the exception that the flying time is somewhat earlier.

It is further noted that there is no growth-stoppage during winter. There is a significant difference between consecutive averages for the period 8.9.1959—1.12.1959, while the significance of the difference between the values for 1.12.1959—4.1.1960 is only 97.5 % increasing again to over 99 % for the difference between the values for 4.1.1960—1.2.1960. As far as the summer generation is concerned the significance of the differences between consecutive averages is over 99.95%.

Agapetus fuscipes CURTIS:

Material: 385 larvae from stony bottom in Rold Kilde. The species occurs frequently in this locality, but it is found in considerably greater number in the topmost part of the spring on stones protruding from the water, and here in addition, the fauna is characterised by containing typical representatives of the fauna hygropetrica. The present material of *Agapetus fuscipes* from the last-named locality does not, however, lend itself to growth examinations. It is

sorted on the spot and thus it cannot be expected that the size distribution in the material corresponds to the size distribution in the population of the locality.

The growth-rate was studied earlier by ILLIES (1952) and MACKERETH (1960). NIELSEN (1942) also mentioned the mode of growth. ILLIES found that growth takes place in the period from August—June/July of the following year; it stops in the middle of December and is resumed again at the end of February. MACKERETH, who has studied the growth over a period of 3 years, found that in one year the species apparently grew without interruption throughout the winter, while in another year a growth-stoppage, or at least a retardment took place during winter. His observations otherwise correspond to those of ILLIES. NIELSEN also stated that the species undergoes a growth-stoppage in the winter months, despite the constant temperature conditions. He gave the flying time from June—September, both months inclusive.

Measurements taken by the present author of animals from one of NIELSEN's localities obviously show, however, that the species has a retarded growth in the period from around 1st December to around 7th February (fig. 7). There is no significant difference of the averages during that period, whereas this is the case for all remaining figures.

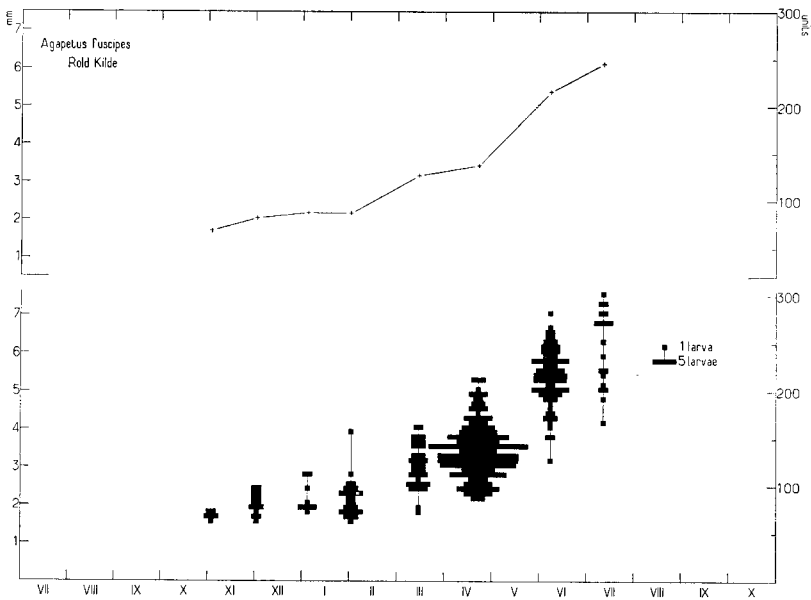


Fig. 7. Growth-curve and size distribution in each sample of *Agapetus fuscipes* from Rold Kilde.

In the present material the first larvae from this locality appear in the month of November, and NIELSEN states that larvae of the first instar are numerous in October. The last larvae were taken in July and the present author has found pupae from June until well into October. However, the imagines are frequent for a much longer period than one is led to suppose from the histograms. The author has found them in the entire period from May to December, although many of them may be assumed to originate from areas situated above the examined locality. Here the ecological conditions are much more varied, from which it may be supposed that the flying time is stretched over a somewhat longer period; in these localities the pupae occur from April—December.

It can be seen that the dispersion within different size-groups is very large, and for this reason a growth-curve does not give a completely true picture of the conditions. The curve only gives the conditions for a part of the population, while the remaining individuals may deviate from this.

Crunoecia irrorata CURTIS:

Material: 401 larvae from Rold Kilde (112 larvae) and Ravnkilde (289 larvae), both places in piles of dead foliage. *Crunoecia irrorata* may be considered a characteristic species from this biotope.

The growth conditions were also studied by ILLIES (1952), while NIELSEN (1942) gave a short description of the life-cycle. NIELSEN's observations correspond exactly to those of the present author, and to the results made; whereas, in the author's opinion, ILLIES' considerations on the growth-rate of the species appear to be somewhat curious.

After examining *Hydropsyche fulvipes* CURTIS which always occurs simultaneously in two generations of different age (as the life-cycle of the species is characterised by two yearly flying times and a period of development lasting one and a half years), ILLIES

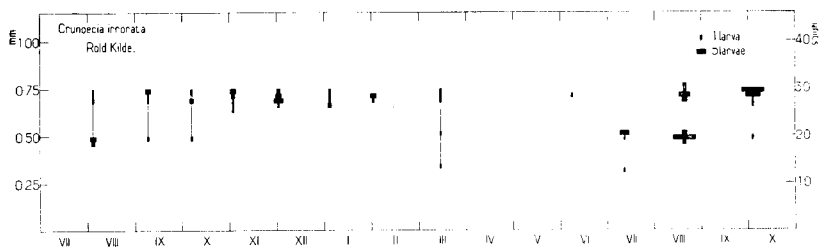


Fig. 8. Size distribution in each sample of *Crunoecia irrorata* from Rold Kilde.

states that by measuring *Crunoecia irrorata* (and other species), he has found that this species simultaneously appears in two (to three?) size-groups. From this he concludes that the species must have the same type of development as *Hydropsyche fulvipes*.

