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by B. Streit

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Experimental investigations on the metabolism of Ancylus fluviatilis (Gastropoda — Basommatophora)

2. Investigations on the incorporation and turnover rates of carbon

by Bruno STREIT

with 30 illustrations and 3 tables in the text

Abstract

Measurements of carbon turnover in *Ancylus fluviatilis* by means of ^{14}C-labelled food were run. It was shown that *Ancylus fluviatilis* must be treated as a three-compartment model in respect to carbon exchange. Material is not transfered directly into the reproductive organs, but stored in the rest of the body forming a mobile reserve pool. Turnover rates for the animal and the organs were determined. A carbon balance for different temperatures and animal sizes is presented.

Table of Contents

1. Introduction

Measurements regarding metabolism and energy flow are currently in the foreground of studies of limnetic ecosystems. Several metabolic balances were published initially for lotic systems (ODUM 1957, TEAL 1957, TILLY 1968, FISHER & LIKENS 1972). Later, partial aspects were investigated increasingly, and meanwhile, the metabolic and energy balances of some benthic stream invertebrates were studied, such as the insect larvae Pteronarcys scotti (McDIFFET 1970), Potamophylax cingulatus (OTTO 1974), and Agapetus fuscipes

(BENEDETTO CASTRO 1975), the amphipod Gammarus pulex (NILSSON 1974), and the pulmonates Ferrissia rivularis (BURKY 1971) and Ancylus fluviatilis (STREIT 1975). In addition, an extensive number of measurements concerning specific metabolic factors are available. On the one hand, these previously published data indicate great differences between the different animals investigated with respect to important ecological characteristic values such as the ratios production/ingestion (K_1), production/assimilation (K_2), and assimilation/ingestion, but also with respect to daily and seasonal activity, or as regards the relation between metabolism and temperature. On the other hand, considering their special structural and functional organization, we should expect an optimal metabolic strategy for each of these species.

In the present report, therefore, we shall investigate the internal carbon turnover of Ancylus fluviatilis using ^{14}C-labelled food, and, using also the data reported in Part 1, construct a balance and a model of the carbon metabolism of this limpet, which is already well known with respect to metabolism and feeding strategy (BERG et al. 1958, SCHWENK & SCHWOERBEL 1973, CALOW 1974, 1975a, 1975b, STREIT 1975). For this purpose, we shall study the carbon dynamics first of the entire animal, and then at the level of the

organ systems. Special attention will be given to carbon reserve pools and the mechanism of diverting carbon to the next generation through the reproductive organs.

2. Material and method

Ancylus fluviatilis were obtained for our experiments directly from two natural habitats, and were put directly into the experimental vessels. The animals from one habitat (Population I) lived in a brook with a maximum summer temperature of 11.8°C; those from the other habitat (Population IV) lived in a brook warm in summer, with temperatures up to 20.8°C. For our feeding and labelling experiments, the diatom Nitzschia actinastroides was filtered off on a membrane filter, and offerred to the experimental animals in 100 ml Erlenmeyer flasks over twelve-hour days as artificial Aufwuchs. Details concerning the rearing of the food animals and the measurement of ingestion, assimilation, and production may be found in Part 1. By labelling the algae with ^{14}C, we could obtain measurements of the incorporation and turnover of carbon in the same manner (see below).

All directly measured basic metabolic values, such as the level of ingestion, the assimilation ratio, height increment, egg production, and ^{14}C-incorporation, were determined under completely uniform experimental conditions. For practical

reasons, the metabolism was determined on the basis of the total carbon content. "Relative" ingestion refers to the percentual ingestion per day in relation to the molluscan carbon content. The same applies to "relative" incorporation, etc. The following values were measured, with the following abbreviations:

I ingestion — measured in terms of the surface area of algae consumed, with a known concentration per unit area

F feces — measured in terms of the feces produced during the feeding experiment

A assimilation — $= I - F$

P production — comprising only the net production of growth (tissue growth) and egg production

growth — measured in terms of height increment, converted to carbon increment for the molluscan body using the formula $y = 6.644 \cdot x^{2.656}$ (STREIT 1975)
(x = length in mm; y = C-content in μg)

egg production — egg capsules deposited

basal metabolism — i.e., respiration, secretion, and excretion, or the total assimilation not used for the net production, as calculated from the following equation:
$I - F = A = P \neq$ basal metabolism

Ink. incorporation i.e., the proportion of labelled food remaining in the body of the animal after a fixed period of feeding with labelled food followed by a fixed period of feeding with unlabelled food

The following abbreviations were used for the most important coefficients: 4

$K_1 = P/I$ production/ingestion

$K_2 = P/A$ production/assimilation

$K_{1'} = Ink./I$ incorporation/ingestion

$K_{2'} = Ink./A$ incorporation/assimilation

The concept of "incorporation" is closely connected with the conventional usage in the physiological literature, where the word refers to incorporation into relatively stable compartments of the body. Because of the very complicated exchange kinetics of labelled carbon (ATKINS 1969, SPECK & URICH 1969, CONOVER & FRANCIS 1973), a more precise characterization is not possible. At any rate, the incorporation of a labelled substance can not be equated with production or assimilation. In isotope investigations, these values can

be ascertained, at best, through a combination of several investigations concerning the uptake and release of the labelled substance (CONOVER & FRANCIS 1973, LAMPERT 1975). For details, see Section 4.2.

To produce ^{14}C-labelled diatoms and green algae,* we filled several 1000 ml Erlenmeyer flasks with 200 ml of nutrient solution and added approximately 400 ml of algal suspension from our chemostat culture. Into this suspension, measuring ca. 600 ml, we injected 40 μC $NaH^{14}CO_3$ from previously prepared 1 ml ampules. The Erlenmeyer flasks were then closed with rubber stoppers and placed on a shaking table for 15 hours under 3000 lux continuous illumination. Afterwards we separated diatoms in a Universal Junior III S laboratory centrifuge (manufactured by Heraeus-Christ Co., Osterode am Harz, Germany) in 10 minutes at 4900 g, and green (and blue) algae in 20 minutes at 6000 g; we floated these with a little Lake of Constance water and transferred them into a flat-bottomed flask. This flask was then filled with water until the extinction at 720 nm (filter S 72, thickness of layers: 1 cm) was between ca. 0.05 and 0.1. On the basis of the measured extinction, we calculated the amount to be filtered off for our experimental vessels by means of a calibration chart.

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*Green algae (Scenedesmus acuminatus) and blue algae (Anabaena cf. planctonica) were used only to gauge the different assimilability of different algal groups. The results are described in Part 1.

The blue algae had to be treated a little differently. The batch cultures, drawn into tubes, were treated with $NaH^{14}CO_3$ while they were still in the exponential phase, and kept in the tubes overnight, exposed to the air. When shaken in Erlenmeyer flasks, this suspension reacted with considerable foam and extensive destruction of the algae. The illumination was kept at a weaker level than for the diatoms and the green algae, at approximately 1000 lux.

Following the centrifugation, to gauge the specific activity of the ^{14}C-labelled algae, we filtered off three 20 ml portions of the suspension on a membrane filter with an 8 μm pore size and a diameter of 37 mm above ignited kieselgur; we used these to measure the carbon with a carbon-hydrogen analyzer, then filtered off three 2 ml portions on a cellulose nitrate filter with an 8 μm pore size and a diameter of 30 mm. These latter filters were put into a plastic counting tube, dissolved completely with 15 ml of a solution of 120 g naphthalene and 4 g PPP (2,5-diphenyl oxazole) per liter of dioxane, and placed in a Packard Co. "Tri-Carb Model 3375" liquid scintillation counter. After a period of at least two hours, during which the light-induced phosphorescence diminished and the sample became tempered, the measurement was taken. The quenching was calculated by means of an external standard (expressed as the AES ratio) using

a quenching chart established previously for the mixture of membrane filter, algae, dioxane and PPO with a known level of radioactivity. This enabled us to calculate the number of disintegrations per minute (dpm).

Aqueous samples were first made basic with 1 ml of concentrated NH_3 and put into the counting tubes in amounts of from 2 to 10 ml. We then added at least the same amount of Instagel (a Packard Co. gelling scintillator mixture, not further described), and later also the very similar substance Unisolve (Zinsser Co., Frankfurt am Main, Germany), and calculated the dpm by means of the AES and a suitable quenching chart.

To measure the activity of the limpets, we dipped them quickly in 10% NaOH to remove the shells, then put them into the counting tubes, covered them over with $\frac{1}{2}$ ml Soluene-100 (a strongly basic quarternary ammonium hydroxide marketed by Packard Co.), and dissolved them overnight in a drying oven at 45°C. We then added 7 ml of a solution of 4 g PPO per liter of toluene (analytical rate), and, again after waiting for at least two hours, evaluated the samples with a scintillation counter.

Egg capsules of Ancylus were deposited almost exclusively on the lateral walls of the experimental vessels. These were removed with a spatula bent at the tip and put into the

counting tubes. They also were dissolved overnight in ½ ml of Soluene-100 and then treated with 7 ml of a solution of 4 g PPO per liter of toluene.

To measure the radioactivity of the different organs in Ancylus, at the end of the experiment, the animals were completely submerged in a mixture of picric acid, formalin, and glacial acetic acid according to BOUIN. After a period of at least a day, they were dissected in some water in a wax cupel, and the organs were put separately into counting tubes, where they were dissolved overnight with ¼ ml of Soluene-100. The intense yellow color of the picric acid produces a strong quenching effect in toluene, so that the operating range of the quenching chart was no longer within the linear range; however, it could be linearized through a double logarithmic transformation, making it possible to determine the degree of action quite precisely even with relatively low AES values (Figure 1).

CONOVER & FRANCIS (1973) have pointed out that very considerable sources of error arise in the ^{14}C-measurement technique if the ^{14}C-exchange rates between the compartments of an experimental system are not considered. In what follows, we shall investigate the balancing relations of equilibrium after centrifugation between the algae and the surrounding water, first for the conditions in the flat-bottomed flasks,

in which the algae were still in suspension, and then in the experimental vessels, where they had been filtered off onto membrane filters. The carbon measurements were obtained with the carbon-hydrogen analyzer mentioned in Part I.

When the algae were transferred from the centrifuge into a two-liter flat-bottomed flask, they initially showed a sharp rise in their carbon content; after a few hours, this reached a saturation level up to ca. 20% above the initial value (Figure 2). The obvious assumption seems to be that the carbon uptake of the algae was restricted during the 15 hours of shaking prior to centrifugation, but that it was made possible again for some time after the addition of Lake of Constance water, until another substrate limitation occurred. However, we can not state which material represented the limiting factor in this case. Because of this change in the carbon content with time, the sample used for determining the carbon in our experiments was filtered off only after about $1\frac{1}{2}$ to 2 hours.

The dpm value found in the algae remained approximately constant throughout the experimental period. This signifies that the specific activity (dpm/μgC) fell sharply at first, then hardly fell any more, as is shown in Figure 3. In this case, according to our visually drawn curve, the decline was only around 6%. This suspension was placed under dim

light after one hour, and it was shaken less than the first suspension. However, even in this case, there was a levelling off after a period of one or two hours.

The experimental vessels were always prepared before noon. To trace the changes in the specific activity on the membrane filters, we took measurements every two hours, and plotted the binomial moving averages in Figure 4 for a 30-hour series of measurements. During the first 24 hours--the period during which most of our experiments were performed--the values averaged 87.9% of the initial value. During this time, the water reached a considerable specific activity.

Strictly speaking, all of these adjustments of equilibrium plotted in our illustrations should have been followed in each experiment and calculated afterwards, since even with largely identical preliminary treatments and experimental procedures, the algal material showed variable relations of equilibrium with respect to the ^{14}C kinetics. However, because this would have been too expensive, we did not do so, and we also did not undertake any numerical correction based on the curves found, except that, as mentioned above, the suspension in the flat-bottomed flasks was further processed, following centrifugation, only after about $1\frac{1}{2}$ to 2 hours.

As a limit of significance for our statistical calculations we chose 95%. Series of measurements in relation to the length of the shell were generally calculated as a double logarithmic regression, which treats the family of points as accidental deviation from a power function* of the form $Y = a \cdot X^b$. We designated an animal with a shell length of 4.5 mm as our "standard limpet". Details may be found in Part I.

3. Results

3.1 Turnover, incorporation, and size of carbon pools

3.1.1 Carbon turnover pattern in the body of Ancylus in relation to time

The considerable seasonal and also short-term fluctuations in the rates of ingestion and production described in Part 1 make experimental studies very difficult, insofar as any numerical value found is useful only when it can be classified within this activity pattern which is so variable in relation to time. Moreover, it is all but impossible to repeat any experiment under equal conditions, since these conditions--which undoubtedly include also the entire previous history of the animal--are not yet clearly known.

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*The term "power function" was chosen in this context following the designation of the allometric relation in this regard by other authors (e.g., ASCHOFF & KRAMER 1971, PROSSER 1973). In mathematical analysis, on the other hand, the use of this term is restricted to functions of the form $y = x^n$ (n natural).

Our investigations with isotopes were usually carried 7
out in such a way that the animals from the field were acclimated to the experimental conditions for four days in the experimental vessels, and then fed ^{14}C-labelled Nitzschia. The subsequent (unlabelled) feeding period varied in length, depending upon the problem under investigation.

To measure incorporation and turnover with the ^{14}C method, we must first know approximately how fast the labelled carbon taken up is released again through respiration, secretion, and excretion. Many of the experiments described below were carried out at 25°C, in order to obtain the highest possible results, which thus would be readily measurable. At lower temperatures, all processes should take place at a correspondingly slower rate.

