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Individual growth and seasonal cycle of Daphnia middendorffiana in an Alpine lake.

I.Ferrari.

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Translated by W.J.P.Smyly.

At the conclusion of a paper devoted to the morphological study, on a systematic and biometrical basis, of Daphnia middendorffiana in the Lago of Campo 4° (2293 m above sea level, Bognanco Valley, Western Alps, Italy) (Ferrari 1967) the opportunity was presented to investigate the problem of the peculiar reproductive biology of this cladoceran from a cytological viewpoint, and to estimate by direct observation the meiotic phenomena of the eggs both subitaneous and resting, and during maturation, the true mechanism of the succession of reproductive phases of different ecological significance. It is noteworthy in D. middendorffiana that there is an alternation of generations by eggs which develop at once and by ephippial resting eggs; the absence of males means that the production of the ephippial resting eggs (pseudosexual) comes about as for parthenogenesis, unlike that which is observed among most species of Daphnia in which the full development of the resting egg in the ephippial case depends on fecundation.

The results of a preliminary investigation on maturation of the egg of D. middendorffiana from Campo 4° (Zaffagnim and Sabelli, 1970) confirm the observations of Schrader (1925) on a pseudosexual stock of Daphnia pulex. The maturation process of the resting egg begins with a single division of equational type with no coupling and with no reduction in number of chromosomes, as in the normal parthenogenetic egg. An oocyte therefore would develop into a subitaneous or a resting egg not on the basis of the type of maturation which takes place but with reference to the nature of the processes in the formation of the egg-yolk.

On the other hand, it was of interest also to study more accurately some parameters of a natural population of D. middendorffiana so as to assess thoroughly the dynamic aspects of its seasonal cycle, namely the succession of the growth stages, the number of generations and the importance of each of them in relation to determining the size of the whole population, the alternations of the reproductive method and so forth.

Numerous samples, for that reason, were taken in the lake of Campo 4°, from June to October in 1968, monthly up to the end of August and every two to three weeks in the last two months. Each time, the collected material was used to proceed with the preparation of laboratory cultures and the individual growth of the animals was observed under a variety of ambient conditions, (temperature, photoperiod, etc.). The experimental data permit the interpretation of the biodynamic events in action in the natural environment, also through the formulation of hypotheses relative to the rhythm and phases of development of the population, verifiable for each successive collection of Daphnia in the lake.

During the summer and autumn of 1969, further samples were taken with about the same frequency of those of 1968; this second series of results was considered necessary to verify some results from the first year and to evaluate the importance of differences between the seasonal cycles of the Daphnia in two consecutive years.

Material and methods

During the first year of research, on each visit to the Lake of Campo 4°, a large volume of water was filtered to retain zooplankton (51 threads per cm) to obtain a qualitative sample representative of the population in all its components, spatial and ecological. The methodological limits of the vertical hauls were ascertained, when repeated over a certain number of profiles, and also to evaluate the biomass of all the population living in the lake. In fact, the distribution of Daphnia, as of the other forms of zooplankton, is largely conditioned by the morphological relationships of the lacustrine surroundings in microhabitats with unusual characters of situation liable to rapid changes in weather and to rapid transfer from one part to another in the biotope. There is observed a type of spatial aggregation in swarms, frequently encountered in all the samples, a phenomenon amply verified also on the other hand in lacustrine waters of sufficient size. The samples were then mixed combining the different types of collection, (vertical profiles at different depths or transverse according to the longer axis of the lake or shorter hauls parallel to the shore) giving in the end enough animals and sufficiently representative of the variety of microhabitats present in the lake. Obviously, collections of this type do not permit the population dynamics to be followed on a quantitative basis, that is to say they do not lend themselves to a study of the antagonistic roles exercised on the modelling of the population by mortality and natality of the various ranks of frequency into which they can be divided : nonetheless, they put at our disposal a large amount of information relevant to the different levels of structure into which the population tends to be organised.

Before starting the summer programme of collecting in 1969 it was decided to institute more rigorous criteria for sampling, fixing a certain number of shore stations and drawing the net - from a certain depth below the water surface - based on bathymetric data, - along a prearranged number of transects of known length. Volumes of the order of ten cubic metres of water were filtered each time. But the results of calculations did not confirm the overdispersed character of the Daphnia distribution of population and could not be generalized to give a value for the numerical density and biomass present in the whole water-mass.

All the animals caught were transported in thermostatic bottles to the laboratory, where the calculations were made and each animal was measured under the microscope: for each individual, length was measured from the apex of the head to the point of attachment of the tail-spine. These measurements are probably indicative also of weight increases in D. middendorffiana. Burns (1969) has found that there is a correlation between the logarithm of length and weight in all stages of development of four species of Daphnia (magna, schodleri, galeata mendotae and pulex): an analagous relation is documented by Edmondson (1955) for D. pulex tenebrosa (= middendorffiana) of Alaska.

After the calculations and measurements, part of the material was preserved in formalin and part was cultured in glass vessels, previously sterilized and containing fresh Chlorella suspended in Lake Maggiore water membrane-filtered (Sartorius MF11009, porosity 0.2μ). Every three to four days, the Daphnia were transferred to freshly prepared culture-medium.

The cultures were kept for the most part at a constant temperature of 10°C and a photoperiod regulated approximately to the seasonal variations in the light:dark ratio in nature (14:10 in July and August; 12:12 in September; 10:14 in October, and so on). As a source of light we used an outfit with six fluorescent tubes of white light : the light intensity on the cultures was estimated at about 1500 lux.

In the collections taken in 1969, we noted in addition the number of subitaneous eggs or embryos carried by each ovigerous female preceding measurement, in order to be able to correlate this important biological parameter with body-size. The calculations, as usual, were made after preservation in formalin and included the aperture of the carapace valve and escaped embryos from the brood-chamber.

Thermal conditions and population dynamics of the animals of the alpine lakes.

To be able to evaluate the kind of changes, thermal, daily and seasonal affecting the water of an alpine pond, it would be necessary to take a large number of samples continuously over a long period of time, in different parts of the lake surface and along vertical profiles into the greatest depths, when these regions achieve such values to make possible the establishment of a clear thermal stratification during times of calm and of strong solar radiation. In effect, temperature parameters show large variability over very short periods of time, because of sudden meteorological changes: a few hours of wind are enough, even in conditions of strong sun, because the surface temperature falls and gives rise to isothermal conditions at values which are very low even in high summer.

On the other hand the study of the thermal budgets of these small lakes is of extreme importance : the establishment, conservation and biological equilibrium of these communities are conditioned to a large extent by the kind of thermal regime which influences the lacustrine water.

The thermometric results collected in 1968 and 1969 in the Lake of Campo 4° are totally inadequate for preparing a temperature budget : surface temperatures were observed in the centre of the lake and at five inshore stations; temperatures were also measured at the principal inflows and outflows, and some in deep water (these latter readings made with a reversing thermometer) (see Table 1 and Table 2). It is nonetheless possible to reach some conclusions orientated relative to the peculiarities of the thermal regime of Campo 4° and to the main line of development of its thermal changes in summer and autumn. In the first place, the surface temperatures in the littoral zone, being more directly correlated with the atmospheric temperature and the general meteorological conditions of the short period of the observations, did not differ as a rule by a four tenths of a degree from that measured in the centre and outflow. The series of temperatures of the water at the inflow are, however, for each date, noticeably lower than those measured at other points. The inflowing water mixing with that in the lake all through the summer serves to keep the water of the lake at low temperatures and tones down the effect of solar heating, levelling to low and uniform value the thermal state of the whole water-mass. The mixing water thus shows an effect of compression on the thermal summer regime, very characteristic of high mountain lakes and seen also in another series of temperature results collected from lakes in the same valley of Campo to which stream waters enter but do not mix. The maximum temperature registered at the surface of lake Campo 4° was 10.7°C in the summer of 1968 and 10.8°C in the summer of 1969. On sunny days, the water of the neighbouring lakes Campo 2° and 3° warmed up to 15°C and over.