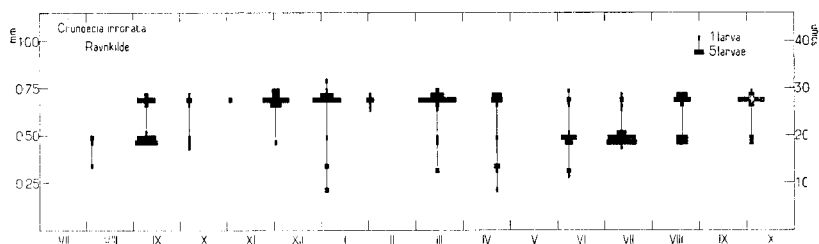


Fig. 9. Size distribution in each sample of *Crunoecia irrorata* from Ravnkilde.

The author's own measurements also show that in many of the histograms the species occurs in several size-groups (fig. 8 and 9). However, these groups are merely the different larval instars showing large size differences – since, according to NIELSEN (1942) the species has few, usually five, larval instars. The present material probably shows the four last instars, characterised by the following head capsule widths;

2nd instar	0.205—0.218 mm
3rd „	0.293—0.328 mm
4th „	0.433—0.510 mm
5th „	0.625—0.790 mm

which approximately correspond to NIELSEN's measurements (NIELSEN 1942, p. 465). Those size-groups which ILLIES obviously regards as different generations should probably be regarded as different instars of the same generation.

Very few animals in the 2nd instar are found in the present material and it is not possible therefore to conclude anything of positive value from these. However, it appears from the histograms on Ravnkilde that animals in this instar occur here in the months of January and April – although it is not very likely that these are larvae from the same year, since the first pupae occur in April and the flying time is even later. Since, however, the flying time lasts well into late summer (NIELSEN (1942) gives August as the last month), it is more probable that these animals are descendants of late-flying individuals from the previous year which have not progressed further. This further indicates that the growth must be slow during the winter.

Larvae of 3rd and 4th instar are also found in early spring, but

from November to (and including) April, 5th instar may be said to predominate. This is in good agreement with pupae in the present material being found from April to August, and the flying time is the period May to August (NIELSEN 1942). That larvae in the 5th instar appear so long before pupation also points to a growth-stoppage during the winter.

Thus, the old generation dies in the course of summer, with the result that larvae of 3rd and 4th instar found in the late summer and autumn months are representatives of the new generation. There is little doubt, therefore, that the species from localities examined by the author are annual and that a growth-stoppage takes place during the winter.

There is very little to gain in drawing growth-curves of material distributed in the histograms in the way it is here; on the contrary, such curves would merely be misleading. Accordingly, this method has been discarded and the growth conditions is evaluated on the basis of the frequency of each instar in each histogram, and not on the basis of the average figures.

Pericoma blandula EATON?:

Material: 562 larvae from moss-growth in Ravnkilde. The species can be said to be characteristic of this biotope. It is not possible to determine the species with absolute certainty, as, with reference to JUNG's work on European Psychodidae (JUNG 1956), full agreement has not been reached between his description of the larvae and the author's material.

The species displays a typical annual life-cycle with a growth-stoppage in the winter period (fig. 10); There does not appear to be

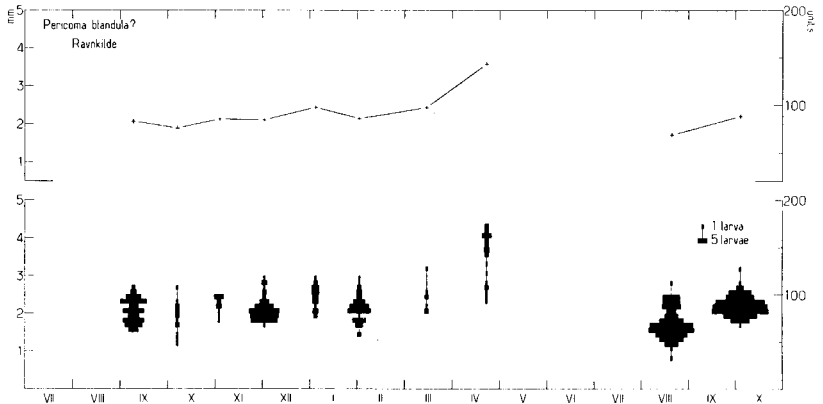


Fig. 10. Growth-curve and size distribution in each sample of *Pericoma blandula?* from Ravnkilde.

any growth from September to February. It is remarkable that the growth should stop so early, but the large number of animals in the sample from September seems to be proof that the growth actually does stop by this time.

Furthermore, it was noted that no animals were found in the sample from August, which again means that the first part of the growth must take place very rapidly. This factor is also seen in the samples from autumn 1960, where the species first occurred on 19th August and where the growth took place so rapidly that the animals had reached the size in which they overwinter not later than 3rd October. In March the growth sets in very definitely, and thus the animals are able to hatch in April and May. No animals were found in June and July.

The significance tests appear to support this pattern. However, the sample from 4.1.1960 lies so high that there is a significant difference between the average for this and the average for December, as well as for February. It is not possible to give any explanation for this fact, but there is little likelihood of a real size difference in the population. On the other hand, it seems that it is the small animals that are absent in the sample, as the larger animals are not bigger than the largest animals from December and February.