We placed several Ancylus specimens on ^{14}C-labelled algae, and after 15 minutes, we removed those animals which happened to have begun feeding immediately, and put them directly into a scintillation counting tube in somewhat sterile-filtered Lake of Constance water. After another 15 minutes, we took them out again and placed them on a membrane filter covered with unlabelled food. They were put into a tube again for another quarter of an hour after 1, 2, 7, and 19½ hours. The ^{14}C passing over into the water was measured with Instagel, and the measured values are shown in Figure 5,

as the amount of ^{14}C released hourly by a single animal. The pattern on the graph implies that a portion of the carbon used for basal metabolism is respired very soon, viz., within the first hour, while a further portion is respired only over a number of days. However, this second portion can not represent carbon permanently incorporated into the tissues, since, as we will show later, the high rate of release after 19½ hours --at approximately 130 dpm per hour--with a total incorporation of 2336 dpm, would correspond to far too high a turnover rate. Instead, these values fit better with the conception of a reserve pool, built up and exhausted again relatively quickly, which would have to be largely devoid of ^{14}C again within a few days.

The feces released in the tubes was filtered off over 8 μm membrane filters measuring 30 mm in diameter and dissolved in the dioxane-PPO-naphthalene mixture. Its ^{14}C-content showed a similar pattern; in this case, however, the maximum was not in the first quarter of an hour, but only after one hour (Figure 6).

Next, let us examine the changes in the release of ^{14}C into the egg capsules in relation to time. For this investigation, we labelled animals at 25°C for 24 hours. We then measured the ^{14}C-contents of the egg capsules deposited during the next 16 days of feeding with unlabelled food, and

also the amount still found in the animal afterwards. Three different representative examples are presented in Figure 7: One animal (circles) deposited one egg capsule with a relatively high ^{14}C-content on the first day of this subsequent feeding period, and three more with relatively low ^{14}C-contents on the following days. Another (dots) did not begin to produce eggs until more than four days after labelling, then deposited eggs showing a largely uniform decrease in ^{14}C-content. Finally, a third animal (triangle) deposited only one egg capsule after more than four days. The egg capsules were all of comparable size, so that a decrease in the percentage of ^{14}C represented also a corresponding decrease in specific activity.

The specific activity of the eggs deposited after several days was still much too high to assume that this ^{14}C had been mobilized again from the permanently incorporated portion. Instead, once again, the values fit only in a model based on the assumption that a portion of the carbon assimilated is incorporated into a carbon reserve, where it is replaced by a further carbon supply in a few days. Since the specific activity also was low in the egg capsules produced only after several days, but high in those produced immediately, we must assume that this reserve pool required for egg capsule

production is in a constant dynamic equilibrium between supply and use even when oviposition is not taking place. Hence, this is probably the same reserve pool from which came the ^{14}C found in respiration after the high initial release of ^{14}C.

In Figure 7, the egg capsules deposited over a period of 16 days contained up to 72.8% of the total ^{14}C-content. In 54 series of measurements with only four days of subsequent (unlabelled) feeding, we found a maximum of 60.5% of the ^{14}C in the egg capsules. If eggs were deposited while the labelling experiment was still in progress, their maximum level of ^{14}C was 56.5% of the total ^{14}C-content at 25°C, but sometimes it also was very little.

In summary, then, we find the following pattern: The specific activity of egg capsules deposited already during the labelling period may be high or low, depending on whether they have already taken up the labelled carbon or not. The specific activity shown by egg capsules deposited after the labelling period decreases constantly with increasing intervals of time. As was shown in Part 1, Ancylus divides the portions used for growth and for egg production into 81.2% for egg production and 18.8% for growth. Hence the percentages obtained with ^{14}C-labelling for the egg capsules were always lower. However, the

incorporation values for the animal do not represent only growth, but the amount of carbon incorporated, which involves the turnover of the tissues as well as growth. Therefore the values found in the above experiment are not inconsistent with the distribution coefficient reported previously.

Now let us test this model in relation to temperature. The mean values, and also the standard deviations and extreme values, of the percentage of the ^{14}C-content found in the egg capsules during four days of subsequent (unlabelled) feeding are plotted in Figure 8. The mean values are grouped, without any distinct tendency, around an average of 29.5%. The maximum values increase gradually from 28.8% at 10°C to 60.5% at 25°C. This uniform rise in the maximum values probably reflects the faster turnover rate of the reserve pool with rising temperatures. At low temperatures, the pool is filled gradually over several days, and the next egg capsules deposited show a relatively low percentage of the ^{14}C-content. With high temperatures and accordingly high turnover rates of the reserve pool, egg capsules deposited shortly after the labelling period may also show a very high percentage. Perhaps this relation to temperature results not only 9
from an increased turnover rate, but also from the fact that the average size of the pool is greater at lower temperatures. At a temperature of from 20° to 25°C, the half-life of the carbon reserve must be about one day.

The size of the reserve pool can be estimated on the basis of the fact that an individual egg capsule contained a maximum of 56.5% of all the labelled carbon found in Ancylus and the egg capsules together, but the limiting value in all egg capsules deposited subsequently also was not much higher. Hence, the pool was largely emptied for this oviposition. Assuming an amount of 37 µg carbon in the egg capsules (the average value for five eggs), this gives a value of fully 6% of the carbon content of an animal with a shell length of 5.5 mm and 615 µg C.

According to the data in the literature, the most likely reserve materials in pulmonates are glycogen and galactogen (polysaccharides composed of units of glucose and galactose, respectively). Only one datum is available for Ancylus fluviatilis, in BERG (1953), and certainly this should not be generalized. However, the value of 5.8% glycogen reported in that case fits well with the pattern of a reserve pool which we have outlined on the basis of the carbon kinetics. Given the very variable C:N values of Ferrissia rivularis (BURKY 1971), it must be assumed that the pool is considerably larger from autumn to early spring, with C:N values up to 4.8, than during the other seasons (minimum C:N ratio, at the end of May, 2.94).

3.1.2 Carbon incorporation in relation to body size

In this and the following section concerning investigations on the rate of incorporation in relation to various parameters, we fixed a uniform subsequent (unlabelled) feeding period of four days. Hence the reserve pool was largely empty of ^{14}C, and the measured levels of activity approximately reflect the permanently incorporated portion.

Once again, like the relative ingestion (cf. Part 1), the relative incorporation (in relation to the carbon content of the body excluding the shell) generally was approximately in proportion to the size of the body. Also the b exponent in the allometric formula was again lower in the autumn (Figure 9). There did not appear to be any direct connection with the experimental temperature.

If the incorporation/ingestion ratio--which we shall designate as K_1' to differentiate it from K_1 (production/ingestion) --is taken in relation to the size of the body, this gives the relation shown for 10°C, 19°C, and 25°C in Figure 10. K_1' decreases with increasing body size. At 10°C, the falling tendency of the curve is not significant, but the absolute values for b and r become greater with rising temperatures, and the decrease becomes significant. A striking feature is the considerable variation of the individual values at all temperatures, indicating that the different animals differed

greatly during the experiment with respect to the percentage of the ingested and assimilated carbon lost through basal metabolism, and the percentage available for storage and growth. According to these charts, an animal containing 10 μg of carbon (corresponding to a shell length of 1.2 mm) showed only a slight diminution of the value for K_1, from 20.9% at 10°C to 9.6% at 25°C; whereas adults containing 1000 μg C (corresponding to a shell length of 6.6 mm) showed a sharp reduction from 17.1% at 10°C to 2.0% at 25°C. Thus juveniles showed a very high efficiency of incorporation in relation to the level of ingestion even at high temperatures. However, since the ingestion rate generally varies approximately in proportion to the body size (Part 1, Figure 19), this signifies that the relative incorporation rates (in relation to the carbon content of the animals) were higher for juveniles than for older animals. Corresponding results were obtained for the production rates at 16°C (Part 1, Figure 26). --All of the above incorporation rates took into account also the carbon transferred into the egg capsules deposited in each case. Therefore the sharp reduction of the incorporation value in adults measured after four days of subsequent (unlabelled) feeding was not caused by the fact that material was diverted into the egg capsules; rather, it indicates a higher rate of general basal metabolism.

3.1.3 Carbon incorporation in relation to the temperature and season

We shall now examine the relation between carbon incorporation and temperature, and how carbon incorporation is related with production. Once again, we investigated the values for mid-August separately from those for the period from late August to mid-October. For this second period, we made separate calculations for the old and the new generation; the statistical material did not permit any satisfactory conversion for our standard limpet.

In Figure 11, the mean values of the series in the spring and early summer, together corresponding to the normal activity defined in Part 1, are represented by dark circles, and the highest points, representing a maximum incorporation analogous to the maximum activity defined previously, are connected by a solid line. Thus the highest relative carbon incorporation value was found at 16°C, and amounted to 6.8% in relation to the carbon content of the animals. In the autumn, for both the old and the new generation, the maximum point shifted into a higher temperature range, from 22°C to 25°C. Because there were not as many series available in this case as there were for the values before the end of August, and also because they did not vary as much, we grouped them together. The smoother curve for the autumn is in accord with the corresponding findings for ingestion.

Because of the great variations in activity, it is difficult to coordinate the measured incorporation rates with specific rates of production. The maximum incorporation for the spring, as ascertained above, and the maximum production in the spring, as found in Part 1 (Figure 29) are compared in Figure 12 (shaded area in the upper graph). In the lower graph, the average incorporation values for late summer and autumn are compared with the average production in the late summer and autumn. The autumn incorporation rate shown here corresponds to the arithmetical mean between the points for the old and the new generation in Figure 11. These graphs show that the incorporation values were usually clearly higher than the production values. Therefore the difference corresponds to the portion incorporated in addition to the production, which maintains the dynamic equilibrium between construction and degradation of the tissues. This is at approximately the same level as the production itself. In relation to the carbon content of the animal, in a moderate temperature range, this was about 2%, corresponding to a half-life of 34 days. This does not take into account the fact that, naturally, during the course of the four days of subsequent (unlabelled) feeding, some of the ^{14}C originally incorporated had already been released again.

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The still quite high incorporation rates also at low temperatures, in contrast to practically no production, must correspond to the assimilation of reserve material. The ratio of the incorporation rates to assimilation is plotted in Figure 13. At temperatures of about 13°C and up, the incorporation rates were almost always somewhat higher than the production rates (K_2), corresponding to the portion incorporated in addition to production, as required to maintain the dynamic equilibrium. At lower temperatures, the ratio of incorporation to production was considerably higher, with the incorporation of carbon representing up to 46% of the assimilation. A comparison with Figures 10 a - c shows that the extensive individual differences contrast with considerably smaller differences between series.

3.1.4 Influence of food supply and temperature on activity and the size of the reserve pool

As we have already explained in Part 1 and in Section 3.1.2 above, Ancylus fluviatilis shows considerable seasonal variations in the rates of ingestion, incorporation, and production. It therefore seemed appropriate to investigate whether the food supply can determine the level of activity. In what follows, then, we shall describe investigations on the metabolism of the species under starvation conditions.

We kept 20 animals at 19°C beginning in May. After 24 days, we removed their food; we measured the carbon contents of three animals at the beginning of the starvation phase, and of another three animals after 4, 8, 12, and 16 days of starvation. We also gauged the production of feces and egg capsules. The results are compiled in Figure 14. During the first 8 days--if indeed we may accept the almost linear pattern of this section of the curve as truly exponential--the carbon content of the animals fell by 4.2% each day. However, the decrease from the eighth day to the sixteenth was only 1.8% daily. After the sixteenth day, all of the experimental animals were dead. Thus a carbon content of about 50% must represent the lower limit reached by the living animals. Incidentally, a reduction in weight to 50% of the initial value seems to be the limit of survival also for other poikilotherms (FLOREY 1970, KAUSCH 1972).--BERG et al. (1958) also found a reduction of the respiration value with several days of starvation; however, their values can not be compared with the present values. 12

A comparison of these values regarding the degradation of organic substances during starvation with the values for basal metabolism (assimilation minus production) calculated in Part 1 (Figure 30 and p. 487) and with the values for respiration reveals that the 4.2% daily reduction measured at the

beginning of the starvation phase at 19°C practically corresponds with the values for respiration (3.8% converted according to BERG, assuming an RQ of 0.812), but not with our calculated value for basal metabolism (30.3% at 19°C). This is also an indication that the high value for basal metabolism occurs only with simultaneous nutrition.

We observed a notable amount of fecal production only during the first four days, an observation already made also by CALOW & FLETCHER (1972) at 10°C. Egg capsules were produced until the end; initially, however, one capsule per animal was deposited every two days, whereas at the end this fell to one every four days. Simultaneously the average number of eggs per capsule diminished from 4 to 2. However, the average carbon content of the egg capsules was not less than that shown by animals with a corresponding number of eggs not subjected to starvation.

Hence, according to these findings, the animals remained active even when their food was taken away, and continued to deposit eggs until they were exhausted, which happened at about 50% of their original carbon content. We carried out another series of investigations to clarify the behavior of the rates of ingestion and production when food was provided again after several days of starvation.

We obtained 12 animals in nature at the end of May and placed them in experimental vessels at 19°C. Four of these were fed after 3 days, another four after 6 days, and the remaining four after 9 days. Two of the animals--one which had starved for 6 days, and one which had starved for 9 days--died in the first six days after food was provided; the rest survived. All of the animals showed largely the same high rates of ingestion when food was provided again as were shown during this season also by animals not subjected to starvation.

However, as soon as feeding had been started again, they also produced egg capsules with a smaller number of eggs, and thus also a lower average carbon content, per capsule. This situation persisted, without any tendency to increase or diminish, until the end of the experiment 24 days later, so that the average number of eggs for the animals with three, six, and nine days of starvation was 4.0, 3.7, and 3.2 respectively. As shown in Figure 15, egg capsules with only two eggs began to appear already after six days of starvation, while after nine days, capsules with only a single egg began to appear.

When limpets were placed in the experimental vessels with food in the late spring, for a long time they deposited ca. 5 eggs per capsule; this value fell to 3 eggs per capsule only after a long period of observation. Evidently at this 13

point the available reserves were exhausted. It appears that under starvation conditions, this process of exhausting the reserve material is merely accelerated. Under our experimental conditions, a recovery did not occur.

The ratio of egg capsule production to growth in the starvation experiments of Figure 15 averaged 78.5%, but this was not significantly different from the previously reported value of 81.2% (cf. Part 1, Figure 24). Separately, the values found were 78.3% after 3 days of starvation (4 animals with n= 21 measurements altogether), 84.6% after 6 days of starvation (n= 18), and 72.6% after 9 days of starvation (n= 10).

