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Some data on ecological physiology and bioenergetics
of a cold-water stenotherm *Perlodes intricata*
(Plecoptera)

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With 4 figures and 3 tables in the text

Little is known about reactions of cold-water stenotherms to environmental conditions, and in particular about their bioenergetics. *Perlodes intricata* (Pictet) 1842, a large and fairly common predaceous invertebrate seems to be an important component of food webs in mountain streams. Up to now, only its taxonomy and ecology has been studied. The data presented here are fragments of more extensive comparative investigations on reactions of larvae of *P. intricata* and larvae of a eurythermal mayfly *Cloeon dipterum* (Linneé 1761) to environmental conditions (Kamler 1971, in print) and on the energetic balance of *P. intricata* (Kamler, in prep.).

Terrain, material and methods

P. intricata lives in the stony habitats of European mountain streams between about 700 and 1700 m above mean sea level. In the streams of the Tatra Mts it occurred in places where current speeds ranged from 0.2 to 0.7 m/sec (Kamler 1967). Fig. 1 presents data, compiled from literature, on the frequency of observed levels of O₂ content (curve 1) and temperature (curve 2) in Tatra streams. It can be seen that the O₂ content is rather high, mostly reaching 90 to 100% air saturation of water, and temperatures are low, most often from 4 to 14°C. The presence of this species within that range of low temperatures was found, among others, by Pleskot (1951) — curve 3, and Wojtas (1964) — curve 4. Measurements of water temperatures by the author (Kamler 1965, 1971 in print) in Olczyski stream, from which the larvae used for investigations were collected, gave 5 to 7.5°C in summer, and the 24 hourly amplitude did not exceed 1°C. The lowest temperatures (1–2°C) were recorded in spring. The reproductive period of *P. intricata* of the Olczyski stream occurs in July and August and the larvae appear to grow throughout most of the year, attaining a maximal length of about 35 mm.

Experiments were performed in constant-temperature water baths; the temperature was controlled to $\pm 0.015^\circ\text{C}$. Lethal O₂ concentrations were determined by placing single larvae in ground-glass stoppered bottles. Two temperatures each with five levels of O₂ concentration were tested, and each temperature/oxygen concentration combination was repeated ten times. The initial and final O₂ content of the water was determined by the Winkler' method, the final when the animal died. A blank experiment was used to check the precision of the determinations, yielding a standard deviation of 0.02 mg/l O₂. Respiration was recorded by means of Klekowski's modification of a constant-pressure microrespirometer (Klekowski 1968); this respirometer is sensitive to 0.1 μl . The animals were put individually into a respiration chamber together with a pebble; there was no water movement inside the chamber. The length of the larvae was determined to 0.1 mm, wet weight of the living larvae to 1 mg, and dry weight to 0.01 mg after drying in 50°C over NaOH. The calorific value was determined by means of a microbomb calorimeter described by Phillips (1964), applying the technique recommended by Prus (1968). The calibration and estimation of accuracy of the apparatus used was performed by burning 19 benzoic acid samples; the coefficient of variation was 2.18%. Thirty determinations of the calorific value of larvae with wet weight from 25 to 325 mg were performed. The material was classified into twelve weight classes at the intervals

of 25 mg. The ash remaining after the burning was weighed. Besides this, five determinations of ash content were carried out in a muffle furnace at 550°C. The elements of the energy balance were measured at 5°C for 3–4-day intervals simultaneously for ten animals. The animals were kept alive in small vessels with constantly aerated water together with pebbles; they were fed with Tubificidae. There was no water flow in the vessels.