Simulium ornatum MEIGEN:

Material: 874 larvae taken in Rold Kilde (477 larvae) and Ravnkilde (397 larvae), both places from stony bottom. The species is characteristic of this biotope and is only found here.

The growth conditions for the black flies has been studied earlier by HYNES (1961) and DAVIES & SMITH (1958), but none of these authors have studied *Simulium ornatum*. Further, PETERSEN (1924) has made a study of black flies including the life-cycle of *Simulium ornatum*. HYNES did not distinguish between different species of the family but only stated that in the population he studied there were at least three species. DAVIES & SMITH studied *Prosimulium hirtipes* FRIES and *P. inflatum* DAVIES, but only published histograms for the first-named species. It appears from HYNES' studies that there are three flying times per year, one in early spring, one in mid-summer, and one well into the autumn. However, it need not necessarily be the same species which hatches every time; as stated, his material includes several species. DAVIES & SMITH found that in low lying localities (around 180 m above sea level), the hatching of larvae takes place on two occasions during spring, whereas only one hatching takes place in higher lying localities (around 300 m above sea level). Unfortunately, the authors did not examine the growth-rate throughout the course of one whole year, but only from November to May.

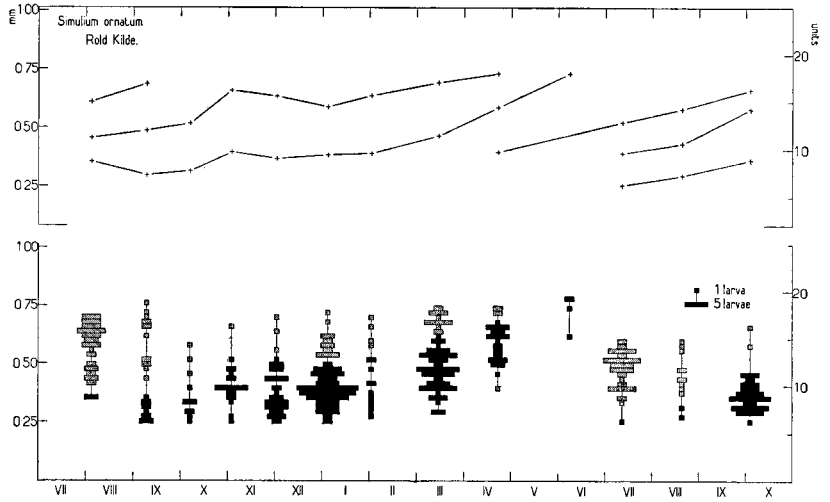


Fig. 11. Growth-curve and size distribution in each sample of *Simulium ornatum* from Rold Kilde. Spring generation stippled, summer generation black and autumn generation cross-hatched.

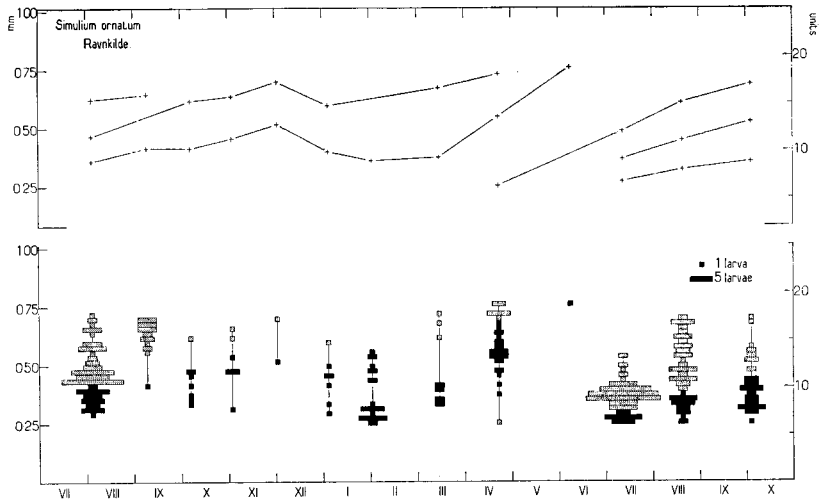


Fig. 12. Growth-curve and size distribution in each sample of *Simulium ornatum* from Ravnkilde. Spring generation stippled, summer generation black and autumn generation cross-hatched.

However, as their histograms show that very small larvae occur in November, it may be assumed that an autumn generation also appears, from low lying as well as from higher localities. Conditions in the low lying localities accordingly correspond very well with HYNES' observations. PETERSEN likewise gives three flying times for *S. ornatum*.

The present author's study also shows the same picture, as one flying time occurs in April, one in July, and one in September/October (fig. 11 and 12). The next question is, how widely these different generations enter into a common cycle or whether there are more or less separate populations. With this species it has also been found necessary to divide each histogram into several others, each corresponding to a generation. This division was made according to the same principles as for *Baëtis rhodani*.

Beginning with the early spring generation which has a flying time from February—April, it can be seen that their progeny could utilize the favourable summer period to such an extent that the fully grown insects would be ready to fly the same autumn. The generation with a flying time in May—June starts a new generation which is just too late to complete its development the same autumn, but must overwinter as rather large larvae. This generation, therefore, has a flying time as soon as the growth sets in in spring. Finally, there is the generation originating from animals which have a flying time in late summer and autumn. This generation overwinters as rather small animals, and in all the winter months these are shown as a large top at the lowest part of the histograms until they begin to grow in spring, this generation has its flying time in May—June. It may be assumed that *Simulium ornatum* has a biennial life-cycle with three generations, as follows:

1. generation lasts from Feb./April—Sept.
2. " " " Sept.—May/June.
3. " " " May/June—Feb./April.