It was noticeable, then, that the animals maintained intensive production of egg capsules, and even remained fully active, when food was no longer available to them. Even the distribution coefficient of about 81.2% to 18.8% remained unchanged. Thus the respiratory pattern outlined by WIESER et al. (1970) for the land pulmonate Arianta arbustorum, in which the level of activity is determined primarily by endogenous control factors, seems to apply also to Ancylus. In the above example, the activity was accordingly set at a high level and remained at this fixed setting until death by starvation. However, the carbon content of the egg capsules deposited diminished together with that of the adults.

Therefore the food supply does not effect the level of activity, but it influences the level of the reserve pool, and thus also of the egg capsules.

If the animals are kept at temperatures below 13° in the spring, they often show a low rate of production, reaching a phase of higher activity only months later, depending on the individual. They then also live to an old age. Above ca. 13°C, however, the limpets shift spontaneously into a higher level of activity, deposit many egg capsules, and die a few months later (depending on the temperature). Thus it appears to be the case that above 13°C, these animals are no longer able to reduce their metabolism, and allow it to continue unchecked even under starvation conditions. This would accord with the fact that the animals from the brook warm in summer (Population IV) already die off in July (STREIT 1974, 1975), whereas those of Population I, with a maximum summer temperature of 11.8°C, do not perish until the autumn. At 7°C or below, the animals soon pass into a relatively dormant state, or die. Then no developmental cycle can continue.

3.2 Incorporation and turnover of carbon in the organ systems

3.2.1 Anatomy, morphology, and morphometry of the organs

Anatomically, adult and juvenile individuals of Ancylus differ in two respects, which must have effects on their metabolism: First, the juveniles still completely lack the female reproductive organs, which are very striking in adults; 14
secondly, the relative size of some of the other organs is characteristically altered (Figure 17).

The gonad (ovotestis), which produces oocytes and spermatozoa, grows disproportionately to the rest of the body. In small animals, it is an unobtrusive organ at the posterior end of the visceral sac. In large animals, it has attained approximately the same size as the stomach. This positive allometry is represented in Figure 18a. The b exponent, at 1.592 with a high correlation coefficient $r = 0.959$, is significantly higher than 1.

In contrast, the radula shows very negative allometry, with a b exponent of 0.679 (Figure 18b). It is constructed with its maximum possible extension in juveniles, extending to the posterior end of the visceral sac. In adults, it remains visible only as a small appendage of the head (Figure 17b).

The remaining feeding organs and digestive organs all show approximately isomorphic growth. This is shown for the stomach and the length of the gut in Figures 18 c and d.

All of the organs discussed so far maintain the same geometrical shape throughout ontogenesis, so that analogous carbon contents should correspond to these allometric relations of size. A different situation prevails with respect to the midgut gland. Its linear dimensions likewise indicate approximately isometric growth (Figure 18e), but this organ seems to be rather flattened in larger animals, so that the relative carbon content is somewhat reduced (cf. Table 1, p. 15).

Another group of organs, which must be classified under the female reproductive system, show still other growth characteristics. These organs include, on the one hand, the large, lobular albumen gland, as the main source of nutrients for the eggs, and on the other hand, other appendages (the mucous gland, etc.) which sometimes also contribute material for the egg capsules, and which we may designate collectively as the "other female reproductive organs". In animals with shells measuring less than about 2 - 3 mm, the female reproductive organs are no longer visible even with a stereomagnifier, being present only as a tiny primordium. During the course of ontogenesis, these organs gradually push back from the anterior left side of the visceral sac, between the foot and the digestive tract, towards the posterior,

showing very strongly positive allometry (b exponent for the albumen gland = 3.142). The albumen gland, always somewhat farther advanced in this process than the other organs, finally reaches the posterior margin, near the gonad. However, there is no really definite maturation size. Certainly below a shell length of about 3.9 mm these organs are not able to produce egg capsules. At this length, however, in exceptional cases, we observed oviposition, but then the capsules contained only one or two eggs. This was the case especially with animals reaching maturity only in the summer or autumn, and not with those which were already fully grown in the spring. At a shell length of 5 mm, the albumen gland usually has already reached the gonad, and the animals deposit eggs regularly. Later, however, it may still grow disproportionately, extending forwards and backwards to all unoccupied regions of the visceral sac, as facilitated by its plastic morphology. The albumen gland and the other female reproductive organs showed largely the same type of reaction, with respect to the ^{14}C-incorporation analyzed later as well as with respect to growth characteristics.

In adults, the size of the albumen gland shows a pattern of seasonal variation. In Figure 18f, "J" indicates the regression line which applies generally to juveniles up to 5 mm shell length, while line "1" represents the values

measured in adult animals of Population I from the spring until early July. The albumen glands of animals with a corresponding shell size were smaller already in August ("2"), and even smaller in September and October ("3"). This gradual degeneration corresponds to an analogous diminution of the capacity for egg production, as will be shown later.

The only other organs contained in the visceral sac in addition to those mentioned so far are the excretory duct of the gonad (ovotestis) and the vesicula seminalis. Farther to the right, as an offshoot of the male copulatory organ, embedded in the midgut gland, is the coiled end of the flagellum.

All other organs not yet enumerated were grouped together under the designation "Resttier" (i.e., "remainder of the animal"). This includes the entire foot, the head with the buccal mass, the salivary glands, the ganglia, the heart, the kidneys, and the male reproductive organs. We also included the radula in this group. This arbitrary, but practical kind of classification has proven useful for physiological investigations also with other animals (cf., e.g., the "Restkrebs", "remainder of the crayfish", for Orconectes in SPECK et al. 1972).

In order to have a basis for calculating the incorporation rates and turnover values, we measured the carbon content of these organs. The values were obtained from organs fixed with Bouin's fixative. Preliminary investigations revealed no significant differences in carbon content between fixed and unfixed material.

In all organs and organ systems, the percentage of carbon changes to some extent with the length of the shell. 16
In Figure 19, based on the values of 11 measurements using 1 to 3 animals each time, we have calculated a linear regression and its 95% confidence region for the "remainder of the animal", although this does not mean that the statistical relationship may be viewed as linear. For the other organs, the (statistical) estimated values for a juvenile measuring 2 mm and an adult measuring 6 mm are compiled in Table 1.

Organe 1	2 mm	6 mm
Resttier 2	71,2%	62,5%
Mitteldarmdrüse 3	18,4%	12,9%
Magen 4	5,1%	3,6%
Darm 5	4,9%	3,4%
Gonade 6	0,4%	2,3%
Flagellum		1,6%
Eiweißdrüse 7		6,1%
Restl. weibl. Fortpflanzungs-Organe 8		7,6%
	100,0%	100,0%

Table 1. Percentage of carbon in the different organs of Ancylus fluviatilis, for animals measuring 2 mm and 6 mm in length. Key: 1- organ 2- "remainder of the animal" (Resttier) 3- midgut gland 4- stomach 5- gut 6- gonad 7- albumen gland 8- other female reproductive organs (Read commas as decimal points.)

3.2.2 Incorporation of carbon into the vegetative organ systems in relation to the season, temperature, and body size

In Figure 19, the carbon content of the "remainder of the animal" was shown as percent of the content of the entire animal. This percentage decreased only slightly with increasing body size. To calculate the incorporation of ^{14}C into the "remainder of the animal" we selected 60 animals from a temperature range of from 13°C to 25°C, since there were no significant differences due to temperature in this range (see below). In Figure 20, the result is shown again as a linear regression against the length of the shell. According to this graph, for juveniles measuring approximately 1 mm, incorporation into the "remainder of the animal" corresponded with the carbon percentage. However, as the animals grew larger, an increasingly smaller percentage was conveyed into the "remainder of the animal"; i.e., the visceral complex gained increasing importance in the dynamics of metabolism with increasing age.

To ascertain whether the percentage incorporated into the "remainder of the animal" is related to the experimental temperature, we calculated the estimated value of each series for our standard limpet. These values are presented in Figure 21; values for the spring animals are shown as dots, those for the autumn animals as circles. The graph indicates no difference

between spring and autumn animals. We also found no distinct trend between temperatures from 10°C to 25°C. The arithmetical mean in this range was 48.05%. However, below 10°C, the incorporation percentages were higher, and at 5°C, they reached approximately 62%.

We also tested relations to temperature for the midgut gland, the gut, the stomach, and the radula, but no definite relations were detected. Instead, incorporation into the reproductive organs was depressed at low temperatures, as will be shown in the following section. The carbon thus saved was then available for the "remainder of the animal". We also found no seasonal variations for the vegetative organ systems. In what follows, therefore, regarding these other vegetative organs, only the relation between the percentage incorporated and the length of the shell will be examined.

The values for incorporation into the midgut gland and into the gut showed a tendency to a slow, but significant increase with increasing shell length; those for the stomach were practically constant, or decreased very slightly (Figure 22). However since, according to Table 1, the percentage of carbon in these organs fell slightly, this means that the specific incorporation was higher in older animals, and thus that the carbon turnover in these organs was accelerated. The radula, which was included with the "remainder of the animal"

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in our investigations, showed lesser incorporation values with increasing shell length (Figure 22), consistently with its decreasing relative size; however, this was only a very small part of the general decrease in the percentage incorporated into the "remainder of the animal".

3.2.3 Incorporation of carbon into the reproductive organ systems in relation to the season, temperature, and body size

The gonad is located at the posterior end of the visceral sac, and produces spermatozoa and oocytes in adults. Its incorporation rates, as percent of the amount incorporated into the entire body of the animal, are shown in Figure 23 in relation to shell length, for animals measured in the late summer and autumn. The rising percentage of the total incorporation with increasing body size corresponds with the positive allometry of this organ shown in Figure 18a. The values measured in the spring were always higher, yielding the regression line

$$y = 0.629 \cdot x^{0.730},$$

where $y = {}^{14}C$-incorporation in percent and x = shell length in mm, with $n = 69$ measurements and a correlation coefficient $r = 0.433$.

To ascertain whether the percentage of ^{14}C incorporated is related to temperature, we calculated a double logarithmic regression for the percentual values against the length of the shell, and noted the (statistical) estimated value for

an adult animal measuring 6 mm, separately for the spring and autumn animals, in Figure 24. Each series of points was connected by a visually estimated curve. Whereas the spring animals showed a distinct increase due to temperature from approximately 1.0% at 5°C to 2.5% starting with 13°C, this was not found in the case of the autumn specimens. Hence in the spring, the gonad participates in metabolism most intensively in that temperature range in which oviposition also takes place with full intensity, as shown by a comparison with Figure 24 in Part 1. The generally lower values in the autumn correspond to the considerably lower need for sex cells in the animals of the previous year, which are then already quite old. Juveniles also showed higher incorporation rates in the spring, with an estimated value of 1.20% for a limpet measuring 3 mm in length (standard deviation $s = 0.22\%$ with $n = 12$), than in the autumn, with a corresponding value of 0.56% ($s = 0.09\%$ with $n = 17$).

As shown in Figure 25, also the albumen gland and the other female reproductive organs showed an increase in percentual incorporation corresponding to positive allometry. The course of the curve is evidently multiphase, as we have already shown for the morphometry in Figure 18f. To test the incorporation into the albumen gland in relation to temperature, we entered the arithmetical mean for adults

measuring 5 mm and up in each individual investigation series, 18
separately for spring and autumn specimens, in Figure 26. For the sake of clarity, we noted the standard deviation and the number of experimental animals only for the animals of spring and early summer. This graph shows considerably wider variation than the graph for the gonad. However, it does not show any difference between spring and autumn animals. All of these values represent percentages of the total amount incorporated by the animal. Since this total amount incorporated by the animal was lower in the autumn, the absolute incorporation values in the autumn were correspondingly lower. The mean plateau value of the visually drawn trend line was 7.2%. However, at 5°C, the incorporation rate was only about 2%.

The other female reproductive organs showed the same tendency and range of variation as the albumen gland, but their percentual values averaged 1.36 times higher (arithmetical mean at n = 56; extreme values: 0.41 - 4.23). Thus the gonad, albumen gland, and other female reproductive organs showed a different relation to temperature from that of the other organs. Together they incorporated approximately 1.5% + 5.2% + 7.1% = 13.8% more in the plateau range of Figures 24 and 26 (starting from 10°C - 13°C) than at 5°C. This explains the pattern in relation to temperature of the incorporation values into the "remainder of the animal" in Figure 21.

In view of the sharp reduction of the absolute amounts incorporated into the animal with falling temperatures (cf. Figure 11), the additional retardation of the rate of incorporation into the reproductive organs practically represents a shifting of these organs to a minimum metabolism. The other organs can not be similarly restrained, because they are necessary for the life of the animal. The critical temperature probably varies a little at different times, averaging ca. 10°C. At these low temperatures, as mentioned above, we found a rather high ratio of carbon incorporation to assimilation, which was interpreted as a deposition of reserve materials. However, these materials are evidently not conveyed directly into the reproductive organs, but distributed in the rest of the body.

3.2.4 Comparison of the carbon turnover rates in the different organs

To guage the level of the simultaneous anabolism and katabolism in the organ systems, we measured the incorporation values of these organs considered in relation to the amount ingested, and with different periods of subsequent (unlabelled) feeding, at 19°C and 25°C. Organs with a more intensive turnover rate could be expected to show a faster decrease in the incorporation/ingestion ratio over a period of time than organs

with less active metabolism. We performed these experiments during different seasons, so that the level of activity, for both individuals and whole groups of animals, was slightly different. Consequently, the values found should be considered as provisional estimates.

Figure 27 shows the decline of the incorporation/ingestion ratio for various organs of adult animals during the course of 16 subsequent (unlabelled) feeding days (= 384 hours). The chart shows certain irregularities, perhaps actually based on multiphase kinetics (cf. BUCHANAN 1961). On the other hand, obviously a wide range of individual variation is generally found in such experiments (cf. also GRASZYNSKI 1968). The points in Figure 27 are based on the arithmetical means between the 25°C and 19°C series, so that they are representative for a temperature of approximately 22°C. 19

The four points along each of the curves were subjected to a linear regression (not a semi-logarithmic regression as shown in Figure 27), and the estimated values for one hour and for 384 hours (= 16 days) of subsequent (unlabelled) feeding were used as indicators of the decrease in labelled carbon during the first 16 days after labelling. From these values, we calculated the daily turnover rates and the half-life according to a continuous logarithmic reduction by the formula

$$Y = a \cdot e^{-kt},$$

where Y = the ratio of incorporation to ingestion in percent, t = the time in days, and a and k are constants.