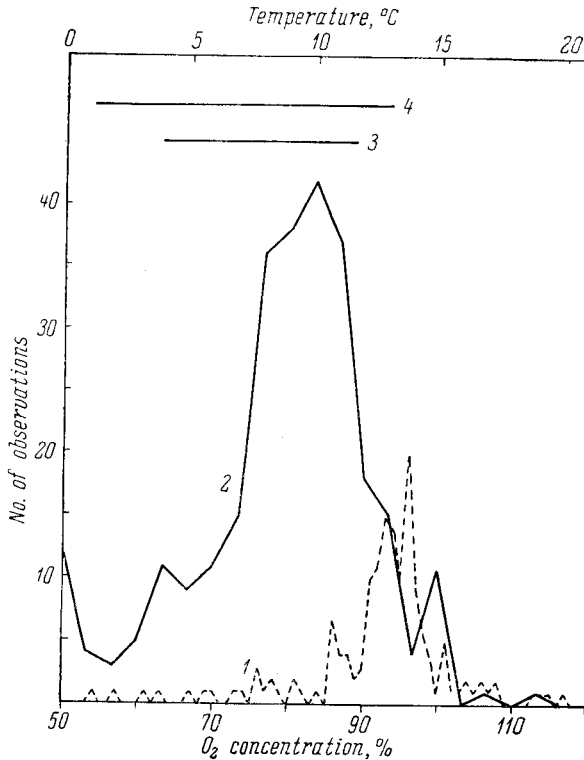


Fig. 1. Distribution of O₂ content (1), and distribution of temperature in Tatra streams (2) (combined from Oleksynowa & Komornicki 1956, 1957a, 1957b, 1960, 1965). Temperature range of *P. intricata* larvae according to Pleskot (1951) (3), and according to Wojtas (1964) (4).

Results and discussion

Larvae of *P. intricata* transferred without previous acclimation from air-saturated water to water with about 26% air saturation died almost immediately (Fig. 2) and were almost unable to exhaust the oxygen from it. In contrast, larvae of *C. dipterum* can exhaust oxygen from water with a much lower initial air saturation. *P. intricata* larvae usually died in higher oxygen concentration than larvae of *C. dipterum*, and the areas defined by the 95% confidence interval (mean - *t*.S. E. to mean + *t*.S. E.) did not overlap. The lethal O₂ concentrations seemed to be fairly constant for each species, and no clear relationship with temperature and initial O₂ content was observed.

For the determination of Q₁₀ (Tab. 1), larvae with equal weights were chosen. The measured value of Q₁₀ for *P. intricata* was much lower than that expected from Krogh's "normal curve" for the same range of tem-

Tab. 1. Q_{10} values for respiration rates of *P. intricata* and *C. dipterum* larvae for different temperature ranges.

Species	Temperature range, °C	Q_{10}	
		measured	expected
<i>P. intricata</i>	5.5—10.5	1.43	3.47
<i>C. dipterum</i>	5.5—15.5	2.70	3.14
	15.5—25.5	2.62	2.35

peratures. However, for *C. dipterum* the measured and expected values were similar. In literature there are many data concerning the relationship between metabolism and temperature for various non-cold adapted animals, e. g. an extensive list was given by Winberg (1956). He found a high consistency between his data and Krogh's "normal curve". Schölander *et al.* (1953) proved that their observed values were usually

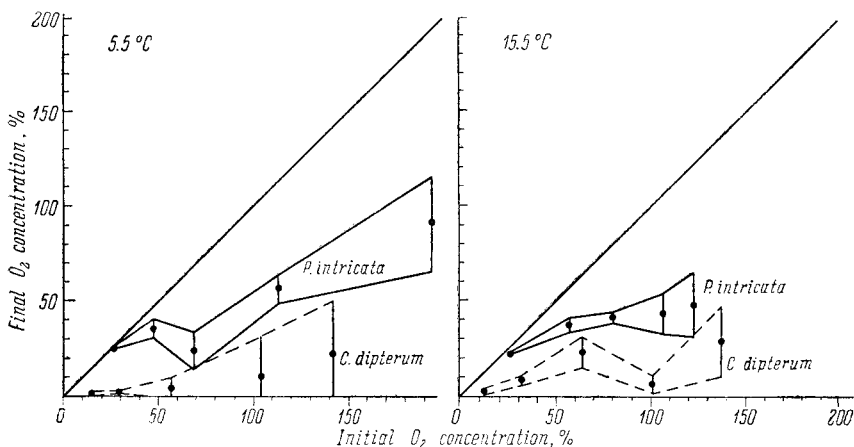


Fig. 2. Lethal O₂ concentrations of *P. intricata* and *C. dipterum* larvae. Points — the means for 10 measurements, vertical lines — 95% confidence interval.

consistent with Krogh's "normal curve" for animals living in constantly warm environments, in cold but fluctuating environments and in temperate climates. On the other hand, in constant cold environments the values of Q_{10} for arctic fish and Crustaceans were lower than those expected from Krogh's "normal curve". A survey of sixty four Q_{10} values presented by Morgan & Wilder (1936), Istenič (1963), Knight & Gauffin (1966) and Pattée & Rougier (1969) for cold-adapted Plecoptera and Ephemeroptera species from temperate climate show that all the Q_{10} values except two are much lower than those expected from the "normal curve". Thus it turns out that animals adapted to cold environments and so less vulnerable to temperature changes than might be expected from the "normal curve", can live also in temperate climate. This is not surprising, if we remember that the "normal curve" is an empirical construction and was not based on measurements of cold-stenotherms.