The reason for this somewhat complicated cycle may, to some extent, be found in the shape of the growth-curves which show that there is a growth-stoppage in the winter. This is the explanation for the two generations being stretched over so long a period. Since the summer temperatures do not become very high there can be no question of a growth-restriction in summer due to too high temperatures.

Ancylus fluviatilis O. F. MÜLLER:

Material: 220 animals, all taken from stony bottom in Rold Kilde. The species is a typical stony-bottom form.

The growth-rate has been studied earlier by GELDIAY (1956) and HUNTER (1953). Both authors found the species to be annual, as it grows in the course of summer and autumn, has a growth-stoppage in winter, and resumes growth again in the spring. From here on egg-laying takes place and the generation dies off.

However, this pattern is not in full agreement with the results of the present author's own measurements. On consulting the histograms (fig. 13), it will be observed that several of these show a marked bimodality, and since this is also valid for the winter collections, it is obvious that more than one generation occurs simultaneously.

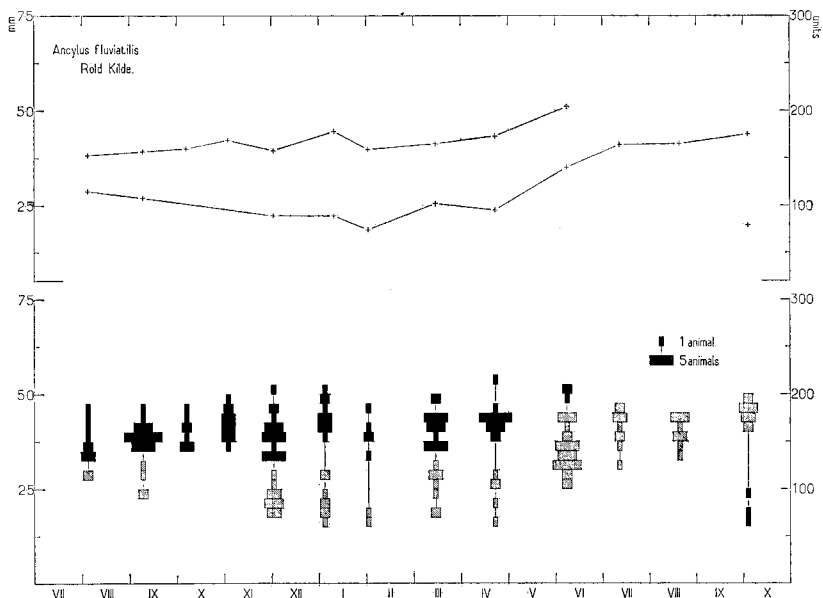


Fig. 13. Growth-curve and size distribution in each sample of *Ancylus fluviatilis* from Rold Kilde. The younger generation cross-hatched and the older generation black.

A further help in clarifying the problem is found by an examination of the growth-lines. On examining the animals from samples of 2.7.1960 and 19.8.1960, it will be seen that these animals have only one growth-line, while the larger animals from 22.4.1960 and 7.6.1960 have two. Consequently, these two groups may have gone through one and two winter periods respectively, and the species must, therefore, be biennial in the locality examined. HUNTER points out that a growth-stoppage, and formation of growth-lines owing to unfavourable growth conditions during other times than winter, may

sometimes take place (HUNTER 1953, p. 633) – but this can hardly be the case here.

Therefore, the histograms should also be divided, as were those for *Baëtis rhodani* and *Simulium ornatum*.

The life-span of the species is, thus, as follows: The eggs are hatched in the course of the summer, and by the first summer the animals have attained a certain growth before the growth-stoppage in late summer, when the animals have reached a length of approximately 2.5 mm. In March/April the growth begins again, so that during their second summer the animals attain a length of approximately 4 mm. At this size they overwinter for the second time, and finish their life-span in the course of the next spring. During the last summer, propagation takes place, but whether there are animals also capable of propagating in the first summer is a matter of conjecture. However, the possibility of this cannot be excluded. If the one-year old generation does not propagate, these two generations which always appear simultaneously become two populations independent of each other. It is a curious fact that the main part of the growth takes place in early summer, while it is almost stopped in mid and late summer. Early summer is the only period when the difference between the averages for each following collection is significant for the large as well as for the small generations.

Asellus aquaticus LINNÉ:

Material: 567 animals (300 ♀♀ + 267 ♂♂) collected from a large spring at the western part of Tystrup Lake in Zealand, where the species appears in large number and in extremely ochrous ooze.

In order to measure this species it was found necessary to divide the specimens into the two sexes and measure them separately; the reason being that, at least with regard to the fully grown animals,

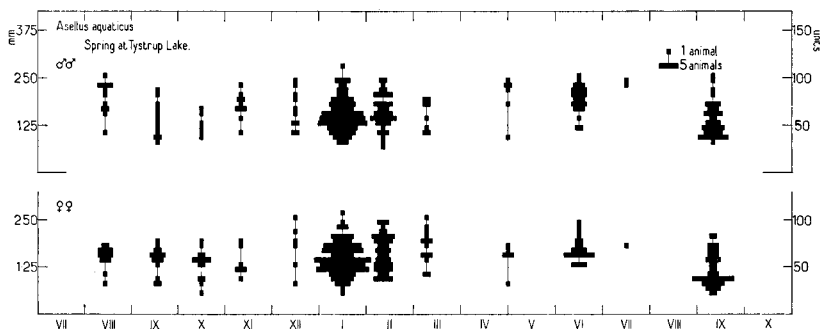


Fig. 14. Size distribution in each sample of *Asellus aquaticus* from Spring at Tystrup Lake.

a rather large size difference exists between the two sexes. However, even with this separation it is not possible to obtain an impression of the life-cycle and growth conditions of the species from the drawn-up histograms (fig. 14). This is probably due to the fact that the animals have too long a breeding period, and it is possible that there are several generations following after each other. It could look as though one generation is over by midsummer (June—July) – as in these months the animals are rather large – followed in September by a marked preponderance of small animals. Apart from this, however, all size-groups are more or less evenly represented throughout the year.