This yielded a half-life of 14 days for the entire animal. Within this period, then, half of the carbon originally incorporated is replaced. To check the reliability of these values, we also compared the average incorporation values during four days of subsequent (unlabelled) feeding with the production value; this gave 34 days as a half-life for the entire animal, as already calculated above (Section 3.1.3). Probably the difference compared with the 14 days is largely explained by the fact that in the first case, the turnover rates were calculated beginning with the first hour of the subsequent feeding period, and thus the rapid turnover of the reserve pool with a half-life of only one day led to the calculation of a very high overall turnover. In the second case, however, the incorporation rate measured on the fourth day was used as the point of departure, so that the rapid turnover of the reserve pool was not greatly considered. Using the carbon values for the organs in Table 1, we could apply this second method also to the organs. The two independent methods of calculation yielded practically the same order of turnover rates for the different organs. The different series of possible turnover rates based on these calculations are expressed in Table 2 by the different ranges of the half-life periods shown. The table reproduces the different numerical

Organ	Halbwertszeit in Tagen 1	Durchschnittliche tägliche Turnoverrate 2 in %	*Helix pomatia* µg O_2/mg TG · h
Gesamttier 3	14	4,8	—
Ganglien 4	—	—	2,53—4,00
Mitteldarmdrüse 5	7— 8	9,7	1,39—2,78
Darm 6	7— 8	9,1	2,56—2,70
Niere 7	—	—	2,05—2,24
Magen 8	6—13	8,5	—
Radula 9	9—12	7,8	—
Gonade 10	7—16	6,7	—
Restl. weibl. Fortpfl. 11	12—14	5,1	ca. 1,03
Eiweißdrüse 12	13—15	4,8	1,17—1,20
Resttier 13	24—27	2,7	ca. 0,85

Table 2. Half-life and daily rate of carbon turnover in Ancylus fluviatilis at 22°C, compared with the tissue respiration of Helix pomatia at 28°C according to KERKUT & LAVERACK (1957). All specimens were adult animals. TG = dry weight. Key: 1- half-life in days 2- average daily turnover rate 3- entire animal 4- ganglia 5- midgut gland 6- gut 7- kidneys 8- stomach 9- radula 10- gonad 11- other female reproductive organs 12- albumen gland 13- remainder of the animal (Read commas as decimal points.)

values for the mean turnover rate from the first to the three hundred and eighty-fourth hour of subsequent feeding (first method). By way of comparison, the table also shows the tissue respiration levels found for certain organs by KERKUT & LAVERACK (1957) in Helix pomatia at 28°C. Comparison indicates that the daily turnover rate runs parallel to the tissue respiration.

These values show that our separation of the "remainder of the animal" from the other organs was justified even from a functional point of view, since the "remainder" had a considerably lower rate of carbon turnover. The turnover rates

of the stomach, the gut, and the midgut gland were about three times as high. The low exchange activity of the female reproductive organs is striking, as these were always measured in adult specimens; yet the values for tissue respiration reflect the same tendency. 20

3.3 Balance and model of the carbon metabolism of *Ancylus fluviatilis*

A balance for normal activity of adult animals is presented in Figure 28, revealing the position of different ecological cardinal points: The maximum ingestion and assimilation are at 25°C, the maximum production and incorporation at 16°C - 19°C. For production and tissue turnover, incorporation has to occur in this temperature range. At lower temperatures, where incorporation considerably exceeds production, a large portion of the material taken up is stored. Below approximately 8°C, much of the material taken up is used neither for basal metabolism nor for tissue increment or oviposition, so that both the ratio of incorporation to assimilation and the ratio of incorporation to production are at a maximum in this temperature range. If the reserve pool is already full at low temperatures, possibly the animal switches to a lower level of activity; at present, however, we can only speculate about this problem.

The ratio of the different metabolic parameters to one another (K_1, K_2, A/I, etc.) seems to remain approximately constant throughout the year, so that seasonal metabolic variations are manifested only in fluctuations of the values recorded in Figure 28. In juveniles, however, the balance is much more favorable in relation to production, and the correlation with temperature also is less marked.

The metabolic values during normal activity for 21
juveniles with 10 µg C (1.2 mm shell length) and adults with 1000 µg C (6.6 mm shell length) at three temperature levels (10°C, 19°C, and 25°C) are compiled in Table 3. In this table, we presupposed a level of ingestion exactly proportionate to the size of the body (cf. Part 1, Figure 19) and fixed the assimilation ratio (A/I) of the juveniles at the same level as that of the adults. Moreover, the same type of relation between the production rate and body size found at 16°C (Part 1, Figure 26) was ascribed also to the other temperatures. The percentage values listed in lines 1 - 6 are in relation to the carbon content of the animal. Thus, with a relative ingestion of 16.6% at 10°C, a juvenile with 10 µg C consumes 1.66 µg C per day, whereas an adult with 1000 µg C consumes 166 µg C. Various ecological coefficients, expressed as percentage values, are listed in lines 7 - 11.

	1 Jungtiere (10 µg C)			2 Adulttiere (1000 µg C)		
	10 °C	19 °C	25 °C	10 °C	19 °C	25 °C
1. I Ingestion	16,6 %	50,5 %	81,1 %	16,6 %	50,5 %	81,1 %
2. F Faeces	7,9 %	17,8 %	28,3 %	7,9 %	17,8 %	28,3 %
3. A Assimilation	8,7 %	32,7 %	52,8 %	8,7 %	32,7 %	52,8 %
4. P Produktion 3	3,3 %	6,6 %	5,8 %	0,8 %	2,4 %	1,1 %
5. Wachstum 4	3,3 %	6,6 %	5,8 %	0,7 %	0,4 %	0,2 %
6. Eiproduktion 5	—	—	—	0,1 %	2,0 %	0,9 %
7. A/I	52,5 %	64,8 %	65,1 %	52,5 %	64,8 %	65,1 %
8. K_1 = P/I	19,8 %	13,1 %	7,2 %	4,6 %	4,8 %	1,4 %
9. K_2 = P/A	37,8 %	20,2 %	11,0 %	8,7 %	7,4 %	2,1 %
10. $K_{1'}$ = Ink./I 6	20,9 %	12,6 %	9,6 %	17,1 %	6,0 %	2,0 %
11. $K_{2'}$ = Ink./A	39,8 %	19,4 %	14,8 %	32,6 %	9,3 %	3,1 %

Table 3. Metabolism of Ancylus fluviatilis for juveniles with 10 µg C (= 1.2 mm shell length) and adults with 1000 µg C (= 6.6 mm shell length) at 10°C, 19°C and 25°C. The values shown in lines 1 - 6 are in relation to the carbon content of the animal (exclusive of the shell).
Key: 1- juveniles 2- adults 3- production 4- growth 5- egg production 6- Ink.= incorporation
(Read commas as decimal points.)

K_1, (incorporation/ingestion) and K_2, (incorporation/assimilation) relate to the carbon incorporation measured by the ^{14}C method, and their average is somewhat higher than that of K_1 and K_2. It is striking that all values in lines 4, 5, and 8 - 11 are higher for juveniles than for adults. This difference is more marked at high temperatures than at low temperatures.

In Figure 29, we have attempted to construct the simplest possible functional model for carbon turnover in the body of Ancylus which can explain the data found above. The quantitative data should be accepted only as provisional estimates. They relate to an adult animal in summer at

approximately 22°C, and indicate relative daily carbon values in percent of the carbon content of the body. The numbers shown in parentheses were calculated indirectly from the other values; the value of 2.5, corresponding to the amount of mucus released by the pedal gland, was taken from the literature (CALOW 1974). At lower temperatures from autumn to spring, the reserve pool was larger, and the basal metabolism was not so high compared with the other metabolic values, but the data available were insufficient for reliable estimates.

As the illustration shows, the animal takes up in 22
food approximately 40% of its own carbon content, from which it assimilates approximately 25%, while the remaining 15% is released again in the feces. Since a large amount of the labelled carbon was detected in the water soon after it was taken up by the animal (cf. Figure 5), we must assume that the carbon is initially conveyed to a pool with a very fast turnover rate (POOL S_1). This pool is linked with a large pool with 2% input and 1.8% output (POOL S_2). Although it is still problematical to coordinate these pools, which we have inferred on the basis of the carbon kinetics, with specific structures or compounds, probably the large pool (S_2) corresponds mainly to tissue turnover or average protein turnover. A third pool, which functions as a reserve pool and perhaps

corresponds to the glycogen content, is connected with the small pool, with 10% input and 9% output (POOL S_3). The existence, size, and turnover rate of this pool are deduced from the kinetics of the release of ^{14}C into the egg capsules, on the one hand (cf. Figures 7 and 8), and on the other hand, from the fact that the amounts of labelled carbon conveyed to the reproductive organs are not adequate for the amounts found in the egg capsules. The surplus of about 1% (10% minus 9%) remaining in this pool is conveyed, through a transfer of material, into the reproductive organs producing nutrients--particularly the albumen gland--usually every few days. Different numbers of eggs per capsule may be released, depending on how much nutrient material is available for this process. (This is symbolized by the boxes to be filled.) MAY (1934) already concluded from his experiments that the polysaccharide serving as a reserve material in the egg-capsules of Helix pomatia--galactogen--is prepared from glycogen in the rest of the body shortly before oviposition.

The temperature factor acts directly on the metabolism, on the one hand, but on the other hand, it also affects central control organs, where, together with the season, it determines the rate of activity and oviposition. Once the metabolism of the animal is set at a high rate by endogenous factors, it can no longer be depressed by exogenous factors (starvation

conditions: cf. Section 3.1.4). On the whole, exogenous factors (temperature, food) seem to be relatively unimportant for metabolism compared with endogenous factors; the same conclusion was reached also by BERG et al. (1958) on the basis of extensive respiratory measurements on Ancylus fluviatilis. This creates a considerable margin of uncertainty for quantitative descriptions of the metabolism of this animal (and probably also other pulmonates; cf. STREIT 1975).

The metabolic strategy of Ancylus fluviatilis has to satisfy various requirements. Juveniles have a high rate of mortality (STREIT, report in preparation), so that it is advantageous, especially at the beginning of the life of an individual, if these animals show a high growth rate (cf. Table 3, line 5). This is achieved by gearing the incorporation of carbon into the organ systems to their carbon content (cf. Figures 19 and 20 in this connection); adult animals, in contrast, incorporate carbon chiefly into the visceral organs, where it is locked into pools with high turnover rates (Table 2). Large animals must resort often to such mobile reserve pools--corresponding to POOL S_3 in our model (cf. Figure 29)--in order to defray the frequent interruptions in feeding necessitated by their greater needs (as a result of local overgrazing), and also for the sake of egg capsule production, which involves approximately 5 - 15% of the

animal's carbon content for each oviposition (cf. Part 1). This leads to very variable carbon values in different animals of the same shell length, as BURKY (1971) has shown also for Ferrissia rivularis.

These animals do not have a distinct storage phase followed by oviposition; instead, in the late autumn and also during other seasons, they show higher carbon values at low temperatures, leading to egg capsules rich in carbon when the temperature rises (cf. BURKY 1971). However, when the internal carbon supplies are exhausted by starvation (Figures 14 and 15) and, starting in the summer, by natural processes (STREIT 1975, Figure 25), the egg capsules contain less carbon, and therefore exhibit a smaller number of eggs per capsule.

The individual animals have no need to coordinate their developmental cycle, and thus the different size classes often show a considerable range of variation in nature (STREIT 1974, Figure 2). At low temperatures oviposition is restrained in favor of growth by a reduction of activity in all the reproductive organs (STREIT 1975, Figures 24 and 26). This normally prevents oviposition from being scattered too widely over the course of the year, since animals which are not yet adults also mature in this period. Hence the final size of the animals may vary considerably, and will generally be greater in brooks reaching only a low temperature in summer.

The reduction of the production and incorporation rates at high temperatures is much less in juveniles than in the adult animals (Table 3). This should be viewed as a direct adaptation of the juvenile metabolism to the higher summer temperatures to which the juveniles are exposed in a typical developmental cycle.

4. Discussion

4.1 Metabolism in relation to temperature

According to BERG (1952) and STARMUEHLNER (1961), the highest temperature at which Ancylus occurs in nature is around 25°C. This corresponds with the present experimental findings, which also indicated 25°C as the tolerance limit. At higher temperatures, especially in the spring, the animals perished very soon, and showed a general reduction of activities (Figure 28). We found 7°C as a minimum temperature for growth, which also corresponds well with observations in nature (SCHWENK & SCHWOERBEL 1973).

The curves for incorporation and production showed 24
a maximum at 16°C and 19°C, respectively (Figure 28). Above these temperatures, the amount which has to be mustered for basal metabolism seems to weigh so heavily that they decline again, in spite of increased rates of ingestion and assimilation. Such optimum production curves have been found

also for sockeye salmon (Oncorhynchus nerka, BRETT 1971), where it was also demonstrated that a limitation of food causes the optimum point to shift to a lower range of temperatures. In the marine polychaete Nereis diversicolor, the optimum is at different temperatures for different size classes (IVLEVA 1970).

The coefficients K_1 and K_2 showed a sharp rise to an optimum point at 13°C, followed by a less abrupt decline (cf. Part 1, Figure 30). This pattern is basically the same as the pattern found by KERSTING (1973; cf. also RINGELBERG 1973) for Daphnia magna. For that species, however, the optimum point for K_1 was around 40%. Hence Daphnia utilizes the amount consumed better than adult Ancylus--and still better compared with the juveniles--for net production. In Nereis diversicolor, K_2 shows a tendency to decrease with rising temperatures (IVLEVA 1970).