There would seem therefore to be a strong inflow of cold water into lake Campo 4°; this fact implies also a rapid renewal of the whole water-mass : estimating at 0.1 m^3 per second (an estimate certainly subject to error) the rate of the main inflow which comes down from the snowfield at times of flood, the whole water volume of the lake (80.000 m^3) would be renewed in the course of about ten days.

Table 1. Temperatures (°C) observed in the lake of Campo 4° in summer - autumn, 1968.

Dates	Surface centre of lake	Deep water	Surface inshore	Inflow
29-VI-1968	3.0	3.5 (m 5.20)	3.0	-
26-VII-1968	8.9	8.0 (m 6.00)	9.5	-
12-VIII-1968	-	-	8.2	-
26-VIII-1968	10.6	9.1 (m 5.50)	10.7	-
5-IX-1968	-	-	7.0	-
28-IX-1968	-	-	7.0	4.8
17-X-1968	-	-	7.0	-

Table 2. Temperatures (°C) observed in the lake of Campo 4° in summer - autumn, 1969.

Dates	Surface centre of lake	Surface inshore					Outflow	Inflow
		Station N	Station NE	Station SE	Station S	Station W		
14-VII-1969	7.5	-	8.5	-	-	-	-	5.0
1-VIII-1969	10.4	10.8	10.4	10.7	10.2	10.5	-	-
22-VIII-1969	9.6	9.7	10.3	10.2	9.7	9.5	9.6	5.7
16-IX-1969	-	8.1	8.0	8.0	8.0	7.9	8.0	4.5
10-X-1969	-	8.4	9.3	9.2	8.1	8.2	7.9	6.2

Only in late summer, when the inflow of cold water from the snowfield has diminished and the time of water renewal has lengthened does it become possible to observe the fuller oscillations in respect to the variability of the meteorological conditions. But in the meantime, the effect of solar radiation on warming of the lacustrine water will already be appreciably reduced with regard to the seasonal maxima and will be able to settle down only with difficulty, even only temporarily, at temperatures higher than those of the early summer. In particular one decisive fact must not be overlooked in the containment of thermal fluctuations, whether daily or seasonal, and that is the considerable volume of the water in this lake which has a maximum depth of 7.4 m and a mean depth of 4.5 m (Tonolli 1947).

In its main lines, the annual thermal history of the lake tends to repeat itself with a certain regularity. It is possible to envisage a succession of temporal phases according to a certain general scheme. During the period of the thaw which extends over several weeks (even up to the second ten-day period of July, as in 1968), the water-temperature stays at very low values, the calories provided by the already strong solar radiation being converted into calories of melting and not contributing to raising the thermal level of the water. Then there follows the summer thermal maximum which starts more abruptly in years in which the spring warming has been delayed and hence the end of the ice-thaw. The process of cooling in late summer, fairly slow because of the large thermal inertia of this water-body, is followed finally by further cooling, more abrupt which foreshadows renewed ice-cover (month of November).

The thermal regime, in lakes like this, has a decisive influence on the expression of the biological processes which organisms complete and hence to impress a certain rhythm of growth and a certain duration of their cycle of development.

It has been observed that the more abundant planktonic forms in these waters belong to Diaptomidae (Copepoda) and Cladocera, especially Daphnia, while other groups as Cyclopidae (Copepoda) and Rotifera are at times well represented (Tonolli 1949). Each of these groups has its own ecological requirements which are seen in chronological differences in their entrances and collapses in their respective biological cycles and also in participation in competitive phenomena which in the course of their development assume diverse significance. It is known for instance that in alpine lakes copepod nauplii appear before the juvenile stages of cladocerans : this fact has been confirmed in the early summer collections in the lake of Campo 4°.

Successively, as high enough thermal levels become established and have started the normal processes of development for the whole population, each of these will preserve specific growth rhythms, while, at the interior of each group, the differences between the growth rates of different organisms will always be minimised. Highest temperatures will indeed accelerate the growth processes of each individual organism. This brings about a very strong reduction in the initial chronological distance between the various phases of development; the thermal state, at least in this period, works on the population to instigate a massive and explosive development, with consequently a drawing together and almost super-position of levels of organisation within a structure which tends to be simplified.

In the lake of Campo 4°, the thermal conditions work as factors of unification on the line of development of the population also for other reasons. We are dealing with an environment which also in its morphometric characters, presents relative ecological stability in respect to environments of more modest dimensions: this avoids the ensuing confusion of situations with sharp pulsations, in one way or another in the populations, which are commonly found in small alpine lakes (Tonolli, 1954). It has already been stated that Campo 4° is a cold lake, certainly one of the coldest lakes found in the Val Bognanco, mainly because of the rapid water renewal assured by the inflow of melt-water. All this results in lengthening of the seasonal biological cycle, and it has been possible to suggest, besides a certain orientation of the periodicity with which it was necessary to effect the collection of zooplankton.

Individual growth of Daphnia middendorffiana.

In the life of a Daphnia there are two successive and distinct stages : there are preadult or prereproductive stages and adult or sexually mature stages. The number of the pre-adult stages varies from stock to stock even of the same species of Daphnia : this is one aspect of the important role played by genetic factors. But other factors also have an important rôle in the natural environment in determining in which stage the animal reaches sexual maturity, in which stage, that is, the first eggs are laid. Green (1954 and 1956) considers that it is the size of individuals at birth which influences the number of moults necessary to reach maturity. In essence, the animal would reach sexual maturity when its body length had surpassed specified values of critical size for the so-called puberal moult. Zaffagnini (1965b) made a thorough analysis of the preadult growth phase of Daphnia magna and concluded that there are a variable number of larval or juvenile stages (from two to four normally) and a second phase of two stages, preadolescent and adolescent, as defined by Green (1956). The two phases are distinguished by a different inclination of the growth curve and are separated by the prepuberal moult, whose critical value is also related to the fact that, in the females, they develop characters bound up with the incubation (convexity of the dorsal margin of the carapace and strong development of the first two abdominal processes) (Zaffagnini 1965 and 1965b).

In this way, the prepuberal moult is always placed in time at the same distance from the moult to sexual maturity and, like this, would be connected with the gaining of a certain body-size and a certain stage of development. The succession of moults, as carried out in the first two stages of growth, seem on the whole to be strictly linked to the differentiation of sexual characters and would be a specific character individually possessed by the organisms.

The series of preadult moults studied in D. magna is not encountered so clearly in all other species of Daphnia: Green (1956) indicates in D. ambigua and D. obtusa that eggs are first laid in the fourth instar, which means that in these species there are only three moults before maturity. Anderson (1932) finds that in some individuals of the same D. magna a much higher number of preadult stages, up to seven or eight : in these cases it is difficult to imagine that the second growth phase continues to be characteristic and definitive to the usual two stages included between the prepuberal moult and the puberal, and that all the supernumary moults are inserted in the first phase. Zaffagnini (1965b) refers to abnormal moults induced by changes in internal factors (metabolism) and of ambient factors (alimentation).