Further, the number of animals in the different samples does not help to make the picture any clearer as their content were very varied and obviously somewhat arbitrary.

Thus, under the circumstances, it was not possible to draw up growth-curves for the species.

DISCUSSION

In the foregoing section the growth-rate has been described for each species. In this section a comparison will be made between the results referred to in the previous section, and the factors which could have an influence on the growth conditions will be discussed and related to these results.

It is characteristic of many of the growth conditions examined that the growth is stopped, or at least retarded, during the winter (fig. 15—18). A growth-curve has not been drawn for *Crunoecia irrorata* (fig. 8 and 9), but from the histograms it can be assumed that the growth of this species also is strongly inhibited, if not completely stopped, during the winter.

In the case of *Ancylus fluviatilis* it is further seen that the growth also stops in midsummer. The only exceptions are *Brachyptera risi* and *Leuctra hippopus*, who not only grow throughout the winter but grow so rapidly that in the course of the winter months they complete one generation.

When seeking an explanation for this, it should first be realized that the observed growth-rates have a certain relation to the seasons of the year. Factors having an influence on growth must, therefore, be sought amongst those varying with the seasons. Of these, the temperature conditions are often taken as being the most important ecological factor; it is intended therefore to begin with these, in order to evaluate their possible bearing on the shape of the individual growth-curves.

It has been known for some considerable time that the temperature conditions of springs are very constant throughout the year. This is also valid for the springs studied in the present work, even though, in some places, temperature amplitudes of up to 10°C have been demonstrated. As a result of these constant temperature conditions it has been thought that the life of the spring animals should be independent of the seasonal fluctuations, and that the period of propagation should be greatly extended to stretch over the whole year (BREHM 1930). However, THIENEMANN (1925) gives a limitation for insects – as these should have their flying time in the early spring – and this, to some extent, is confirmed by the author's own studies. As is seen from the fore-going account, however, some insects do hatch during the summer.

From the author's examinations it appears that the spring species lead a considerably more regular life than the handbooks cited lead one to suppose, as is shown by the growth-curves (fig. 1–14). Even though there is a certain variation around the means which form the basis for the curves, there is very little doubt that each species has a rather precise year-cycle.

The brook animals under discussion have also been dealt with in relation to the dependence of the growth on temperature conditions. Here, ILLIES (1952) advocated the „Entwicklungsnullpunkt” - theory, which in brief, is as follows: As the temperature of the stream gradually falls in autumn the growth comes to a halt for one species after the other, just at the stage where the temperature reaches a critical point for each species – the so called „Entwicklungsnullpunkt”. The growth is then resumed when the temperature again reaches this level in spring.

ILLIES, who has studied the growth of a series of Ephemeroptera, Plecoptera, Trichoptera and Coleoptera, found, curiously enough, that for some Ephemeroptera the growth stops at one temperature in autumn but starts again at a lower temperature in spring. Since these observations are not in full agreement with the theory, he explains the phenomenon by assuming that the „Entwicklungsnullpunkt” sinks during winter. Considering, however, that there are different amounts of energy (in the form of heat energy) at their disposal during these two periods, ILLIES had difficulty in seeing how the animals are able to continue growing in the lower energy present during spring (although they do also receive energy other than heat energy from the surroundings – this is to be further discussed below). Therefore, ILLIES assumed that it is not the heat energy as such which has an influence on the growth, but rather certain temperature stimuli which interrupt and start this.

ILLIES did not go on to say how many animals he had measured,

and since there is not a single point in his diagrams (neither on the growth-curves nor the temperature curves), it is somewhat difficult for the reader to decide just how accurately the curves are drawn. However, he did state that his material of, e.g. Ephemeroptera, consisted of 135 imagines and 1,237 nymphs. This material is distributed over 15 species, of which ILLIES measured at least 5 species, and possibly 7. If only this material was used for the measurements it is evident that his curves are grossly inaccurate, and consequently it is difficult to decide with any degree of certainty when the growth stops and begins.

With regard to animals studied in the present work, and the correspondence between their growth-curves and the temperature conditions, it is striking that the two species found in moss-growth have a typical growth-stoppage in winter – while the temperature is, so to say, constant throughout the year (fig. 15 and 16). It seems that here an „Entwicklungsnullpunkt” does not exist, or if it exists is not determined by temperature. On examining the other growth-curves (fig. 17 and 18), the same picture is evident. Only on the stony bottom of Rold Kilde and in the piles of dead foliage in Ravnkilde is there a temperature amplitude of more than 5°C (fig. 17–19). However, in neither of these localities is there any connection between growth-stoppage and start and the temperature – and not at all when one compares the growth-curve of the same species in localities with more constant temperature conditions (fig. 18 and 19).

In other localities (e.g. moss-growth in Rold Kilde), however, the temperatures are so constant that it would be risky for a species to live here if it had a temperature-dependent „Entwicklungsnullpunkt” lying within the temperature range of the locality.