It is perhaps a common phenomenon that the Q_{10} values for cold-adapted animals can be lower than those measured in the same temperature ranges for warm adapted animals. The survey of patterns of temperature acclimation offered by Prosser (1961) can lead to such a conclusion. According to him, the most frequently observed pattern of acclimation is that defined by him as translation and rotation (IV A C₁). In this case the curve representing the log stabilized rate of the process for cold-adapted animals runs higher than the similar curve for warm-adapted ones (the process is more intensive at the same temperature), but its slope is less steep, giving therefore a lower Q_{10} . However, Prosser offers examples of different patterns of acclimation, too. Klekowski *et al.* (1971, in print) observed an exceptionally high Q_{10} in a maritime Amphipoda, *Paramoera walkeri*, inhabiting the Antarctic coastal waters, in temperatures close to 0°C.

The relationship between wet weight (W , mg) and length (L , mm) was studied on single individuals of *P. intricata* (Fig. 3). One linear relationship was obtained on double logarithmic scale, covering a range of wet weights from 27 to 400 mg. Thus the relationship can be defined by a single formula, $W=0.266 \cdot L^{2.09}$, where the standard error of the regression coefficient b is 0.072, 95% confidence interval is 1.95 to 2.23, $n=116$. Analogously, for the dry weight range from 0.12 to 78.81 mg (Fig. 3) $W=0.020 \cdot L^{2.38}$, $S. E. \pm 0.064$, 95% confidence interval 2.26 to 2.51, $n=110$. The fact that the relationship between weight and length can be described by single formulae for both wet and dry weight is not surprising, as *P. intricata* is a hemimetabolic insect, and the whole experimental material belonged to the same population. The values of the regression coefficient b are much below 3 which means that the shape of the growing larva changes to a more elongated form. This seems to be a fairly common phenomenon; e. g., among the 18 values of b for the weight-length relationship quoted by Arabina & Gavrilov (1967) for freshwater invertebrates, 8 values of b are below 2.5, and only one, the highest, is equal to 3.0. The low values obtained of $S. E.$ of the regression coefficient b tend to prove the reliability of our data. The value of the b coefficient for the dry weight-length relationship is significantly higher than for the relationship between wet weight and length; the 95% confidence intervals do not overlap. This suggests that the % dry weight of wet weight tends to increase with increase in size of larva. The percentage dry weight of wet weight computed from the above formulae is, e. g., 14.3% for 9 mm larvae and 20.7% for 30 mm larvae.

No change of calorific value of dry matter was found within the whole weight range from 25 to 325 mg, and thus the mean value 5.578 cal/mg of dry matter was employed for calculations (Tab. 2). It must be noted, however, that the youngest larvae, below 25 mg and the oldest ones, above 325 mg, were left out of the study. The calorific value of organic matter also does not change as the larvae grow from 25 to 250 mg, but for the largest ones, from 250 to 325 mg, it is higher and the difference is statistically significant at 1% level (t -test). This suggests physiological changes related to the forthcoming metamorphosis and reproduction. Prus (1970) compiled calorific values of organic matter for 84 species of aquatic animals (Tab. 2). If we compare his data with our findings for *P. intricata*, it can be seen that the latter are fairly high but still within the range of values quoted by Prus. The organic matter content in dry matter (%) determined by