The effects of temperature and food on growth in daphnids are sufficiently well-known. As a general rule, it is known that the response to periods of scarcity of available food, growth is slowed down and there is also a reduction in the growth increment to body-size at each moult and a smaller reproductive potential. (Ingle, Wood and Bauta 1937; Hall 1964). Low temperatures also have the effect of reducing growth, prolonging the intermoult periods and favouring in general greater longevity (MacArthur and Baillie, 1929; Hall 1964).

The growth curve described by Edmondson (1955) for Daphnia pulex tenebrosa of Lake Imikpuk (Alaska) has a two-fold interest; firstly, it refers to the same species which lives in the Lake of Campo 4°; secondly, it has been constructed from measurements made on animals immediately after collection in the lake. Edmondson, from study of histograms based on the distribution of of frequencyoof classes of body-size, singled out a certain number of artificial groupings corresponding to different growth stages, thus following the growth of the Daphnia in an arctic lake through the first nine stages. The study of the growth curve suggested the existence of two preadult phases, quite distinct, made up of two stages each, follwed by an adult phase characterised by a reduced growth rate. The author moreover found that the smallest egg-bearing animals belonged, from their dimensions, to the sixth instar. There were therefore at least five preadult stages.

Another kind of question arises, though strictly related to that previously raised, if one studies the growth of a Daphnia after she has reached maturity in relation to the parthenogenetic reproductive activity. The reproductive capacity (expressed as relation between number of young born to one Daphnia in the course of its adult life and duration of the reproductive period) depends either on the temperature (which controls the duration of an instar and hence the time in which a subitaneous egg can start to divide and complete the embryonic stages) or on the level of food, which controls clutch-size and hence the number of neonates. Natality is influenced also by the age of the mother : in this connection, Green (1954) in a study of D. magna found a direct correlation between maternal body-size and production of parthonogenetic eggs, at least in the first adult instar. The same author has observed that the neonates of the third brood were on average larger than those of the other stages either preceding or succeeding : this fact is reflected in the diversity of growth rates of the offspring from successive egg-layings.

The puberal moult need not lead to the laying of subitaneous eggs but can be followed at once by the appearance of ephippia if in the preadolescent stage the differentiation of the resting egg was initiated. This will be deposited in the ephippium at the start of the first adult stage. The formation of the ephippium is always at any rate the response to a stimulus induced by the formation of the resting egg. In its turn, this last process sets in motion a stimulus on the nature of which there are differing opinions: some (Slobodkin 1954) attribute production of resting eggs to decreasing levels of food : others (Stross 1969: Stross & Kangas 1969) associate the start of diapause with photoperiod and temperature. The time which elapses between the action of the ambient stimulus and the appearance of the ephippium is relatively long and corresponds to about two intermoult periods. In the ephippio-genetic process in D. middendorffiana, the pseudosexual egg, from its formation to deposition in the ephippium passes through the same succession of phases which characterize the maturation of the [anfigonico] egg of a common heterogenic Daphnia. In the moults which follow that which coincides with the appearance of the ephippium, there is released at the same time in the exuvia of the animal, from that time preserved in advance the "record" of that event, in the morphology of the dorsal margin of the carapace. If the ambient stimulus has not continued to act in stimulating the formation of resting eggs, the moult may be followed by deposition of subitaneous eggs. If however the external conditions do not change from those present at the first ephippial production, others will follow but the moult with formation of the ephippium will be followed by a stage "in white" and the new ephippium will appear only with the succeeding moult.

The growth of Daphnia middendorffiana was followed in individuals hatched from ephippia or born from eggs collected in the lake and then kept at a constant temperature of 10°C. Many cultures were prepared in which animals of different size developed, to start with in natural surroundings.

Ephippia collected in the samples of June and July 1968 hatched in the laboratory and neonates (27 altogether) were accurately measured. Their carapace length ranged from as little as 900 µm to 1 mm. One of these exephippial individuals hatched on 1 August measured at birth little more than 900 µm; five days later it had moulted to the second instar with an increase in length of 250 µm. The second moult after another five days added on another increment of about 300 µm. The third stage lasted six days and ended with the prepuberal moult by which time the length of the daphnid surpassed 1750 µm.

Other exephippial births in the laboratory were observed up to the prepuberal moult and daphnids that, at the start of rearing, could with certainty be placed in the second moult having lengths from 1150 to 1200 µm. The critical value of the prepuberal moult was always reached after a varying number (always more than three) moults; in general, duration of stages tended to increase with age.

The most interesting information obtained from these first laboratory results was that at a temperature of 10°C, the duration of the first growth phase was never less than 15 days.

Using higher temperatures (18°C) this period was greatly reduced and the duration of a stage changed from 5-6 to 2-3 days. It was however difficult to rear D. middendorffiana in these conditions : the most intense metabolism induces fast growth rates which are not sustained by the adaptive capacity of the animal and cause usually early mortality. In one instance the whole first reproductive phase of a Daphnia was followed at ambient temperatures : after three moults, the animal at one week after birth had doubled in size and reached the prepuberal moult.

With animals born in the laboratory from exephippial catches in the lake on 26 August and 5 September 1968, individual growth was studied up to the reaching of sexual maturity (Table 3). Of three *Daphnia* born on 6 September one reached maturity with the sixth moult in 33 days, the other two in the seventh in 40 and 42 days respectively. Separately from these three, another 2 *Daphnia* were reared, born also on 6 September which laid subitaneous eggs at the seventh moult, about 40 days after birth. One other *Daphnia* born on 12 September laid subitaneous eggs at the sixth moult on 15 October (No. 6 in Table 3).

The moult at sexual maturity was observed from deposition of a considerable number of eggs, and is certainly associated with the abundance of food. At birth the length of all six animals was around 1.050μ; the first growth phase included rather a high number of stages (from 4 to 5); they show modest increments in size from one stage to the next. The second preadult phase always has only two stages, each of which lasts appreciably longer than those of the first phase.

Table 3. Results for individual growth of six exephippial individuals of *Daphnia middendorffiana* reared at 10°C; l_m, l_p, l_s : length in μ at birth, after the prepuberal moult and after the moult to sexual maturity; t_1 and t_2 : duration in days of the first and of the second growth phases; i : number of stages; E : number of subitaneous eggs deposited at the first laying.

No	l_n	l_p	l_s	t_1 (i)	t_2 (i)	E
1	1.030	1.820	2.230	22 (4)	11 (2)	5
2	1.030	1.880	2.290	27 (5)	13 (2)	5
3	1.030	1.820	2.350	28 (5)	14 (2)	5
4	1.060	1.880	2.530	27 (5)	12 (2)	9
5	1.060	1.820	2.350	28 (5)	13 (2)	6
6	1.060	1.880	2.290	21 (4)	12 (2)	6

A culture was prepared with three daphnids developed from subitaneous eggs and born on 18 September : their length at birth was 1.150μ; neonates hatched from ephippia were thus longer than those non-exephippial (Table 3). Table 4 gives some data related to their growth.