Thus, it appears impossible to explain the growth-rates by means of temperature conditions, and it seems doubtful whether the observed temperature amplitudes play any kind of a rôle regarding the fauna.

Concerning other ecological factors which go hand in hand with the seasons, light should be considered the most important. However, with regard to field observations, there is some difficulty in determining the relation between the light and the fauna. BARNES (1952/53) experimented with two barnacles in order to examine the effect of light on the growth, but this did not produce any definite results.

On the other hand, there is the possibility of a degree of indirect effect – as light plays an important part in the production of plant nourishment. In addition to the temperature of the water, or rather, the heat energy content, plant nourishment material is an important source of energy for the fauna, and the influence of this factor on the growth should not be overlooked. However, it has been difficult to

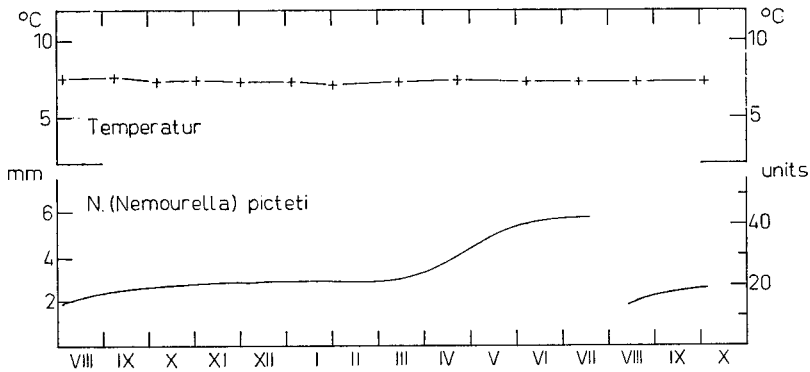


Fig. 15. Temperature-curve for the station with moss-growth in the spring Rold Kilde and an idealized growth-curve for *N. (Nemourella) picteti* from this station.

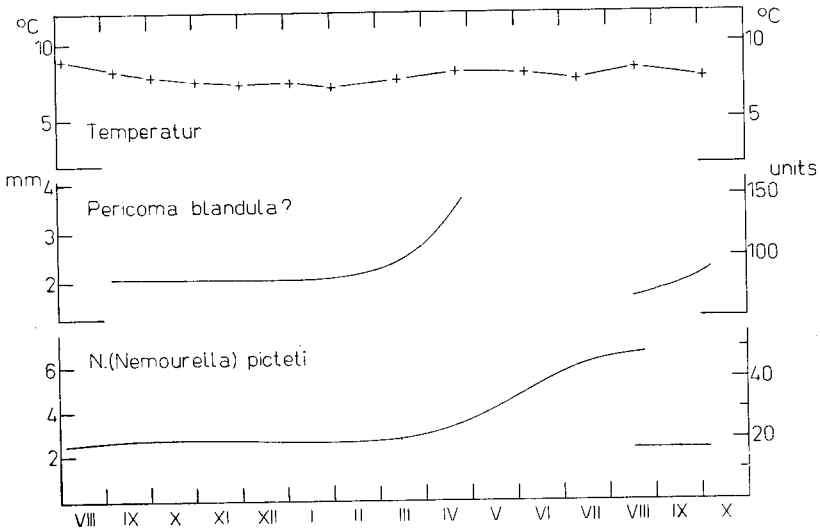


Fig. 16. Temperature-curve for the station with moss-growth in the spring Ravnkilde and idealized growth-curves for the animals collected at this station.

obtain an impression of the production of plant nourishment material in running water, although certain experiments in that direction have been made.

Thus, in many studies it is seen that the composition of the microflora on the stones of the stream vary with the seasons (e.g. BUDDE 1928 and 1932, RAABE 1951 and SCHEELE 1952). In recent years ODUM and co-workers have experimented with measurements of

the production of organic material in streams, especially in springs. ODUM (1956) gives a curve showing the extent of production during the course of the year, based on figures from England published by BUTCHER, PENTELOW & WOODLEY (1930). The curve shows that production rises very rapidly in February and March and reaches its peak in late summer; from here it falls very steeply in November. It is, of course, not really possible to apply these observations to the localities under consideration in the present work. However, the curves does show one general feature of the production in running water, viz. that while the production rises steeply during spring, it falls in autumn owing to its dependence on the amount of light. The possibility cannot be overlooked, therefore, that the production of organic material with the help of sessile algae influences the animals' mode of growth.

However, it is difficult to conceive how those animals not living on the stones' covering of algal growth should, nevertheless, be dependent of the organic material produced by the algae. This holds true for, e.g. species from moss-growths, from dead foliage and species from the stony bottom which are detritus eaters (i.e. *Leuctra hippopus* and *Simulium ornatum* – of which *Leuctra* does not have a growth-stoppage during winter). It is possible, however, that *Simulium ornatum* obtains a certain supplementary diet consisting of loose pieces of algae, but, in addition, the content of detritus in the water must rise steeply during the year, when those leaves which are deposited on the top area of the spring in autumn begin to decompose.

Those animals that live directly on the fallen foliage will, of course, also profit from this – as in the case of *Crunoecia irrorata*. According to NIELSEN (1942) this species lives on dead leaves (at least, leaf remnants dominate in the intestine contents), but it is possible that micro-organisms constitute the actual diet, and these are not so easily observed in the intestine contents. In either case, the species must surely have better nourishment conditions in the autumn when the leaves can be more easily digested by the animals.