In experiments at a fixed temperature with animals freshly obtained from nature, Ancylus fluviatilis from bodies of water reaching 18°C in the summer show higher respiration values than those from waters reaching only 11°C. BERG (1953), who observed this phenomenon, referred to it as inverse temperature acclimatization. In the present study, these two limiting summer temperatures are comparable with those of Populations IV and I. We have mentioned that the adult

phase passes much more rapidly in Population IV than in Population I, ending already in July. Moreover, the metabolism of adult animals in our experiment expired quite intensively and rapidly starting at approximately 13°C. In Population I, however, the animals lived much longer (until October-November), since increased temperatures did not cause them to shift to higher levels of activity. According to the results which we have found, the different metabolic activity of these two populations should not be described in terms of (inverse) acclimatization; rather, it is based on the fact that their metabolism was not left unchecked until they reached a higher temperature (starting at 13°C), and worked itself out in ingestion, assimilation, and production, as well as respiration.

4.2 Compartment models for carbon metabolism*

The use of tracer material has rendered the study of the metabolism of aquatic organisms much more susceptible to measurements, yet the interpretation of these measurements has often led to serious errors (CONOVER & FRANCIS 1973). 25
Consciously or unconsciously, the organism has often been

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* I wish to thank Dr. LAMPERT at this point for the opportunity to read the manuscript of his study.

viewed as consisting of a single metabolic compartment in which the labelled material is introduced, uniformly mixed, and released again as excretion after a period of some length (Figure 30a). In the case of ^{14}C as a tracer, LAMPERT (1975) demonstrated experimentally for Daphnia pulex that a two-compartment model must be assumed in order to describe the carbon kinetics (Figure 30b). According to his model, the carbon first enters a "metabolic pool" (S_1) representing a very small portion of the entire animal, but showing a very high turnover rate. This pool S_1 constantly exchanges oxygen (sic) with a considerably less active, but quite large "structure pool" (S_2). These pools can not be coordinated directly with definite organs or chemical compounds.

In the present study on Ancylus fluviatilis, we were able to explain the carbon kinetics only by assuming a three-compartment model. The "metabolic pool" (S_1), the "structure pool" (S_2), and an additional reserve pool (S_3) are represented schematically in Figure 30c. In such a model, the assimilation is ρ_{01}, the total amount of carbon released (basal metabolism) is ρ_{10}, and production is the result of two anatropous carbon exchange processes, viz., $\rho_{01} - \rho_{10}$. Since in the ^{14}C kinetics ρ_{10} always follows ρ_{01} after a certain time lag, the production, as

the difference between these two values, can not be measured directly. For this reason, we designated the amount of labelled substance found in the body of the animal as the incorporation value, elaborating its relation to production subsequently. For Ancylus fluviatilis, we found that the material conveyed into the egg capsules was diverted chiefly from pool S_3. Generally a reserve pool with this type of linkage is capable of compensating, as a buffer, for a varying food supply, or a varying requirement--e.g., for the intermittent egg production. Thus, the more the uptake and release of material fluctuates, the more distinctly visible the importance of a reserve pool becomes. However, the similar conditions of LAMPERT's (1975) investigations, with only medium-length experimental periods, brought it about that a two-compartment model was already sufficient to explain the observed carbon kinetics. In a certain sense, the reserve pool S_3 is the ecologically "interesting" pool, since it is most capable of compensating the fluctuations of environmental factors.

Finally there are a very great number of pools with different turnover values and very complicated interconnections, but it is appropriate to find for each experimental pattern the minimum number of pools necessary for a sufficiently exact description of the system. Theoretical constructions of

three-compartment models have already frequently been used in connection with biochemical and ecological problems (VON BERTALANFFY 1953, FRANCIS & CONOVER 1973), but in practise this conception often fails because the data do not permit any complete description of the exchange rates and the size of the pools.

Often the kinetics also of other elements besides carbon indicates the existence of several pools, although 26
in most cases these are probably not related to the carbon pools. In the case of phosphate, for example, frequently at least two compartments with different types of action are differentiated (e.g., BARSDATE et al. 1974 for bacteria).

4.3 Carbon turnover rates

So far, turnover measurements have been obtained chiefly for homoiothermal animals, by introducing amino acids labelled with ^{15}N or ^{14}C into the body, or by providing uniformly labelled food. It has been shown that the protein half-life periods are related to size. Thus, for the total protein content, half-life periods of 80 days in man and 17 days in the Rat were found. The half-life for the liver is approximately 10 days in man, 6 - 7 days in rats, and 1.7 - 4.5 days in mice (SPRINSON & RITTENBERG 1949, SPECK & URICH 1969). In river crayfish, SPECK & URICH (1969) found

half-life periods of 8.5 - 25 days for the visceral organs and approximately 115 - 118 days for caudal muscles and hemolymph proteins, by injecting ^{14}C-leucine into the pericardium at 13°C. However, every tissue contains pools with very different turnover rates, so that these average amounts have only formal value (BUCHANAN 1961, GRASZYNSKI 1968). Assuming a Q_{10} of 2, the half-life periods for the river crayfish correspond at mammalian temperature (38°C) to the turnover rates for mice, whereas the oxygen consumption with the same method of calculation is approximately 10 - 15 times smaller (SPECK & URICH 1969). If the values of 7 - 16 days found in the present study for the half-life of carbon in the visceral organs of Ancylus are likewise converted with a Q_{10} of 2 for 38°C, they also correspond approximately (with 2.3 - 5.3) to the values for the liver of a mouse. Strictly speaking, such conversions are not permissible, because the poikilothermal animals studied in our investigation are no longer viable at 38°C, so that it is arbitrary to assume a Q_{10} at this temperature. Therefore poikilotherms which do tolerate this temperature should be investigated for such comparisons. Moreover, the values for Ancylus should have been compared also with a mammal of the same body size, which of course does not exist. Nevertheless, it is a notable fact

that the considerably lower basal metabolism of the poikilothermal animals (measured as O_2 turnover) is in contrast to a level of constructive metabolism which, in view of the temperature difference, is not reduced. Probably the relatively high intensity of their constructive metabolism is advantageous to these animals, and facilitates sufficiently rapid production processes even at their low environmental temperatures.

Summary

27

Diatoms (Nitzschia actinastroides) were labelled with ^{14}C, filtered off onto membrane filters, and fed to specimens of Ancylus fluviatilis. By measuring ^{14}C-release into the water and incorporation into the entire animal, into the organs, and into the egg capsules, we gained a conception of the dynamics of carbon turnover in the body of the animal.

At 25°C, the assimilated carbon was largely released again within the first few hours through respiration (Figure 5). The specific activity of egg capsules deposited shortly after the labelling period was high, but that of eggs deposited several days later was lower (Figure 7). The variations in the specific activity of successive egg capsules and of the reproductive organs revealed that the carbon for the egg

capsules is first stored to a great extent in the rest of the body (in the midgut gland, etc.), and then mobilized as needed. We have outlined a three-compartment model of the carbon balance of Ancylus fluviatilis to explain the carbon kinetics revealed by our measurements (Figures 29 and 30).

The amount incorporated into the different organs depends on the relative size of the organs, which may change as they grow (Figures 17 - 19, 22, 23, and 25; Table 1, p. 15), and also on their actual activity compared to the rest of the body. The visceral organs generally show increasing carbon turnover activity with increasing body size (Figure 20). At low temperatures (below 10°C), the metabolic activity of the reproductive organs is reduced disproportionately (Figures 24 and 26). At 22°C, the average half-life of the carbon was approximately 14 days. The turnover rates of the midgut gland and of the gut were about three times as high as those of what we termed the "remainder of the animal" (i.e., apart from the visceral organs; Table 2, p. 20).

We have compiled a balance for carbon metabolism in relation to temperature for juveniles in Table 3 (p. 21), and for adults in Table 3 and Figure 28. The values shown are averages for spring and summer (cf. STREIT 1975). At low temperatures, we found a low level of production (growth

and oviposition) coupled with a relatively high rate of incorporation, indicating chiefly the accumulation of reserve material. In higher temperature ranges, incorporation corresponded to production plus the additional amount required for turnover. Juveniles showed higher rates of production and incorporation than adults, especially at high temperatures (Figure 10). In adults, much of the carbon is locked into pools with high turnover rates in the visceral organs, but this does not occur in juveniles.

Seasonal, as well as short-term variations in the level of activity are controlled endogenously, but their general pattern may be controlled indirectly by seasonal factors (perhaps photoperiodism). A high level of metabolic activity can not be reduced even by starvation; the animal continues to produce egg capsules until it dies. However, first the size, and then the number of the egg capsules are reduced. High levels of activity may be induced in the spring by subjecting the animals to temperatures of at least 13°C. At 7°C or below, a developmental cycle can no longer continue.

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Translation of non-English titles

1. Energy metabolism. In: GAUER ... (ed.), Human physiology, Vol. 2

2. Ecology and productive biology in Agapetus fuscipes CURT. in Breitenbach, 1971 - 1972

3. Biophysics of dynamic equilibrium

4. Manual of animal physiology

5. Concerning the distribution of radioactivity among the organs of the river crayfish Orconectes limosus RAFINESQUE (Cambarus affinis SAY) following injection of ^{14}C-glucose into the hemolymph

6. Metabolism and nutrition of fish. In: Manual of animal nutrition, Vol. 2

7. The energy flow in a population of Daphnia magna. Thesis

8. Chemical and biological investigations regarding galactogen. Biological section: The annual cycle of galactogen and glycogen reserves in Helix pomatia. Sixth treatise

9. Experimental investigations on the nutritive biology and mode of life of the freshwater limpet Ancylus fluviatilis (O.F. MUELLER 1774; Gastropoda Basommatophora)

10. Protein turnover in the organs of the river crayfish Orconectes limosus

11. Storage of materials in the river crayfish Orconectes limosus. Annual cycle and distribution among organs

12. Biological investigations in warm brooks in Iceland, Central Europe, and Madagascar (Preliminary communication)

13. Population dynamics of Chaetogaster limnaei limnaei in a population of Ancylus fluviatilis

14. Experimental investigations regarding the metabolism of Ancylus fluviatilis (Gastropoda - Basommatophora). 1. Ingestion, assimilation, growth, and oviposition

15. Seasonal control of the respiration of Arianta arbustorum (Gastropoda)

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Illustrations

(Commas may represent decimal points.)

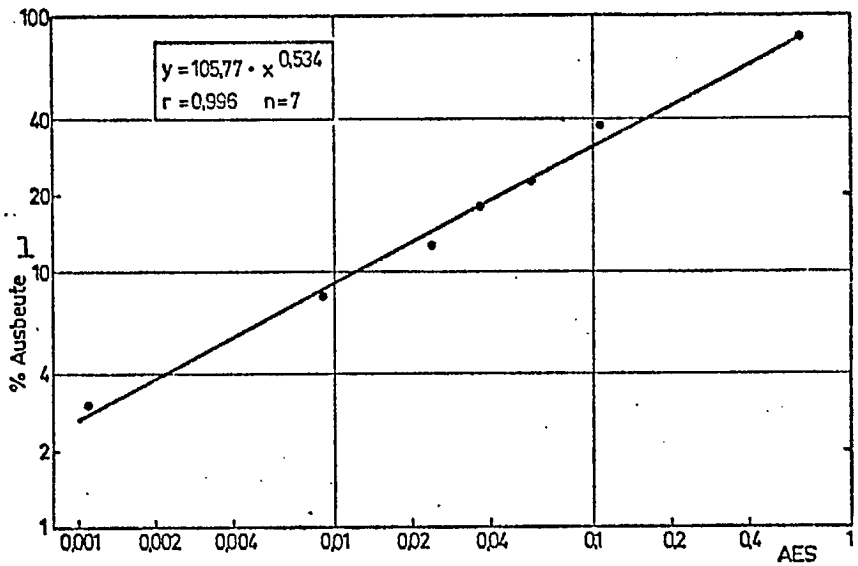


Figure 1. Quenching chart for a mixture of picric acid, formalin, and glacial acetic acid according to BOUIN with a solution of 4 g PPO per liter toluene.
Key: 1- % yield

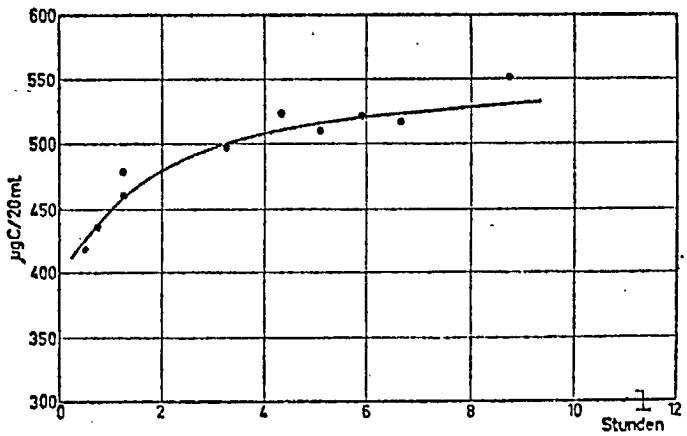


Figure 2. Increase in the carbon content of the algal suspension following centrifugation and the addition of Lake of Constance water
Key: 1- hours

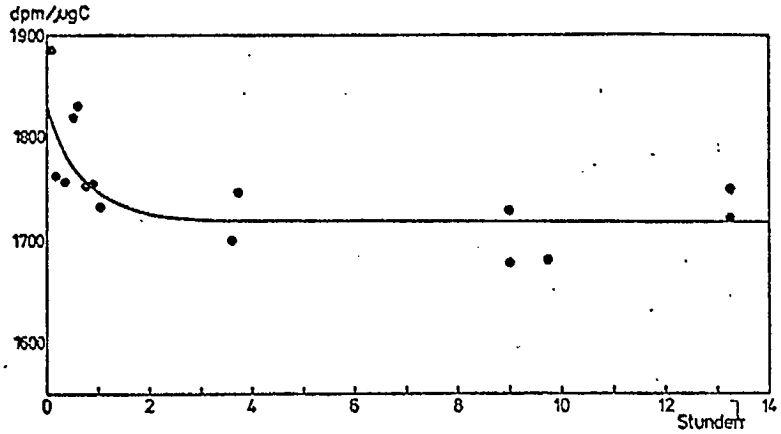


Figure 3. Decrease in the specific activity of the algal suspension following centrifugation and the addition of Lake of Constance water.
Key: 1- hours