Table 4. Results for individual growth of three non-exephippial individuals of Daphnia middendorffiana rared at 10°C; l_m, l_p, l_s : length in μ at birth after the prepuberal moult and after the moult to sexual maturity; t_1 and t_2 : duration in days of the first and of the second growth phases; i : number of stages;

No	l_n	l_p	l_s	t_1 (i)	t_2 (i)
1	1.170	1.880	2.350	13 (3)	18 (3)
2	1.170	1.760	2.290	14 (3)	11 (2)
3	1.120	1.880	2.230	18 (4)	10 (2)

Individuals No. 2 and No. 3 reached the puberal moult after five and six instars respectively, with deposition of resting eggs in the ephippium showing that sexual maturity had been reached. Daphnia No. 1 reached the critical dimensions of the puberal moult at the end of the fifth instar, but laid resting eggs in the ephippium only after another instar lasting seven days; this last moult was not accompanied by increase in length of the carapace.

A group of ten daphnids collected from Campo 4° on 28 September 1968, all beyond the prepuberal moult, were reared singly in glass dishes. A first series of measurements were made on 2 October, the series following after a number of exuviae, equal to the number of reared animals, had been released : the experiment was helped by the fact that, for two complete stages, there was no mortality. In such a way, one can calculate a mean duration for the stages of the last prereproductive phase and the value of the relative growth events. On 2nd October the ten daphnids ranged in length from 1,820 to 2,000μ. It is necessary to allow ten days for shedding of all exuviae : on 12 October carapace lengths were measured again and this time ranged from 2,110 to 2,300μ. With successive moults all the animals eventually reached sexual maturity : between 12 and 21 October, 7 daphnids laid subitaneous eggs and their lengths varied from 2,470 to 2,590μ; the lengths after the moult of the other three were from 2,350 to 2,410μ and they had ephippia. The mean values for length of carapace (standard deviations in parenthesis)up to the 2nd, the 12th and the 21st October were 1,938 (± 58), 2,210 (± 50) and 2,500 (± 151)μ respectively. These daphnids, to complete the last two reproductive stages, took a very much longer time than that required by animals kept in culture from birth; had larger increments in body-size at each moult; also size at sexual maturity was notably bigger. One could envisage that in the natural environment, during the first stages of development they acquired dimensional levels higher than those in the artificial medium, conserving afterwards this advantage right through the whole preadult period. The lower values in dimensions regarding length of carapace of ephippial animals seems to be related to particular morphological adaptations which the animal undergoes with the appearance of the ephippium.

The results relating to the stock of ten daphnids referred to blockages in individuals developing, not being able to maintain the moults, because of the conditions of the medium, compared with the continuity of those having antecedents in the lake; this requires the working out of certain premises of biological and ecological rules concerning single organisms during their stay in natural water. These premises and in the first place the nutritional history of each individual will condition and decide the type and the rhythm of growth in successive times. These results then reflect more nearly the reality of biological events in the lake than of those collected in the first cultures : not only that but they make it possible to evaluate the "naturalness" of the level of the living conditions provided for the animals reared in the laboratory from birth.

A culture of animals in the stage immediately preceding the puperal moult was started with exephippial animals collected on 26 August 1969 to verify the critical dimensional limits corresponding to the attainment of sexual maturity in individuals collected from their natural environment after only a few days. Thirteen daphnids were reared at 10°C and a photoperiod of 14:10 on 27 August; size limits (minimal and maximal) were 2170 and 2470μ and none of the daphnids carried eggs. In the course of eight days all moulted once and at each moult they laid, except one, subitaneous eggs. The increase in length of carapace was clear enough: the attained size at this moult lay between 2410 and 2760μ, the mid values being from 2350 to 2590μ. With the new moulting cycle, the young, which completed their embryonic development in the brood-chamber, were released from the body of their mother whose size increase was always less than that observed in the preceding moult.

Finally, reproductive phenomena were studied and associated rhythms of increase.

The Daphnia referred to in Table 3 released their young 7 to 8 days after deposition of eggs. From adult or pre-adult daphnids collected on 5 September 1968, a series of cultures were started at different temperatures : at 18°C, the interval between moults was 3 to 4 days, while at 10°C the intermoult period was more than a week.

Other cultures were set going with material from the last two collections of 1968 (28 September and 17 October). On the 2nd of October, 123 large-sized daphnids (exephippial daphnids which had already produced several broods of neonates from subitaneous eggs) were transferred to glass-bowls at 4°C in total darkness. After 15 days only two thirds of the animals had moulted: this event, in all cases, was followed by the appearance of an ephippium and was preceded by the liberation of the young which had developed from the last deposition of eggs to develop at once.

On the 20th October, 46 daphnids with ephippia were isolated from field collections three days before the temperature of the lake reached 10°C and at a photoperiod of 10:14. Moults with release of ephippia followed for all the last ten days of the month: at the time of each moult a new ephippium was not observed, nor subitaneous eggs in the brood-chamber. During the first ten days of November, the majority of the daphnids (22 out of the 26 survivors) produced a new ephippium; the others continued to lay subitaneous eggs. From the 11th to the 15th of November the last survivors cast off their second ephippium.

Measurements made on these animals showed moreover that their growth rate was the slowest recorded by the advanced adult stages.

Other results of positive interest can be gathered from numerous cultures with animals collected on the 28th September 1968 and brought to maturity in the course of rearing (always at 10°C). On 2nd October, about ninety daphnids were isolated with a carapace length ranging from 1450 to 1750μm. On 15 October, after two moults, 47 of the longest daphnids of this stock were separated and found to measure from 2050 to 2350μ : none of these had reached sexual maturity. With succeeding moults, 20 of these laid subitaneous eggs and 10 ephippia; the remainder were in the last prereproductive stage. Starting from the 28 October they began to produce respectively the first neonates and the first ephippia; the time required to complete the first adult stage was certainly not less than 8 to 10 days. It was observed that while the ephippial neo-adults measured from 2150 to 2350μ, daphnids with first embryos attained up to 2500μ.

On the basis of results from cultures, especially those with animals which had spent part of their lives in the natural environment, one can attempt to make a preliminary assessment of the growth of Daphnia middendorffiana. But quite a number of considerations have to be dealt with first. More especially, dynamic processes occur in the lake which involve the entire structure of populations with regard to age and size classes; the results from a study of the growth of individuals cannot then be used mechanically as parameters for an interpretation of the dynamics of a natural population. With respect to the modifications of the ambient factors, the response of an individual (which consists of its physiological adaptations to new conditions) is certainly more simple than that of the whole population which will tend to show in a new equilibrium, a different distribution of the frequency classes of age and of size with a certain slowing up - in respect to the rising of the external stimulus - which acts more on the physiological properties of individuals than on the intrinsic properties of the same population, (Slobodkin 1954).

In the second place, the fact of having always furnished an abundance of food for animals in culture does not mean that the artificial means offers all the necessary requisites for normal development and reproduction of D. middendorffiana. A large amount of uncertainty exists also on the chemical nature of the substances responsible for growth, ovulation, embryonic development, etc. in Daphnia. It has been observed that the addition to inorganic media of cultures of Chlorella and Chlamydomonas does not furnish the "growth factors" required by Daphnia for its metabolic needs (Taub & Dollar 1968). It has been noted also that different species of Daphnia show metabolic changes of different entities in relation to the same artificial medium in which they were reared. (Frank 1957).

On the other hand, the water of Lago Maggiore when used as a culture medium has chemical and physical properties clearly different from those of Lago di Campo 4°. It is enough to record that the electrical conductivity was around 130-140 μs at 18°C (from July to October 1967) in Lago Maggiore (Gerletti 1968) while in the water of Campo 4° (results for June and July 1968) the conductivity varied from 45 to 50 μs at 18°C. Comparison between the $N/NO_3/\ell$ content showed concentrations in Lago Maggiore which were three to four times as high as those of Campo 4°.