Even more difficult to explain are the growth-conditions of the two species living in moss-growths. It is stated that *N. (Nemurella) picteti* lives mainly on detritus (HYNES 1941, BRINCK 1949), and it may be assumed that the same is true for *Pericoma blandula*?. There appear to be large quantities of this material in the localities throughout the whole year, and since the temperature conditions do not explain the growth-stoppage it is perhaps possible that the light conditions are primarily responsible.

As the growth-curves show further, the animals from moss-growth and dead foliage have always one generation yearly, and there appears thus to be a marked similarity between the growth conditions in

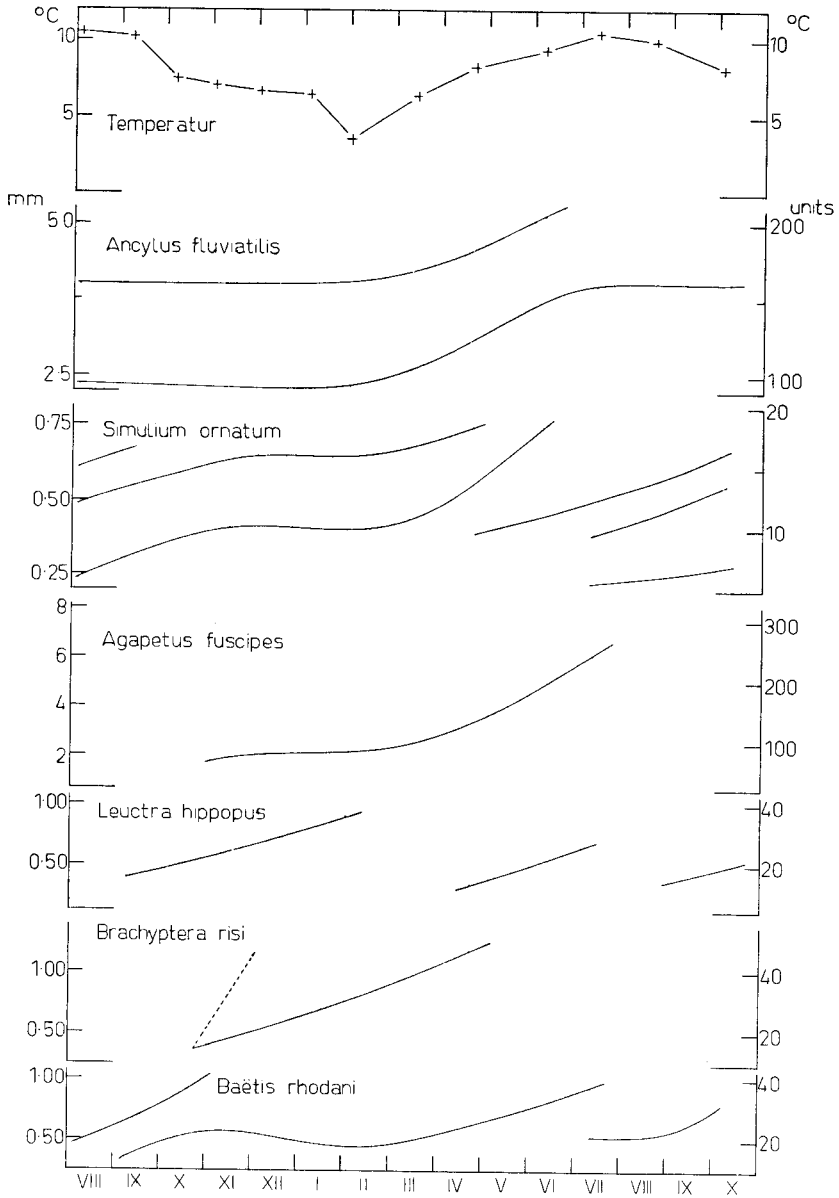


Fig. 17. Temperature-curve for the station with stony bottom in the spring Rold Kilde and idealized growth-curves for the animals collected at this station.

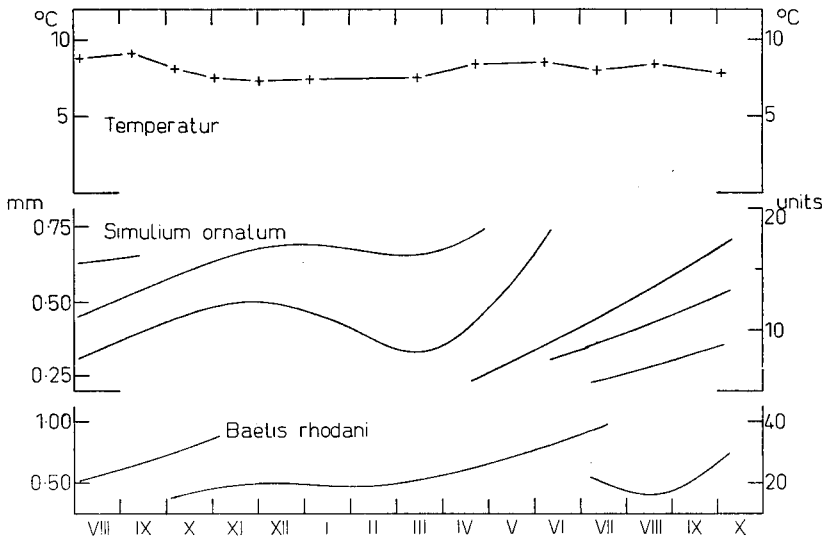


Fig. 18. Temperature-curve for the station with stony bottom in the spring Ravnkilde and idealized growth-curves for the animals collected at this station.

these two biotopes (fig. 8, 9, 15 and 16). In contradiction to this are the animals from the stony bottom in both Ravnkilde and Rold Kilde (fig. 17 and 18) where the majority of the species are characterised either by a very rapid growth (as *Leuctra hippopus* and *Brachyptera risi*) or, in addition, by having several generations yearly (*Baëtis rhodani* and *Simulium ornatum*) – which was not the case for any of the animals examined from the two other biotopes. *Agapetus* is, however, comparatively slow in its initial growth rate, and *Ancylus*, being biennial, is even slower. Furthermore, it can be seen that the growth-stoppage in winter is somewhat shorter for the stony-bottom species than for the remaining species. While the growth-stoppage for the stony-bottom species appears at the earliest in November (the exception is *Ancylus*), and only in individual cases lasts through to March (but usually finishes in February), for the species from moss-growth it begins in September and continues until February/March.