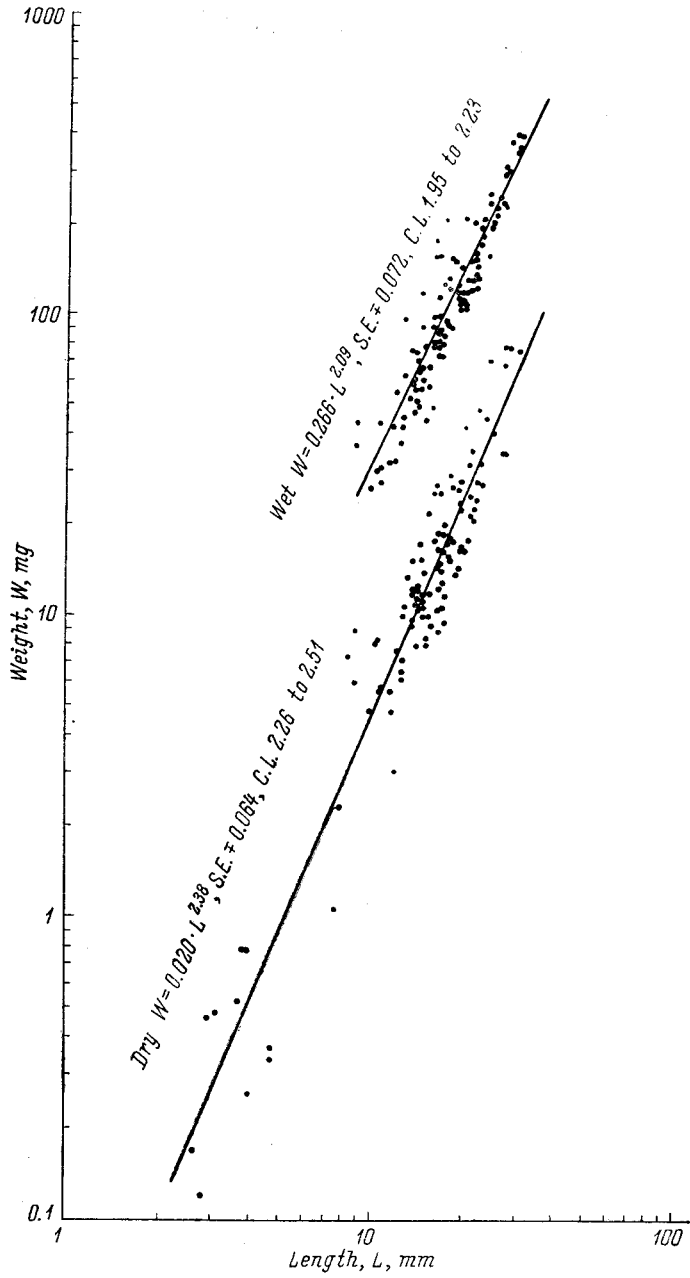


Fig. 3. Relationship of wet and dry weight to length of *P. intricata* larvae.

Tab. 2. Calorific value and per cent of organic matter in dry matter in two weight groups of *P. intricata* larvae.

Animal	Calorific value		Organic/dry matter, %	
	cal/mg dry matter	cal/mg organic matter	bomb	ignition
<i>P. intricata</i> :				
25—250 mg	} 5.578 5.528—5.628 (30)	5.890	95.28	93.86
		5.823—5.957 (24)	94.42—96.14 (24)	91.74—95.98 (5)
250—325 mg		6.118	90.82	—
		5.889—6.348 (6)	87.95—93.69 (6)	—
Aquatic animals (P r u s 1970)	—	5.528	—	—
		4.419—6.637		

Means, 95% confidence interval (n).

the ash content in the bomb was higher for smaller larvae; this result is significant at 0.1% level. A comparison of organic matter content for larvae from 25 to 250 mg as determined by "bombing" and by burning in a muffle furnace revealed no significant differences ($t=1.443$).

251 measurements were made to find out the relationship between weight increase (P , mg wet weight/day) and initial weight (W , mg wet weight). The relationship is defined by the formula $P=0.0025 \cdot W^{0.98}$. The high value of the regression coefficient is probably the effect of the fact that the youngest and oldest larvae have not been investigated.

The relation of oxygen consumption (QO_2 , $\mu\text{l O}_2/\text{ind}\cdot\text{ähr}$) to weight (W , mg wet weight) was investigated, Fig. 4. This relationship can be defined by only one formula $QO_2=0.167 \cdot W^{0.86}$; the standard error of the regression coefficient b is 0.046, 95% confidence interval is 0.77 to 0.95, $N=187$. Comparative measurements, not included in Fig. 4, which were carried out in respiration chambers without pebbles, gave respiratory values twice as high. The measurements of oxygen consumption presented here were always made at 11.30 a. m. A control measurement of oxygen consumption for 8 individuals throughout 24 hours revealed no significant difference between average consumption of oxygen as measured at 11.30 and the average for 24 hours ($t=1.307$). 19 measurements of the respiratory quotient gave a mean value of 0.75 ± 0.015 S. E. Thus, the daily cost of maintenance in calories (R , cal/ind. \cdot day) was computed by multiplying the hourly oxygen consumption (QO_2 , $\mu\text{l O}_2/\text{ind}\cdot\text{hr}$) by 24 and by the oxygen-calorific coefficient $4.739 \cdot 10^{-3}$ cal/ $\mu\text{l O}_2$, appropriate for the above RQ .