D. middendorffiana is an animal with peculiar ecological features compared with the more common species of Daphnia: the breeding experiments have among other things shown a very low tolerance for high temperatures. At 18°C mortality is always very high both in the immature stages and in the adults. It is not surprising that the culture medium is found to be substantially inadequate for the nutritional needs of the species. An unbalanced diet is certainly the reason for the retarded growth of all the juvenile phases, characteristic of a notable number of the "supernumerary" stages and of moderate increments in length between one stage and the next: animals kept in culture from birth finally reach sexual maturity with dimensions generally lower than those typical of such levels of increase in the natural environment.

Finally there is to be considered the influence of temperature on the rhythm of the succession of instars. The laboratory cultures were for the most part maintained at a constant temperature of 10°C, a value undoubtedly higher than the mean daily temperature of the water of the alpine lake, even on days of strong solar radiation. Hall (1964) published a table of the durations of an adult stage of D. galeata mendotae as a function of temperature. While 11°C corresponded to a period of 8 days, at 9°C a mature stage was completed in almost 11 days and at 5°C as long as 18 days. It is allowed that the day-night thermal differences in the Lago Campo 4° are generally rather restrained for all the summer-autumn period: nonetheless the role of this factor in the biological cycle of the natural population should not be underestimated.

It is now possible to make an inventory of the more salient features of the individual growth rates of D. middendorffiana in Campo 4°.

1) Size of neonates differs appreciably when those which hatch from ephippia in July are compared with those which develop from subitaneous eggs in September; these last usually have a much longer carapace. Size at birth influences the number of stages needed to reach the prepuberal moult, more than the body dimensions which individuals attain as adults. One observes in fact a variable number of juvenile stages, whereas the second pre-adult phase on the contrary (with just a few exceptions) appears with the fixed number of two moults.

2) In the laboratory, a Daphnia alone has completed the whole pre-reproductive period moulting five times only.

3) Daphnids which at birth have a carapace length of about 1mm tend to be twice as long at the prepuberal moult. At sexual maturity the lengths come to be about two and a half times with respect to the values measured at birth. All this is in reasonably good agreement with the results of Edmondson (1955) for D. pulex tenebrosa and with those of Anderson (1932) and Zaffagninifor D. magna.

For the animals to reach the size limits characteristic of the puberal moult in only four stages, there would still be needed increments in carapace length of 350-400µ at each moult of the preadult period. The calculated increments at each moult (obtained from hundreds of results derived from cultures) are always distinctly lower. It is very probable that also in nature these daphnids pass through at least five stages before becoming adult.

4) Intermoult periods increased with the age of the animals. The relation between the duration of the entire preadult period and duration of one of the first adult stages was near enough to the value of 3 calculated by Hall (1964) in Daphnia galeata mendotae.

5) Males have never appeared in the cultures. That they have no function in this species is shown by the normal maturation of the resting eggs. The complete absence of males differentiates this population from that in an arctic pond studied by Stross (1969) in which males, though rare and non-functional, were present.

Seasonal Cycle of Daphnia middendorffiana.

Summer to autumn, 1968.

Dates of samples :- 29 June - 26 July - 12 August - 26 August - 5 September - 28 September - 17 October.

On 29 June three quarters of the Lago di Campo 4° was covered with ice: a wide area close to the north east shore was open, owing to greater exposure to solar radiation, and here collections of zooplankton were possible along lines parallel to the bank, and also vertical hauls from bottom to surface with a small net towed by boat. Examination of the material did not reveal the presence of daphnids in these samples: but numerous ephippia were isolated from the sediments. On 5 July some ephippia kept in glass dishes at 10°C and a photoperiod of 14:10 started to release the first daphnids. It may be that the dormant phase of the resting eggs was already complete at the time of collection in the lake : certainly the temperature of the thermostatic dishes was decisively higher than that of the Campo 4° water (which was only 3°C on 29 June) and helped to accelerate the development of the embryonic processes of the resting eggs.

It should be borne in mind, in this context, that the birth of the daphnids does not happen contemporaneously in all the ephippia: the last hatching in the cultures was on the 12 July. Evidently then the phenomenon in the lake is protracted for a longer time. It suffices to record that some ephippia collected in the samples at the end of July were hatching in the laboratory in the first days of August.

On the 26th July, 44 daphnids were caught of which 42 had carapace length of less than 1400 µm : one measured about 1760 µm and another, adult was 3060 µm long (Table 5). The population thus was constituted as if entirely from exephippial births of a few days, referable in large part to the first and second stages while some individuals had probably reached the third stage. This interpretation is based on the knowledge from at least three experimentally controlled factors, namely the dimensions of the exephippial neonates, the duration of one stage at fairly low temperatures, and the amount of size increment from one moult to the next. It is necessary in particular to refer to the thermal evolution of the water in the lake in the period 29 June to 26 July, because on this depends both the time at which the main hatch of ephippia in the lake began and also the rhythm of successive phases of development.

Now, allowing that the melting of the ice in Lago di Campo 4° is completed in a short time, presumably during the first ten days of July and that it may be fast enough to initiate a fairly rapid warming of the lake-water, it is evident that some embryos will have a somewhat reduced time in which to complete their development between hatching from the ephippial egg and reaching the first free-swimming stage of life. It is not necessary to assume that the phenomena of renewal in the development of the resting egg begin only from the moment in which, the thaw completed, they experience higher temperatures in the lake. This does not appear to be the decisive stimulus which acts on the hatching of the ephippia; Stross (1966), working with a pseudo-sexual stock of D. pulex, reared in the laboratory, has observed that the end of diapause was activated by the photoperiod, while temperature had no special effect on the phenomenon. Consequently, the presence of daphnids in the third and later stages in the samples of 26 July should be thought of as individuals hatched from ephippia during the period of the thaw, when the water-temperature was on 3-4°C. On the other hand, such low thermal levels act, all through that period, as a basic limiting factor, especially on the duration of the embryonic and of the first preadult stages. If then it is relatively easy, on the basis of information collected from animals reared in the laboratory, to assign to this or that stage of development the individuals appertaining to the different biometrical classes in which they have been distributed, it is less easy, to deduce from the biometrical configuration of the population, a reconstruction of the early history of each class which may not be the same for all the organisms belonging to it (which have nonetheless the same actual dimensions) owing to nonconformity of the events concerning different individuals in the period preceding the date of collection.

The presence of an adult of conspicuous size (over 3 mm) in the collection of 26 July is an important fact which cannot be ignored and intervenes to complicate the study of the dynamics of the natural population imposing restriction of schemes interpreted too rigidly.

The population which is found established in the lake after the thaw is not exclusively represented by young hatched from the autumnal ephippia but also of adults - born probably from ephippia hatched in the previous year - which have managed to overwinter in the water beneath the ice-cover.

Brooks (1964), studying the distribution of the zooplankton in relation to the duration of the ice-cover in lakes in New England, found that some species of Daphnia (and especially those northern species such as retrocurva and dubia besides pulex) have developed the ability to pass long periods of life under the ice, not in the form of ephippial embryos in diapause but as free-swimming adults in which reproductive activity is reduced or totally suspended.

Moreoever, D. longispina in apennine lakes of sufficient depth (Moroni 1962) do not enter diapause completely in the three to four months of their annual cycle, during winter when the water in which they live is covered with a thick layer of ice.