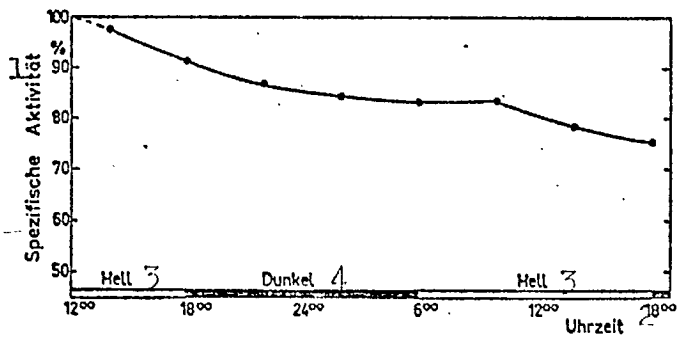


Figure 4. Decrease in the specific activity of the food algae on the membrane filter. Binomial moving averages.
Key: 1- specific activity 2- time 3- light 4- darkness

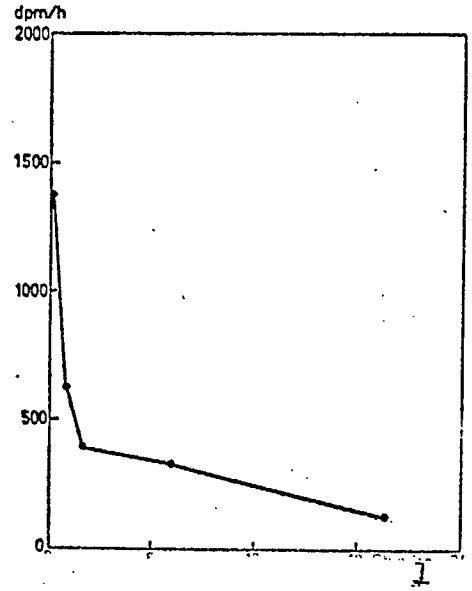


Figure 5. Pattern in time of the release of ^{14}C into the water during the subsequent (unlabelled) feeding period. After 19½ hours, 2336 dpm were incorporated. Temperature 25°C.
Key: 1- hours

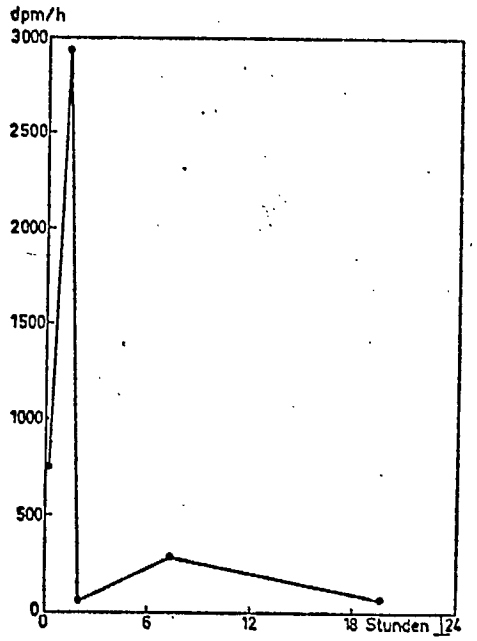


Figure 6. Pattern in time of the release of ^{14}C in the feces during the subsequent (unlabelled) feeding period. Same animal as in Figure 5.
Key: 1- hours

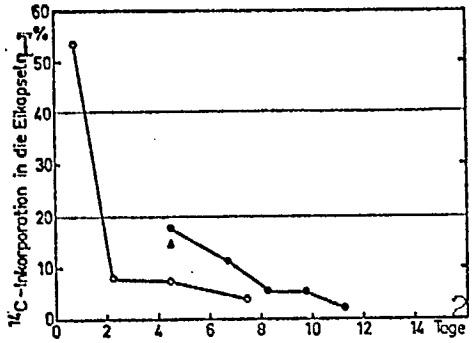


Figure 7. Percentage of the ^{14}C-content in the egg capsules from 3 animals during 16 days of subsequent (unlabelled) feeding. The sum of the ^{14}C-contents of the egg capsules and the animal after 16 days $\hat{=}$ 100%.
Key: 1- ^{14}C-incorporation into the egg capsules 2- days

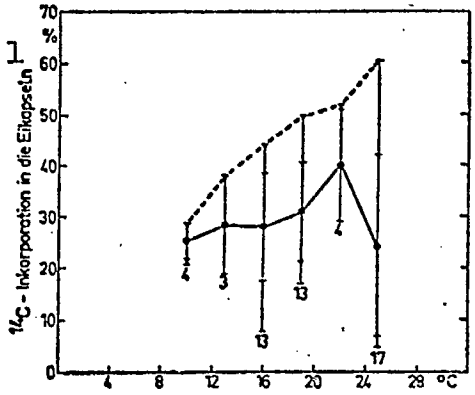


Figure 8. Average ^{14}C-content of the egg capsules produced during 4 days of subsequent (unlabelled) feeding, expressed as percent of the total ^{14}C-content of animal and egg capsules. Standard deviation, extreme values, and number of experimental animals.
Key: 1- ^{14}C-incorporation into the egg capsules

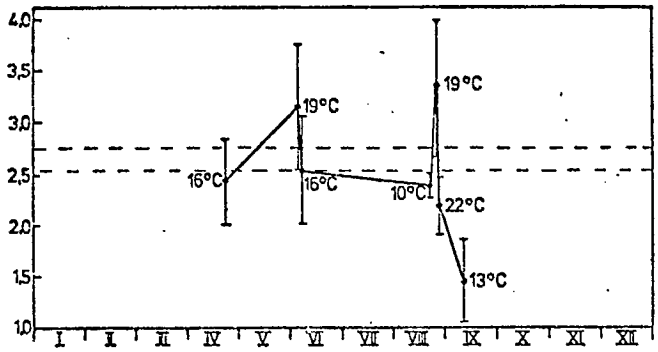


Figure 9. "b" exponent of the allometric equation incorporation = $a \cdot L^b$ (L: length), in relation to the season and temperature. Broken lines: proportionality to the body (limits within the standard deviation of the b exponent)

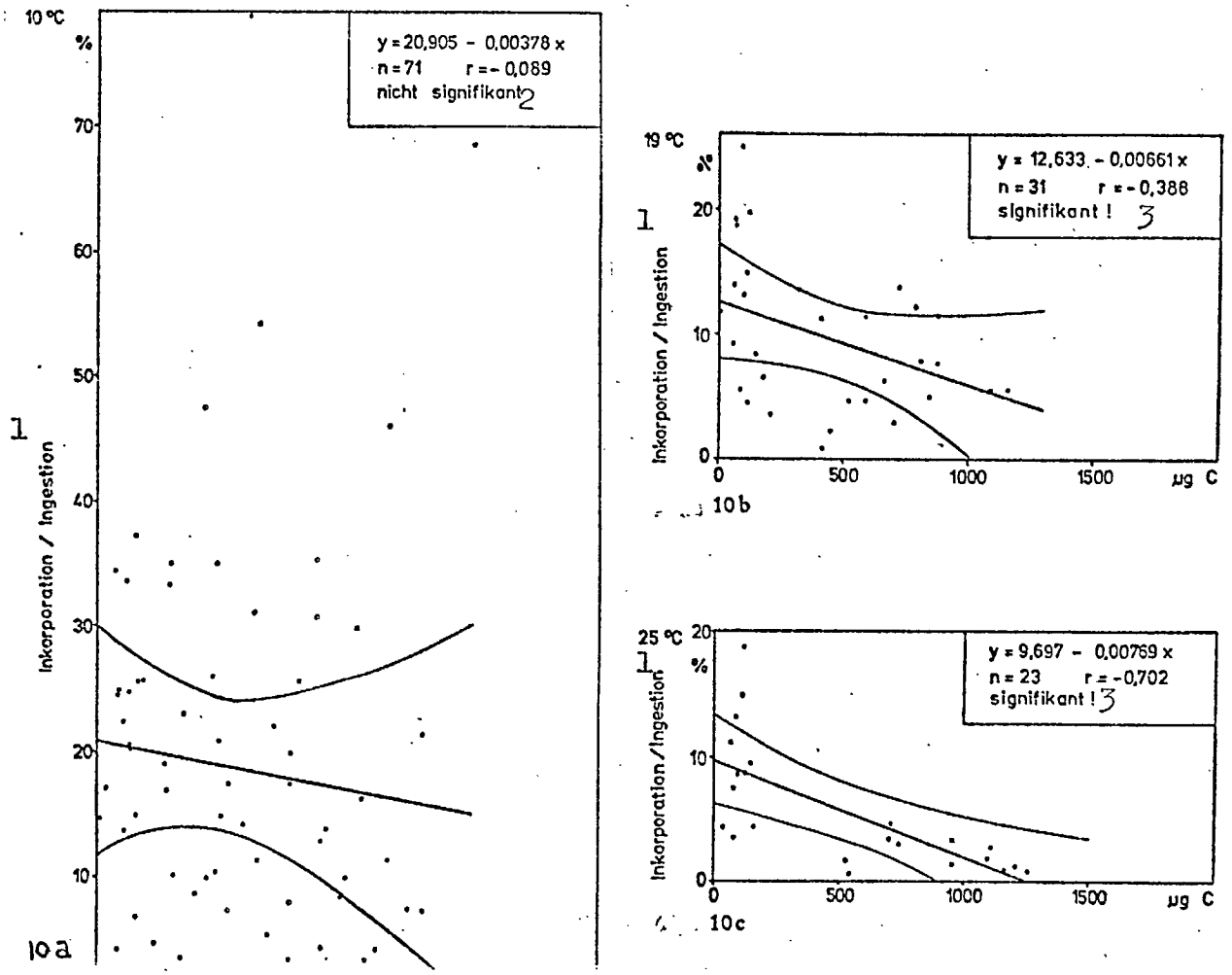


Figure 10. Incorporation/ingestion ratio, in percent, in relation to body size, at 10°C, 19°C, and 25°C.
Key: 1- incorporation/ingestion 2- not significant 3- significant

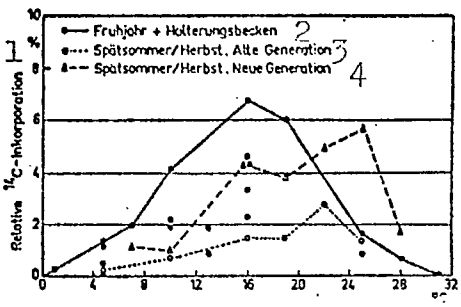


Figure 11. Relative ^{14}C-incorporation in relation to the temperature and season. In the case of the spring animals and those from the holding basins, only the highest points are connected with a line; therefore these should correspond to the maximum level of activity.
Key: 1- relative ^{14}C-incorporation 2- spring / holding basins 3- late summer - autumn: old generation 4- late summer - autumn: new generation

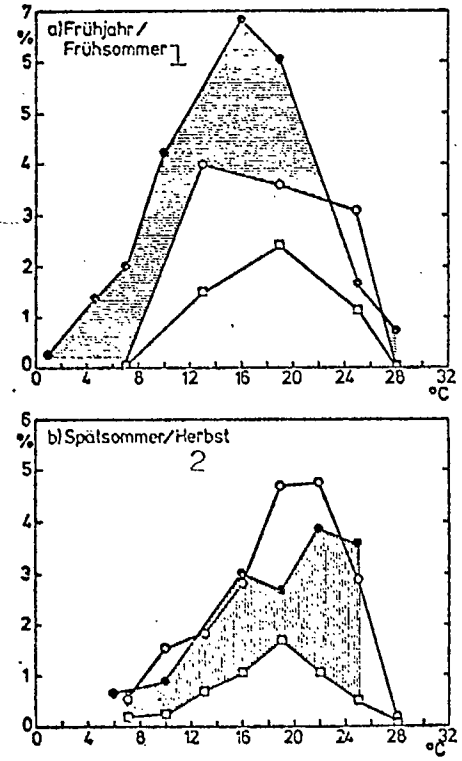


Figure 12. Comparison of relative production and relative incorporation. Dots: spring maximum incorporation (above) and autumn incorporation (below). Circles: maximum production. Squares: average production in the spring, and in the autumn. Shaded area: difference between the incorporation and production values compared.
Key: 1- spring-early summer
2- late summer-autumn

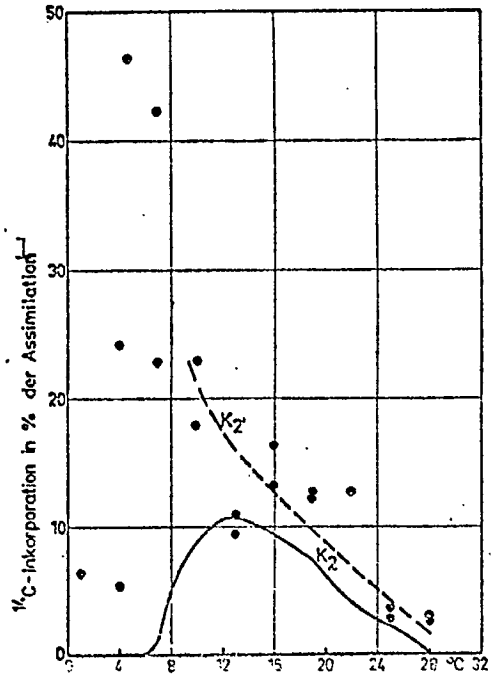


Figure 13. K_2 (= production/assimilation) according to STREIT (1975, Figure 30), compared with K_2, (= incorporation/assimilation). Key: 1- ^{14}C-incorporation in percent of assimilation