Tab. 3 presents production (P , cal/ind. \cdot day), cost of maintenance (R , cal/ind. day) and efficiency of utilization of assimilated energy for growth ($K_2=P+R$, %) for various weight classes of *P. intricata*. The efficiencies are very low. Actually, values of K_2 of the order of 10—20% have been quoted for some species of aquatic animals (*Alysia punctata* — C a r e f o o t 1967; *Ctenopharyngodon idella* — F i s h e r 1970; *Stenonema pulchellum* — recalculated from T r a m a (1972 in print) *Asellus aquaticus* — P r u s (1972 in print), but for herbivores or detritus feeders. For the predator *P. intricata* a much higher value of K_2 might have been expected.

Tab. 3. Daily production (P), daily cost of maintenance (R) and efficiency of utilization of assimilated energy for growth (K_2) of different weight classes of *P. intricata*.

Wet weight, mg	P	R	$K_2 = \frac{P}{P+R} \%$
	cal/ind. day		
25—49.9	0.071	0.432	14.12
50—74.9	0.127	0.672	15.90
75—99.9	0.184	0.898	17.01
100—124.9	0.243	1.116	17.88
125—149.9	0.305	0.326	18.70
150—174.9	0.366	1.533	19.27
175—199.9	0.430	1.734	19.87
200—224.9	0.494	1.932	20.36
225—249.9	0.560	2.126	20.85
250—274.9	0.626	2.318	21.26
275—299.9	0.693	2.508	21.65
300—324.9	0.760	2.695	22.00

The low value of K_2 can be accounted for by either too low values obtained for P , or by too high values of R , or by both. Low P values could be the result of the procedure of weighing, as it was performed twice a week at room temperature, or it might have been caused by the type of food administered during the experiment, or by the lack of water flow in the experimental vessels. F e l d m e t h (1970a) found that *Pycnopsyche lepida*, living in streams with fast current, grew much more slowly when it was kept in still water than in fast-flowing water. The results of growth of *P. intricata* weighing 200—225 mg, taken from preliminary field measurements gave P of about 1 cal/ind. day, which is twice as high as the laboratory value. Then the K_2 value would become 40%. The obtained values of R were high, probably because they were measured in still water. An earlier research (K a m l e r 1969) proved that the frequency of respiratory movements of *P. intricata* is dependent on current velocity and that it attains the highest values in still water. Ventilation involves muscular activity and therefore it requires energy in itself. F e l d m e t h (1970b) found for the above mentioned *P. lepida* living in fast-flowing streams that its active respiratory rate in still water was 4 times higher than the inactive respiratory rate; according to him, this difference is mainly caused by the ventilatory activity, since the locomotory activity in those conditions is negligible. If we divided the values of R obtained for *P. intricata* by 4, then the values of K_2 would become 40—50%. However, if the measurements of oxygen consumption had been carried out in respiration chambers without substrate, then the values of R would have been about twice as high as the actual ones, and the values of K_2 would have been even lower than they are now. It is also possible, that the high cost of maintenance is a peculiar characteristic of this cold-stenothermal species.

Thus the problem, of whether the low values of K_2 which have been obtained can be accounted for by experimental conditions, or if they are typical for the species investigated, still remains unsolved. From this can be seen, that the investigations of bioenergetics in cold-stenotherms from

flowing waters is difficult, and so it is not surprising, that up to date very little has been done on the subject.

It may be added, that besides *P* and *R* measurements, consumption and the unassimilated part of the food were also measured; those results are now being elaborated.