The effect of the low temperatures on longevity and the slowness with which the adult stages are reached, and at temperatures much higher than those of the water in which the daphnids live during the months of ice-cover, are good reasons for the length of the biological cycle of the D. middendorffiana of 3 mm taken in the samples on 26 July and justify the deduction thence that their age is between 8 and 9 months. On 12th August (3rd visit to Lago di Campo 4^{o}) a series of collections from different depths did not catch any of the smallest daphnids; among the others were found one adult with a carapace length of 3170 µm which carried two embryos in the brood pouch : these, in the laboratory, were set free on 16th August, at a temperature of 10°C. The next moult took place on 29th August so that thirteen days were needed to complete one adult stage.

In the course of samples following those of 12th August, also more numerous and hence more representative, there was no further trace of this generation of "winter" Daphnia. The fact of having caught only two individuals does not reduce the significance of their presence. One can assume that the animals which live in the lake from the end of the summer to the following year are rather rare. But, independently of their role in the demographic configuration of the population, these animals assume the significance of a bridging generation between the ephippial individuals of the autumn and the exephippials which hatch in summer and thus guarantee the continuity of the presence of the species in the lake all through the year.

If suspension of any reproductive activity in the lacustrine winter is accepted as extremely probable (especially in relation to the difficulty of finding food), one should also take into consideration the possibility of a renewal of reproductive activity with the return of higher temperatures (it has already been noted that daphnids caught on 12th August carried two embryos: the neonates had the same dimensions as the smallest exephippials born in the laboratory). In this way, some daphnids which overwinter will give rise to individuals which may be confused with the population of the neonates from ephippia; from these the non-exephippials anyhow appear hardly distinguishable, as shown by the use of the somatometric index (ratio between length of carapace and length of spine) proposed by Edmondson (1955) in his demographic study of arctic D. middendorffiana. It can be held that the small offspring from the subitaneous eggs laid by old overwintering daphnids form a modest and almost negligible part of the whole population, both on account of the long maternal intermoult period, characterised hence by a very low reproductive rate, and also because these animals have a life-history conditioned in all its phases to low temperatures and with onset of summer temperatures, will inevitably become senescent and die. For this reason, we will not find overwintering daphnids in the samples after 26 August.

The collection of the 12 August was too sparse to permit the working out of the demographic physiognomy of the Daphnia population on that date. The result of this sampling is certainly related to the character of the uneven spatial distribution of the D. middendorffiana in the lake: a volume of several m^3 of water was in fact filtered along different transects, in virtually the same fashion as on all other sampling visits. Besides the capture of the adults already mentioned, some neonates were also present in the samples of

12th August: the population thus continued to be enriched with new individuals for several weeks after the first arrival of neonates which followed the completion of the phenomenon of thaw. It is difficult however to decide whether these neonates derive from the last ephippia or are the daughters of precociously maturing exephippials: the examination of subsequent samples will help to clarify this point. Nor can it be altogether excluded that they are progeny of overwintering adults.

On 26th August a total of 131 daphnids were caught, of which 79 were taken in "quantitative" samples along the usual vertical profile and 52 by other sampling methods (Table 5). Carapace lengths were measured in the two collections separately; in the following diagram are presented the results for each sampling, the daphnids having been arranged in four size-classes:

Carapace length (μ)	Numbers of *Daphnia*	
	Quantitative sample	Mixed sample
< 1.750	6	5
1.750 - 2.050	14	6
2.050 - 2.350	26	22
> 2.350	33	19

Among the larger daphnids were present also some ovigerous individuals with small embryos in the brood chamber. On 28th August, two days after the site-visit, 7 adults present in the samples were isolated and held in culture at 10°C: their carapaces measured from 2350 to 2650 μ and the number of subitaneous eggs ranged from two to five. The first young born in culture dishes appeared on 5 September. The dimensions reached by the populations in the classes with greater length of carapace and the presence, also in the range of those dimensions characteristic of the puberal moult, of small animals with embryos suggests that the adult daphnids taken on the 26th August are the first exephippials in the lake to reach maturity. The reproductive stage reached in the laboratory would represent the first of the adult period and the date of 5 September can be taken as near enough to the time at which the first non-exephippial daphnids are set free in their natural environment. Moreover, the fact that the smaller size-classes, **those with** the average size of the neonates, were not represented by any individuals, excludes the possibility that the adult with eggs on 26 August might have set free in advance a brood of young. This suggests that in the lake, for a certain period of time, the population does not increase numerically through addition

Table 5. Number of individuals of <u>Daphnia middendorffiana</u> collected in Lago di Campo 4° in the samplings in summer and autumn of 1968. In the first column on the left are given the sizes, corresponding to the measurements of carapace length, in micrometer units (μ.m.) and in μ.

Measurements		26-VII-1968	26-VIII-1968	5-IX-1968	28-IX-1968	17-X-1968
μ.m.	μ					
15	880	3	-	-	-	-
16	940	3	-	1	3	1
17	1.000	4	-	-	12	13
18	1.060	6	-	1	15	38
19	1.115	3	-	1	15	37
20	1.175	5	1	-	5	10
21	1.235	9	-	-	20	5
22	1.295	6	3	-	43	15
23	1.350	2	-	-	42	21
24	1.410	1	1	-	5	12
25	1.470	-	-	-	14	5
26	1.530	-	1	-	12	4
27	1.590	-	-	-	30	4
28	1.645	-	3	1	27	5
29	1.705	-	2	-	13	1
30	1.765	1	8	-	4	2
31	1.825	-	1	-	3	1
32	1.880	-	3	-	2	7
33	1.940	-	4	-	4	11
34	2.000	-	4	1	4	12
35	2.060	-	6	1	2	10
36	2.115	-	5	3	2	1
37	2.175	-	11	1	1	2
38	2.235	-	18	3	2	2
39	2.295	-	8	5	2	2
40	2.350	-	31	10	4	1
41	2.410	-	10	6	1	-
42	2.470	-	6	26	5	-
43	2.530	-	3	20	5	1
44	2.585	-	-	32	7	5
45	2.645	-	2	23	16	20
46	2.705	-	-	12	13	11
47	2.765	-	-	8	15	30

Cont'd

Table 5. Continued.

Measurements μ.m.	μ	26-VII-1968	26-VIII-1968	5-IX-1968	28-IX-1968	17-X-1968
48	2.820	-	-	3	48	56
49	2.880	-	-	-	7	37
50	2.940	-	-	-	19	84
51	3.000	-	-	-	5	29
52	3.060	1	-	-	1	22
53	3.115	-	-	-	-	6
54	3.175	-	-	-	-	-
		44	131	158	428	523