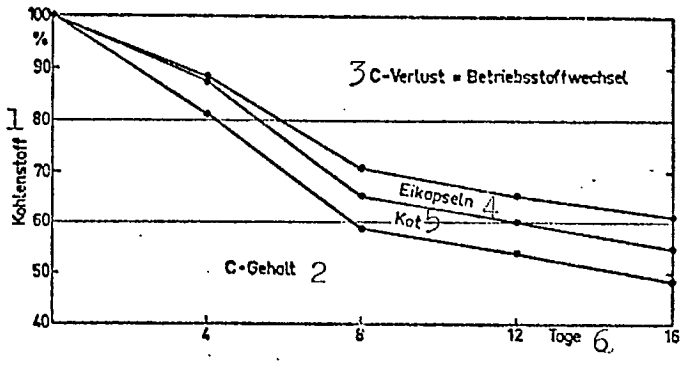


Figure 14. Carbon reduction, feces production, and egg capsule production during 16 days of starvation. Spring, 19°C.
Key: 1- carbon 2- carbon content 3- carbon loss = basal metabolism 4- egg capsules 5- feces 6- days

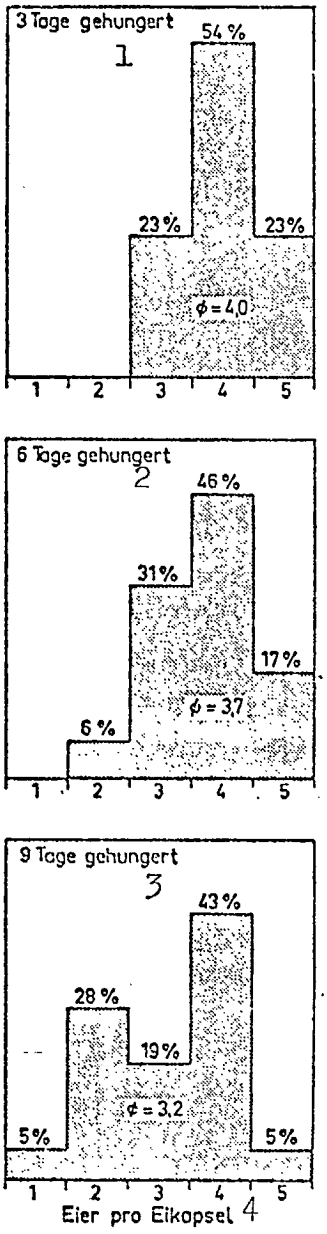


Figure 15. Size distribution of egg capsules after starvation periods of different duration followed by renewed feeding. Spring, 19°C.
Key: 1- 3 days of starvation 2- 6 days of starvation 3- 9 days of starvation 4- eggs per capsule

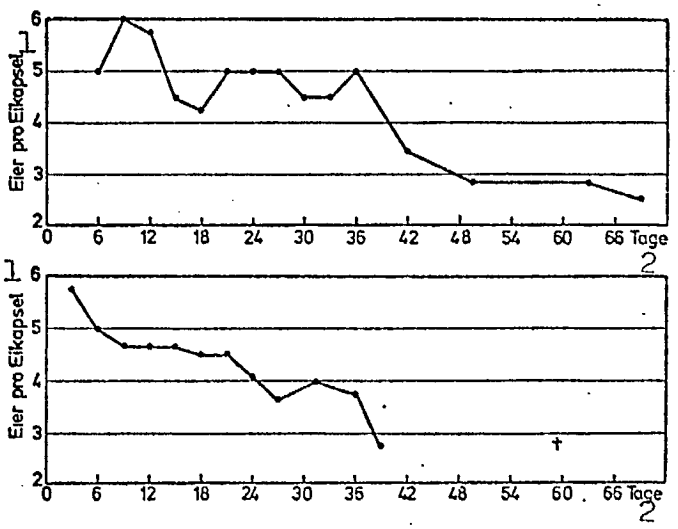
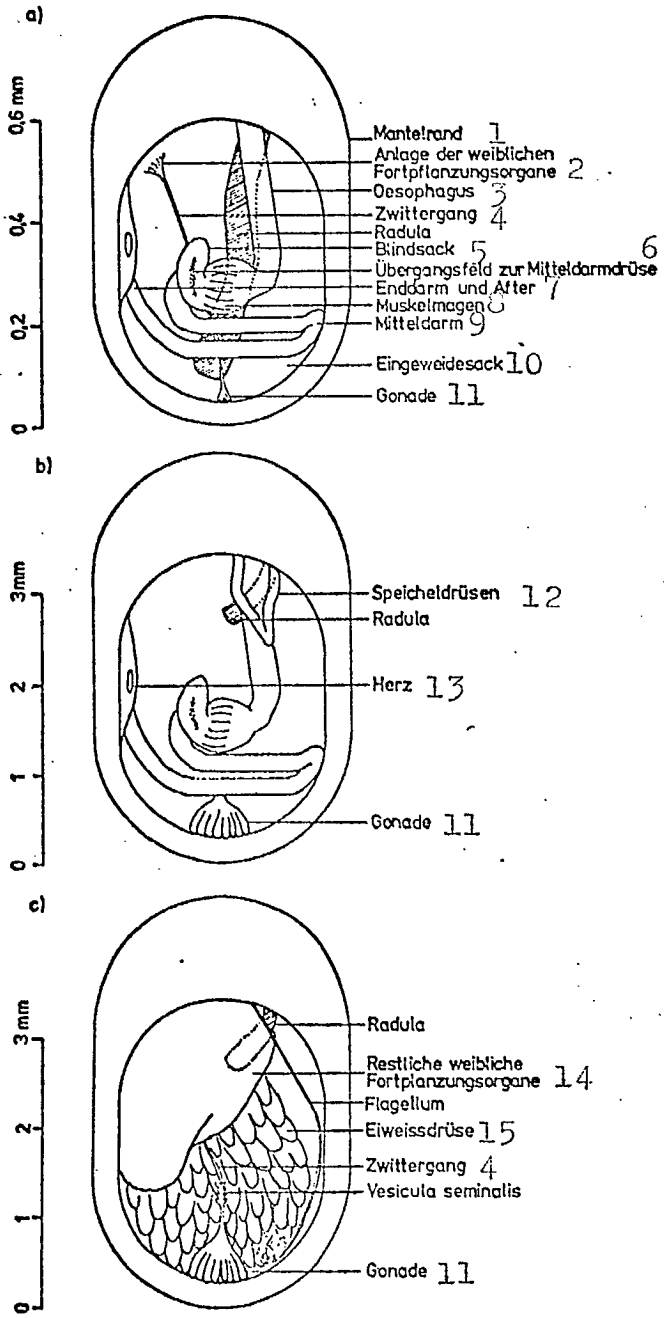


Figure 16. Decreasing size of egg capsules during our experiments. Spring, 19°C. Binomial moving averages for two characteristic experimental animals. --Decreasing tendency significant at 63% and 92%, respectively (test of COX & STUART, two-sided problem)
Key: 1- eggs per capsule 2- days



Key: 1- edge of the mantle 2- primordium of the female reproductive organs 3- esophagus 4- gonoduct 5- caecum 6- area of transition to the midgut gland 7- hindgut and anus 8- gizzard 9- midgut 10- visceral sac 11- gonad 12- salivary glands 13- heart 14- other female reproductive organs 15- albumen gland

Figure 17. Anatomy of Ancylus fluviatilis. Partially schematized. View from above; shell removed, visceral sac open, midgut gland removed. (a) juvenile (shell length 1.3 mm) (b) adult (shell length 6.4 mm): intestinal system (c) same as (b), but intestinal system removed to expose reproductive organs.

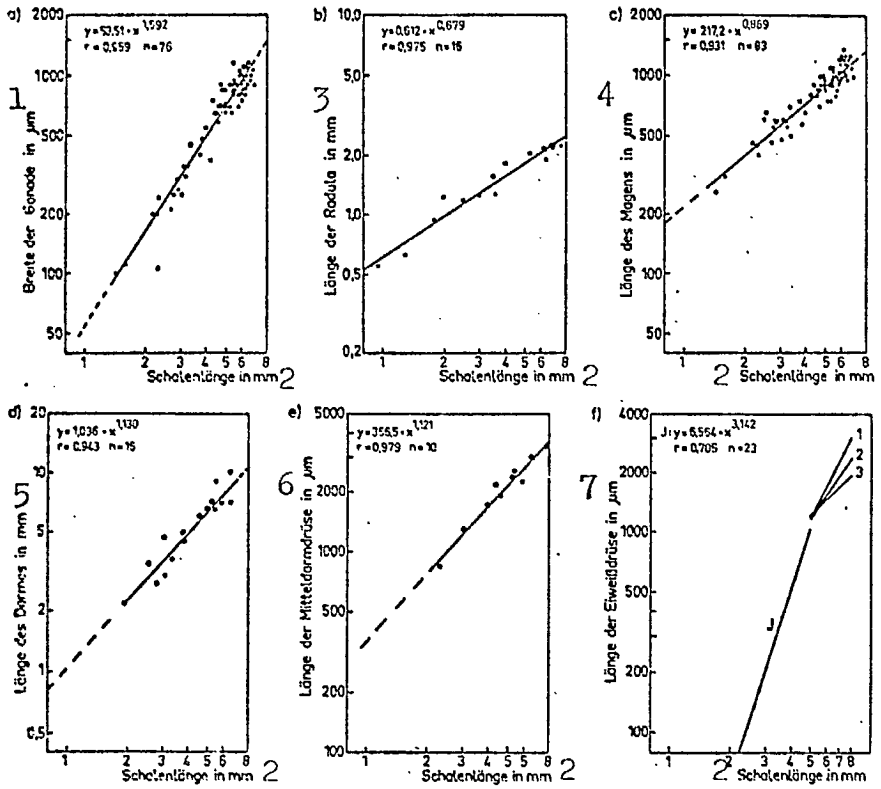


Figure 18. Growth relations of certain organs (values in parentheses refer to the 95% confidence region of the b exponent).

(a)	gonad	(1.483 - 1.701)
(b)	radula	(0.590 - 0.768)
(c)	stomach	(0.794 - 0.944)
(d)	gut	(0.901 - 1.360)
(e)	midgut gland	(0.932 - 1.310)
(f)	albumen gland J	(1.707 - 4.577)

Key: 1- width of gonad in µm 2- shell length in mm
3- length of radula in mm 4- length of stomach in µm
5- length of gut in mm 6- length of midgut gland in µm
7- length of albumen gland in µm

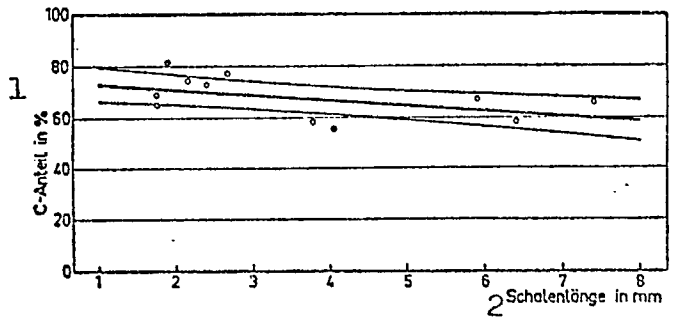


Figure 19. Percentage of carbon in the "remainder of the animal", in relation to the content of the entire animal excluding the shell. Linear regression with 95% confidence region. $y = 73.346 - 2.102x$, $r = -0.518$, $n = 11$. Not significant.
Key: 1- carbon percentage 2- shell length in mm

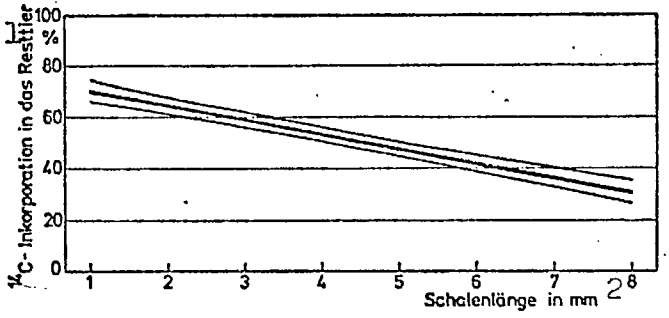


Figure 20. Percentage incorporated into the "remainder of the animal". Linear regression with 95% region of confidence. $y = 76.063 - 5.667x$, $r = -0.765$, $n = 60$. Significant.
Key: 1- ^{14}C-incorporation into the "remainder of the animal" 2- shell length in mm.