It can be seen, therefore, that the stony bottom constitutes a more favourable environment for the single species – provided they are conditioned to life in running water. There can be many reasons for this but one indisputably large factor is that the localities studied lie in open land, freely exposed to sunlight, which again means a lively production of algae. In addition, they lie below the areas of the springs, in which a large amount of detritus is produced, providing the basis for detritus-eaters to be found among the stone fauna.

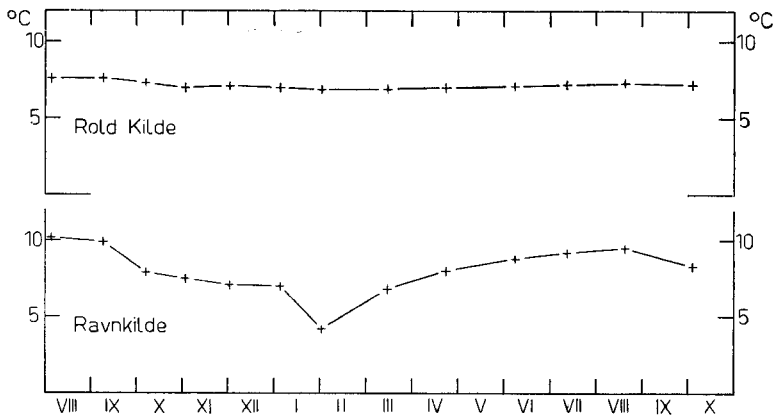


Fig. 19. Temperature-curves for the stations with piles of dead foliage in the springs Rold Kilde and Ravnkilde.

Finally, the movements of the water should be mentioned, which, in connexion with the rich algal vegetation, is responsible for the good oxygen conditions prevailing in the biotope.

When discussing the growth conditions of the single species it appeared that the mode of growth is not necessarily identical in all the localities where it has been measured. It is, therefore, a temptation to claim that the mode of growth is conditioned by the environment, but in order to prove this it will be necessary to demonstrate a connection between the environments concerned and the growth-mode of the species in the different environments. To what degree such differences in the mode of growth become genetically fixed as a racial formation, it is difficult to decide. In practice, a demonstration of a morphological difference between individuals of the different populations is required. An interesting example of how this can be accomplished is found in NIELSEN's demonstration of the presence of more species of the genus *Apatidea* with different life-cycles; these species are thought to originate in the springs where they now live and must be considered as endemisms (NIELSEN 1950 a and b).

SUMMARY

1. The material primarily originates from a thorough study of springs in Himmerland (Jutland) and was collected during thirteen collections in the course of fourteen months. Measurements of the water temperature in the collecting localities were taken at the same time. Measurements of the animals were made with the help of an Edinger Projector.

2. *Baëtis rhodani* has two generations yearly, a long winter generation and a short summer generation. It is possible that these are composed of two generations each. The growth has a stoppage in winter.

3. *Brachyptera risi* has only one generation per year, which, however, is accomplished in a very short time during winter. There is no marked growth-stoppage in winter.

4. *Nemoura (Nemurella) picteti* has one generation yearly, stretched over most of the year and with a distinct growth-stoppage in winter.

5. *Leuctra hippopus* has two generations per year, a longlasting winter generation and a short summer generation. It is not improbable that the material consists of two species, the winter generation being *Leuctra hippopus* and the summer generation *Leuctra fusca*.

6. *Agapetus fuscipes* has one generation yearly. In winter the growth is retarded, or there is perhaps even a stoppage.

7. It has not been possible to draw growth-curves for *Crunoecia irrorata*, but the histograms show that the species has one generation per year. It must be assumed that there is a growth-stoppage in winter.

8. *Pericoma blandula* (?) (determination uncertain). The species has one generation per year, with a long period in winter without evident growth.

9. *Simulium ornatum* has a biennial life-cycle with three generations, having flying times in spring, early summer and autumn. Growth-stoppage takes place in winter.

10. *Ancylus fluviatilis* takes two years to reach full maturity. Two generations occur simultaneously. The growth stops in late summer and the growth-stoppage is lasting the entire winter.

11. It is not possible from the measurements made to ascertain the growth conditions and life-cycle of *Asellus aquaticus*. The reason for this is probably that there are several simultaneous generations, and at the same time, the species has a long breeding period.

12. It is not possible to explain the animals' growth-rate by means of temperature conditions in the various localities where the animals were collected.

13. It appears that the nourishment conditions for certain of the species under discussion can offer an explanation of the growth-rate – at the same time, it is difficult to find a correlation for other species.

14. The stony bottom appears to provide a more favourable environment than the other biotopes examined, as the species living on stony bottom seem to complete their growth in a shorter period than species from other biotopes.

15. The same species do not necessarily have the same year-cycle and growth-rate in different localities.

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