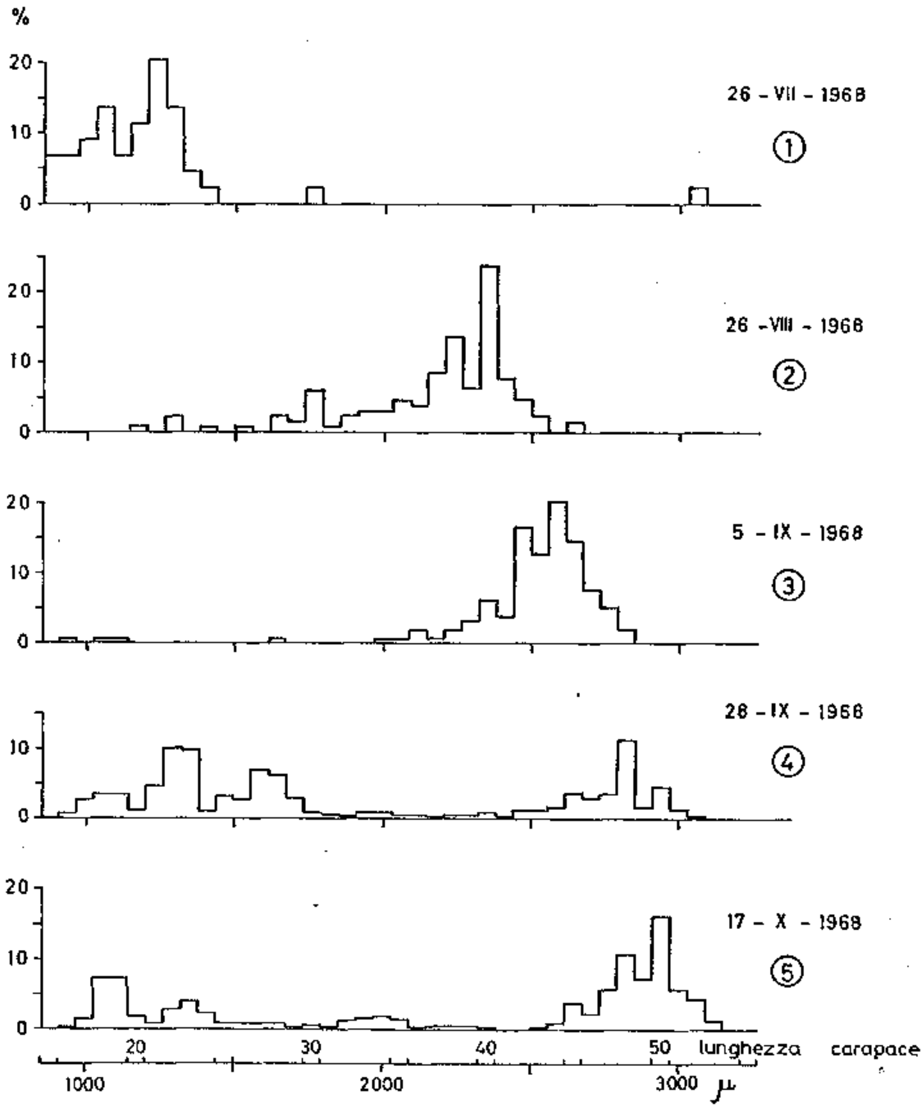

Fig. 1. Percentage composition of size-classes of the populations of Daphnia middendorffiana. The amplitude of each class corresponds to one micrometer unit, equivalent to 58.8μ. Samples in 1968.

of new births. The last ephippia would then have opened early enough for the attainment of sexual maturity by some of the precociously developed daphnids: nor would there be a consistent reproductive activity of over-wintering daphnids, regarding which it has already been assumed would die with the arrival of high summer temperatures.

The population sample of 26 August shows a very wide range of body-sizes: 80% of the constituent individuals have already reached the prepuberal moult while 60% of the entire population are found concentrated in the size range between 2170 and 2410μ. If the contribution of the overwintering daphnids is considered irrelevant and all individuals are assigned to the exephippial generation, then the dimensional structure which appears from these samples can be considered the result of two phenomena which concur sharing in the "formation" of the summer population of D. middendorffiana: namely a pulse of synchronized hatchings, when after the thaw particular ambient conditions favour the discharge of the phenomenon (to this pulse is assigned the greater part of the ephippia); and a succession of hatchings, which precede and follow this moment over a fairly long time, around a month, and in which are concerned without doubt a large number of individuals. Such a process is not altogether implausible when one thinks of the high rate of water renewal of the lake over a long period following the thaw and exactly in concomitance with the development of the growth of the first daphnid instars.

The discharge of water from the outflow is certainly a dynamic factor of great importance in determining the equilibrium of the daphnid population in the course of its formation, since through the more rapid water renewal, it exerts a more intense selective action and the dimensions of the animals living in the turbulent currents are more slender (and hence capacity for active movement lower).

The samples from 5 September (Table 5) give the opportunity to repeat what has already been stated about the time at which the exephippial generation reaches sexual maturity in Lago di Campo 4°. As histogram 3 in Fig. 1 shows, individuals of inferior size are almost entirely missing: the few daphnids of carapace length between 940 and 1115μ are those born by the daphnids which matured ten days earlier; the main part of the population passed to the adult period with the moult which intervened in the period between 26 August and 5 September. In fact, the first pulse of non exephippial births comes in the days immediately after the collection in the cultures at 10°C.

Of the 158 individuals caught on 5 September, only 4 belonged to stages definitely anteceding the prepuberal moult: of these last, 3 were neonates. There is a greater frequency of the classes of size corresponding to the stages of the second pre-adult phase. But the population appears concentrated in a group in a size range exceeding 2470µ which comprises 77% of all individuals. These were not all mature daphnids; indeed, while daphnids of larger size have already passed the first adult stage and have liberated their first brood, a substantial portion of the daphnids over 2500µ have still not reached maturity. The class of greater frequency (length of carapace 2585µ) seems to accord with the mean size level of the puberal moult; this level is without doubt higher than that reached by the daphnids reared in culture from birth. This is confirmed in part also by the histogram for the collections of 26 August, in which the daphnids of the most frequent class (corresponding to 2350µ) are **also not** mature and belongpresumably to the stage preceding the puberal moult.

Comparison of histograms 2 and 3 in Fig. 1, which refer to two collections made at an interval of 10 days, permits the deduction that this period is little more than that necessary to complete one instar in the natural environment at that stage of the evolution of the seasonal dynamics of D. middendorffiana. A modest size increase of the natural population can indeed be observed which is slightly greater than that recorded, corresponding to the puberal moult, in study of experimental populations. Table 6, which groups together some statistical parameters for the exephippial generation alone, allows a more rigorous appreciation through comparing the first summer collections of 1968, both the entity of such mean increment and also the tendency for a progressive diminution of the variability of the size structure of the population.

Table 6. Some statistical parameters calculated on the length of carapace (in μ) of individuals (n) of Daphnia middendorffiana belonging to the exephippial generation, taken in the collections of the summer of 1968; $\bar{x}$ = mean; σ = standard deviation; C.V.% = co-efficient of percentage variation: l.f.i. and l.f.s. = confidence limits below and above the mean.

	26-VII-1968	26-VIII-1968	5-IX-1968
n	43	131	155
$\bar{x}$	1.158	2.167	2.528
σ	170	288	170
C.V. %	14,6	13,3	6,7
l.f.i. 95%	826	1.601	2.195
l.f.s. 95%	1.490	2.732	2.861

The following data have some importance in relation to the reproductive activity of the animals collected on the 5 September. No daphnids carried more than seven embryos in the brood pouch; in the week from 5th to 12th September the egg-bearers (about a hundred) gave birth to over 400 young. The number of neonates was estimated in relation to the number of maternal moults and that is, in practice, to the number of exuviae abandoned in the medium. Thus on the 10 September, 16 moults preceded the liberation of 84 young while the day after, the moults were 19 and the neonates 118.

It can be supposed then that in the lake the first pulse of births from the exephippials occurs during the first ten days of September and that, with the appearance of the new generation, the numerical density of D. middendorffiana in the lake, not taking into account various causes of mortality and of the effect of the outflow, increases abruptly by some four to five times. Other broods produced by the exephippials should follow at intervals of eight to ten days which, with respect to the temperature of the water in the lake in September, represents the more likely duration for the complete development of one adult stage and hence for a normal and complete embryonal development. In effect, immission of new broods of subitaneous eggs going on continuously gives the imperfect synchronisation with which the exephippials have reached sexual maturity.