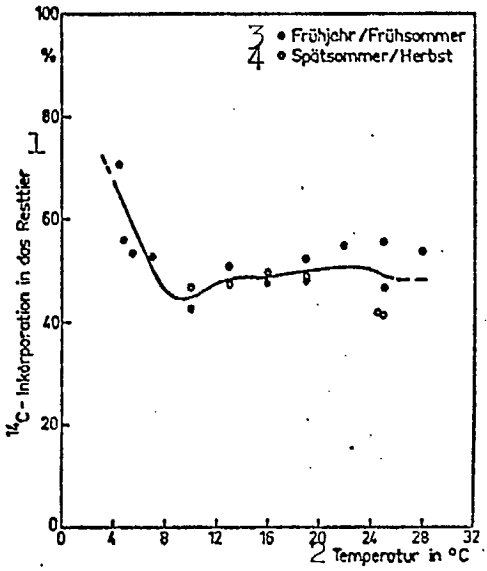


Figure 21. Percentage incorporated into the "remainder of the animal" in relation to temperature, for our standard limpet (4.5 mm length).
Key: 1- ^{14}C-incorporation into the "remainder of the animal" 2- temperature in ^{o}C 3- spring - early summer 4- late summer - autumn

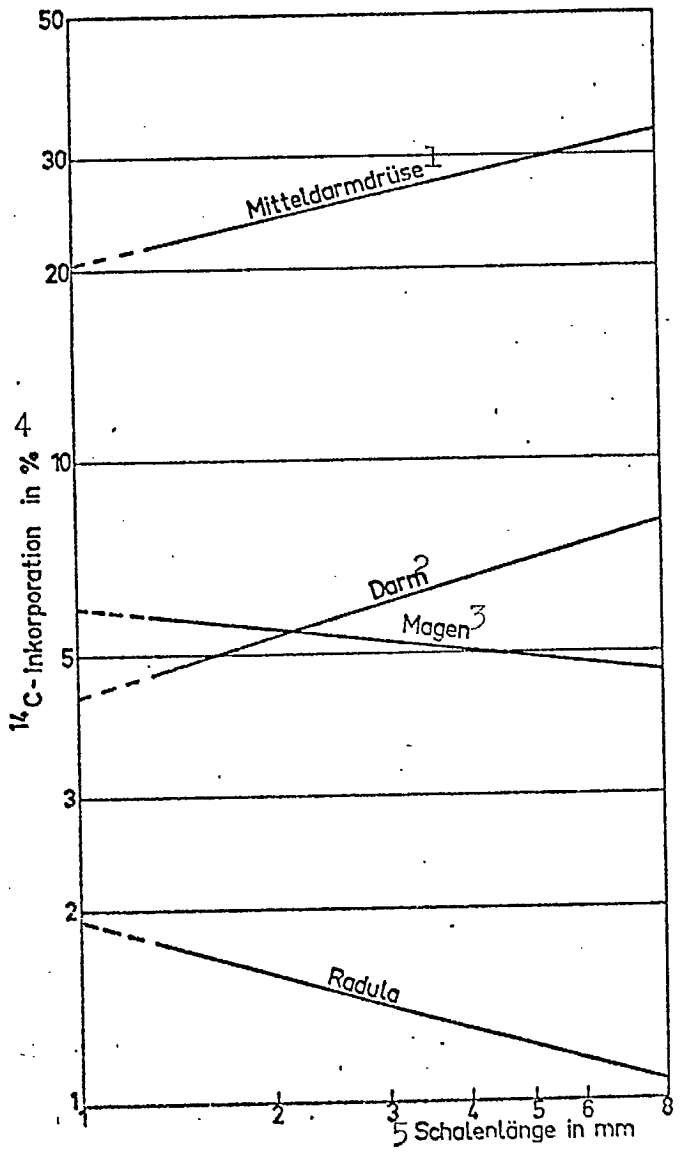


Figure 22. Percentage incorporated into the midgut gland, the gut, the stomach, and the radula. Log-log regression lines.
gut: $y = 4.317 \cdot x^{0.294}$, n= 97, r= 0.302 significant
midgut gland: $y = 20.304 \cdot x^{0.231}$, n= 99, r= 0.423 significant
stomach: $y = 5.900 \cdot x^{-0.110}$, n= 142, r=-0.111 not significant
radula: $y = 1.934 \cdot x^{-0.286}$, n= 83, r= -0.271 significant

Key: 1- midgut gland 2- gut 3- stomach 4- ^{14}C-incorporation in % 5- shell length in mm

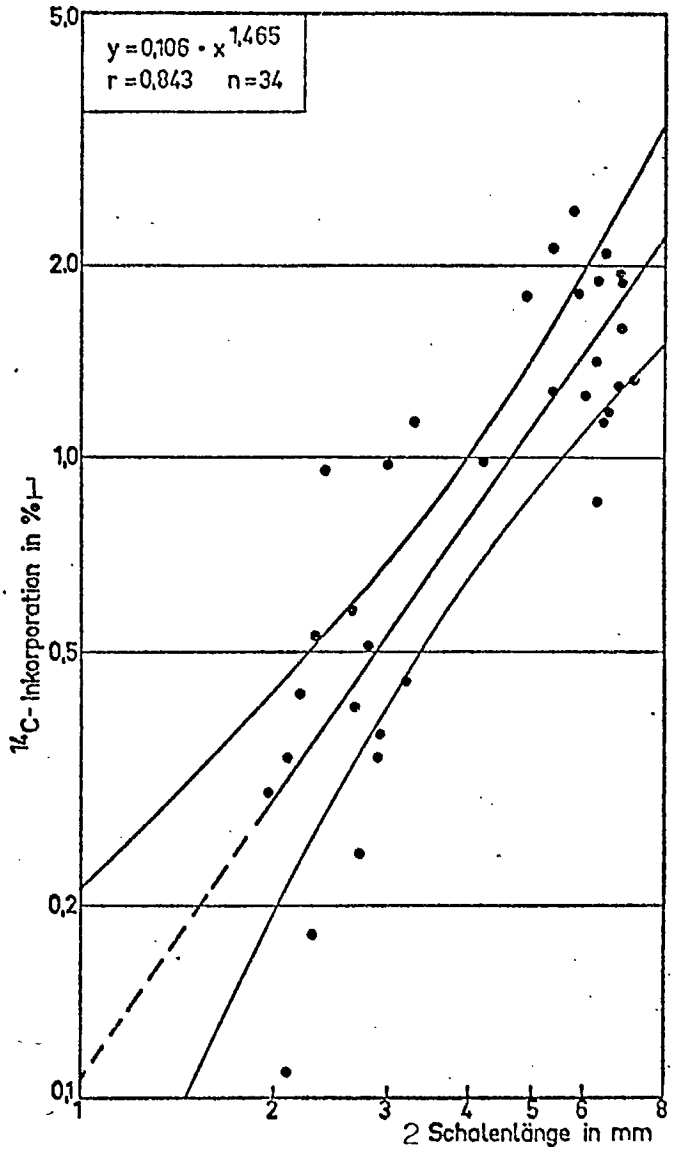


Figure 23. Percentage incorporated into the gonad, in relation to the total amount incorporated into the body of the animal. Values for late summer and autumn. Log-log regression with 95% confidence region.
Key: 1- ^{14}C-incorporation in % 2- shell length in mm

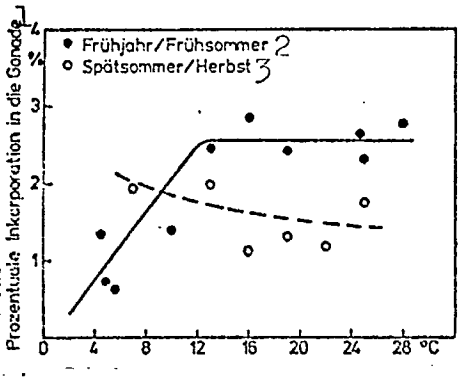


Figure 24. Percentage incorporated into the gonad in relation to the temperature and the season. Values apply to animals with a shell length of 6 mm.
Key: 1- percentage incorporated into the gonad 2- spring - early summer 3- late summer-- autumn

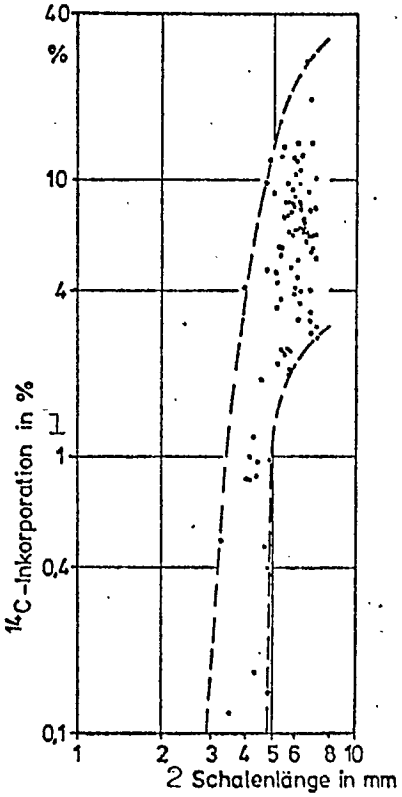


Figure 25. Percentage incorporated into the albumen gland in relation to the total amount incorporated into the body of the animal. The range marked by broken lines comprises the extreme measured values. n = 91.
Key: 1- ^{14}C-incorporation in %
2- shell length in mm

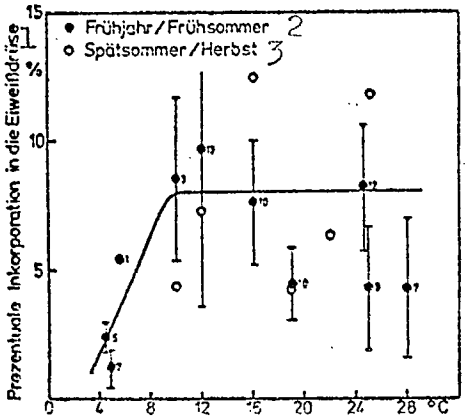


Figure 26. Percentage incorporated into the albumen gland in relation to the temperature and the season. Mean values for adults with shell length over 5 mm.
Key: 1- percentage incorporated into the albumen gland
2- spring - early summer 3- late summer - autumn

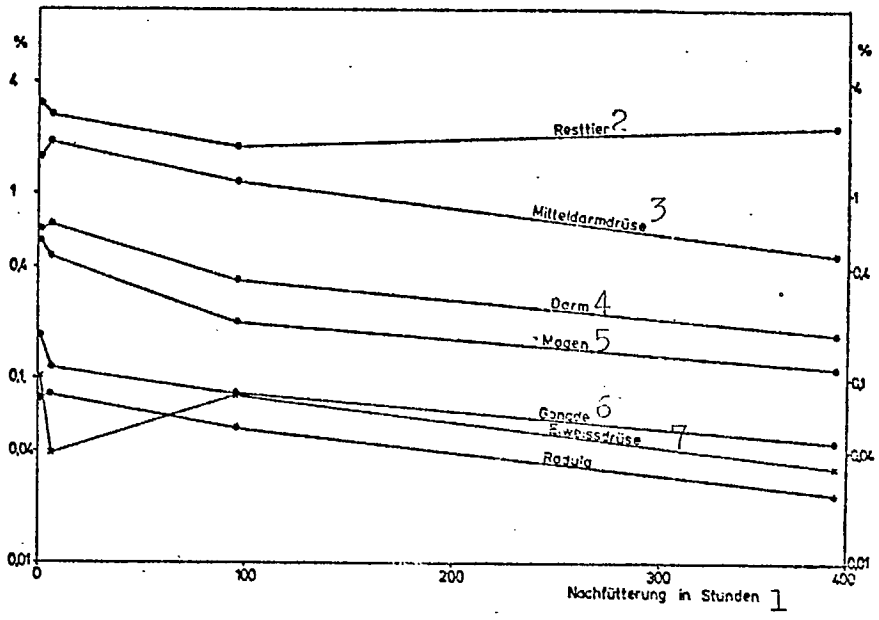


Figure 27. Incorporation/ingestion ratio in percent over the course of 384 hours (= 16 days) of subsequent feeding with unlabelled food, following a labelling period of 24 hours. Key: 1- subsequent feeding period in hours 2- "remainder of the animal" 3- midgut gland 4- gut 5- stomach 6- gonad 7- albumen gland

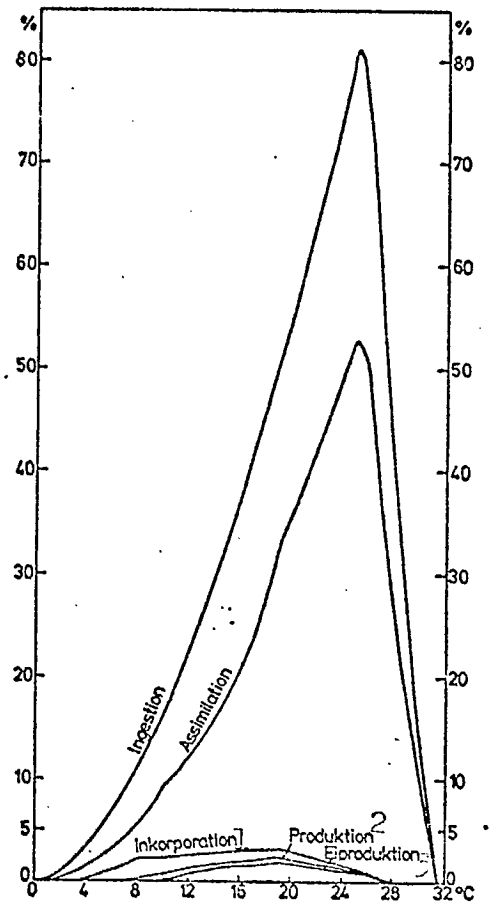


Figure 28. Carbon balance for an adult Ancylus with normal activity, in relation to the temperature. "Incorporation" corresponds to the incorporation of carbon into the body for growth, storage, and turnover.
Key: 1- incorporation 2- production 3- egg production

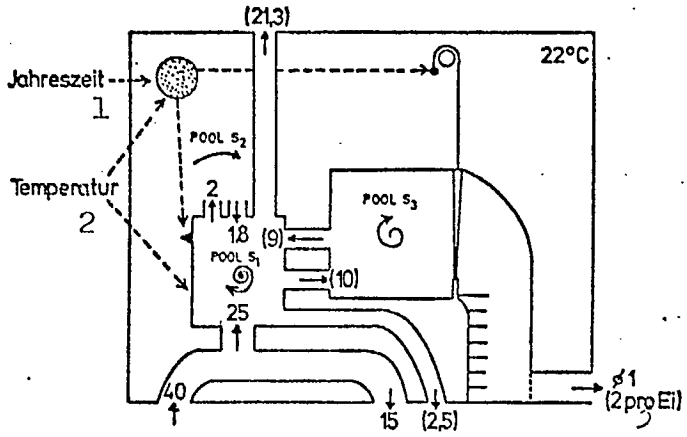


Figure 29. Simplest possible functional model of the carbon metabolism of Ancylus fluviatilis. Values expressed as percent of the carbon content of the body per day. Adult specimen,, 22°C, summer conditions.
Key: 1- season 2- temperature 3- 2 per egg

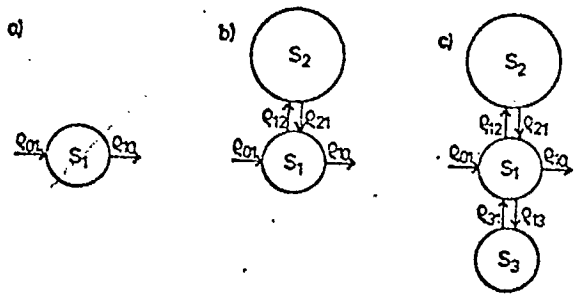


Figure 30. Formal compartment models of the carbon metabolism of animal organisms. (a) 1-compartment system (b) 2-compartment system (c) 3-compartment system (used in this study for Ancylus fluviatilis). (a) and (b) according to LAMPERT (1975).