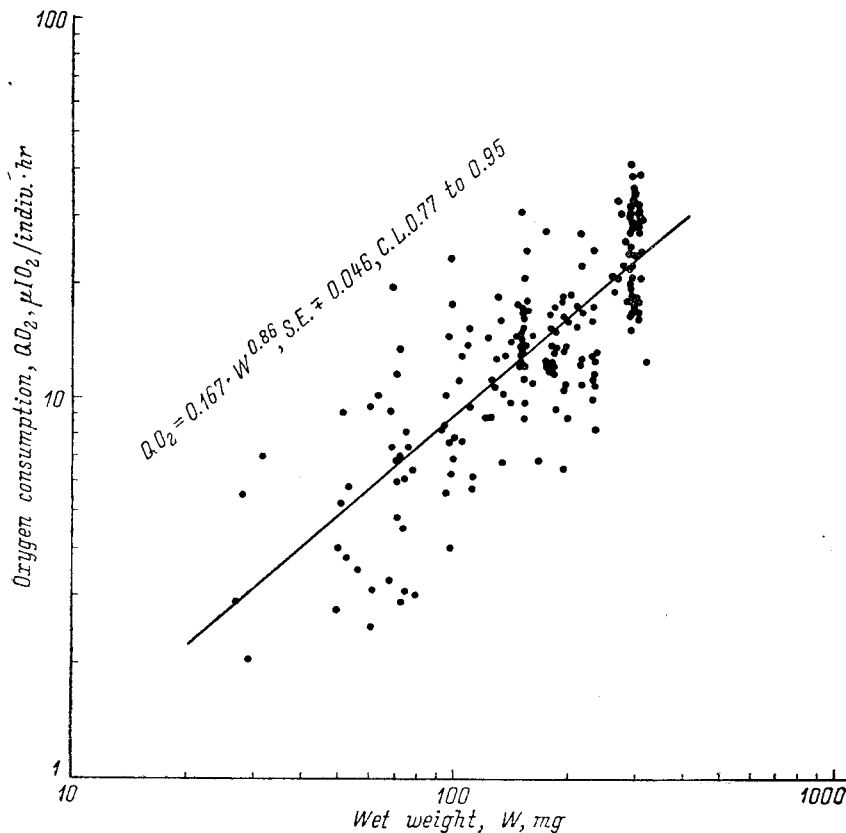


Fig. 4. Relationship of oxygen consumption to wet weight of *P. intricata* larvae.

S u m m a r y

Temperature and oxygen conditions in the natural habitat of *P. intricata* nymphs have been characterized. Lethal O_2 concentrations for *P. intricata* were measured in two temperatures and compared with those of an eurytherm mayfly, *Cloeon dipterum*. The value Q_{10} coefficient is discussed. Regressions of length on weight (wet and dry) are presented as well as calorific values for some nymphal stages. Production and metabolic losses were estimated in $5^\circ C$.

Р е з ю м е

Охарактеризованы температура и содержание кислорода в воде природных местобитаний личинок *P. intricata*. Определена летальная концентрация O_2 для *P. intricata* в сравнении с аналогичными данными для личинок эвритермной поденки, *Cloeon dipterum*. Обсуждается величина температурного коэффициента Q_{10} . Представлены соотношения веса (сырого и сухого) и длины, а также данные по калорийности. Выражена зависимость потребления кислорода от веса тела. Продуктивность и траты на метаболизм определялись при $50^\circ C$.

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Discussion

Edington: Do you consider that the low Q_{10} value for *Perlodes* between 5.5 and 10.5° C provides evidence for a plateau in the graph of the relationship between temperature and respiration rate?

Kamler: No, I do not think. My opinion is that low Q_{10} value is typical situation in this cold-water stenotherm. But I cannot exclude the presence of plateau as I did not measure respiration in small intervals throughout a wide range of temperatures.

Hildrew: 1) Could you specify in more detail your observations on stream temperatures? 2) Low Q_{10} 's are found in animals where environmental temperatures fluctuate widely, cf. the work of R. C. Newell in England on intertidal animals.

Kamler: 1) I presented the frequency of observed levels of temperature (most often 4—14° C). My own measurements in the stream from which the material has been taken gave: summer maximum about 7.5° C; winter temperatures 4° C. The short periods of the lowest temperatures (1—2° C) occurred in spring, due to melting snows. 2) I believe that the pattern of acclimation presented here (low Q_{10} for animals adapted to steady cold environment) is really existing, but other patterns are possible as well. Thank you for Newell.

Pattée: You mentioned the respiratory metabolism measured at 11.30 a. m. — how long did the actual experiment last?

Kamler: Experiments lasted 1.5 hours, started at 10.45 and were finished at 12.15.