On 28 September (Table 5 and histogram 4 of Fig. 1) 428 individual daphnids were caught: 146 had a carapace length of more than 2350μ and of these quite 127 were represented by daphnids with embryos which varied in number from 4 to 8; 15 daphnids had no eggs while 4 carried ephippia (Table 7). Of the non-ovigerous daphnids some were not yet mature and others had probably lost their embryos in the course of various manipulations; not to be excluded also is the possibility that there were some individuals which during the cycle of active production of subitaneous eggs, pass through some stages without deposition of eggs. In the exephippial group, the more frequently occurring class-size from 5 to 28 September ranged from 2585 to 2820μ with an increase of little more than 200μ in the length of carapace. The maximal values of this parameter in the same period were from 2820 to 3060μ.

Table 7. Individuals of Daphnia middendorffiana taken in the samples of 28 September 1968 with body-size greater than 40 u.m. (= 2350μ).

Size (u.m.)	Ovigerous non-ephippial	Ephippials	Non-ovigerous	Totals
40	2	-	2	4
41	1	-	-	1
42	4	-	1	5
43	3	1	1	5
44	6	-	1	7
45	9	2	5	16
46	12	1	-	13
47	13	-	2	15
48	45	-	3	48
49	7	-	-	7
50	19	-	-	19
51	5	-	-	5
52	1	-	-	1
	127	4	15	146

An interesting result is provided below 1800μ by the existence of three size groups (arranged around 1050, 1300 and 1600μ respectively) each separated clearly enough by classes of size of lesser frequency: these peaks evidently correspond to the dimensions characteristic of the first, second and third growth stages and all three were in juvenile stages; so the animals generally would not arrive at the second preadult phase without having moulted at least three times. All the values of length of carapace of the intermediate classes between 1800 and 2600μ are represented, though by the fewest individuals in each class: it is difficult to decide at what size limit, that is at what growth stage are daphnids born from the first eggs laid by the exephippials.

The appearance of the first ephippia marks an important turning-point in the history of the population: the eggs carried by the adults of 28 September will give rise to a new wave of births in the course of the first ten days of October. In the majority of the adult animals, the liberation of young will be followed by the appearance of ephippia.

In the couse of the last site-visit on the 17th of October, 523 daphnids were collected (Table 5). Histogram 5 (Fig. 1) shows that individuals in the size-range 1050 to 1100μ are strongly represented; these are evidently the last of the non-exephippials to appear in the lake in the arch of time in which the mature daphnids have produced the first ephippium. Daphnids exceeding 2350μ in carapace length numbered 302, that is to say more than 50% of the entire catch (Table 8). Of these only one had subitaneous eggs, two were not yet mature, 11 carried ephippia with resting eggs and the rest, 288 in all, forming over 95% of the adult population, had already deposited ephippia. It should be allowed that this event was fairly recent in respect to the date of collection.

If one takes a period of ten days as the maximum inter-moult duration at this stage of the seasonal cycle, one should place not later than the first week in October the moult following the appearance of the ephippium and deposition of resting eggs. On the other hand, this moult had already taken place among a small number of the adults caught on the 28th September. The appearance of the ephippia is preceded by the liberation in the last daphnids to develop of subitaneous eggs which, at least in the exephippials to reach sexual maturity towards the end of August should represent the fourth brood; the ephippium therefore would appear in the older exephippials with the fifth reproductive stage. At this point one can ask whether the daphnids which produce an ephippium towards the middle of October and which form almost the entire population of adults on the 17th October, all belong to the exephippial generation. On the basis of the growth rates, estimated in the laboratory, it has indubitably to be conceded that at least the first non-ex-ephippiales born at the start of September could reach sexual maturity and deposit ephippia roundabout the same period in which occurs the deposition of the first ephippia by daphnids of the maternal generation.

It should be noted also that young daphnids caught in the first week of October, the period in which the last daphnids lay subitaneous eggs, these by 17th October should be at least in the second juvenile stage; at which stage the dimensions are referable in large part to 1050 to 1100μ in the histogram 5 of Figure 1. The carapace length of the final neonates appears therefore to be distinctly less than that of the neonates of the preceding broods. On the other hand, the hypothesis of the existence of a subitaneous line of animals, that is to say they have completely given up the reproduction by resting eggs receives little support from the reality of the situation observed in the natural environment: in the collection on 17 October there was found among 300 adult, only a single daphnid with subitaneous eggs (its carapace was 2940μ long).

With the birth of the last broods of non-exephippials the numerical dimensions of the population reach maximal values of expansion: there is no evidence that the ephippia hatch immediately after being set free from the body of the daphnid nor that diapause ceases earlier than the summer of the following year.

Table 8. Individuals of *Daphnia middendorffiana* taken in the collection of 17th October 1968 of body-size greater than 40 u.m. (= 2350μ). Non-ovigerous p.ef. = daphnids with no eggs which were liberated of an ephippium at the last moult.

size u.m.	Ovigerous non-ephippial	Ephippial	non-ovigerous	non-ovigerous p.ef.	Totals
40	-	-	1	-	1
43	-	-	1	-	1
44	-	-	-	5	5
45	-	2	-	18	20
46	-	-	-	11	11
47	-	-	-	30	30
48	-	2	-	54	56
49	-	3	-	34	37
50	1	3	-	80	84
51	-	1	-	28	29
52	-	-	-	22	22
53	-	-	-	6	6
	1	11	2	288	302

SUMMER–AUTUMN 1969

Dates of visits : 14 July; 1 August; 26 August; 16 September;
10 October.

On 14 July 8 neonates in all were caught ranging in size from 880 to 1000μ. On 1 August, 90 daphnids were taken: besides some neonates there was a majority of animals with carapace length 1150μ, almost certainly in the second stage of growth; also longer animals which measured about 1230μ were probably in their second juvenile stage.

In the samples of 26 August, neonates and daphnids in the earliest stages were completely absent: of the 43 animals caught, 40 had already passed the prepuberal moult and 17 of these were adults with subitaneous eggs. Those with eggs were isolated and kept in culture at 10°C: the first young were born in the laboratory on the 31st August. The dates of this first group of samples (Table 9 and histograms 1 and 2 of Fig. 2) can profitably be compared with those relative to the same period for 1968. Some similarity in line of the development of the seasonal cycle in the two years can be discerned. More especially the fact needs to be stressed that, for a long time after the thaw, the population finds it hard to achieve stability in the lake, notwithstanding continual hatching of new-born individuals from ephippia; in spite of the already high summer temperatures, the mean dimensions of the population (always calculated on the length of the carapace of single daphnids) increased by only 150μ between the 14 July and the 1st August. It does not appear to be the extreme slowness with which they grow in this phase of the cycle of D. middendorffiana which limits the development of the population. While nothing is known of the relations between Daphnia and potential predators, perhaps present at the level of the sediments, one may propose the hypothesis that it may be the water renewal of the lake, particularly rapid all through July, which is the more important factor which prevents a stable establishment in the water of Campo 4° of the animals which come out of diapause.

Individuals of the winter generation of adults have not been found, which confirms the scanty importance which they have in relation to the dynamic events which concern the population as a whole.

In the samples of 26 August the daphnids of larger size have now, although in very few days, passed the puberal moult and laid subitaneous eggs (from a minimum of one up to a maximum of five); sexual maturity is reached when the animal has reached a certain body size; the critical dimensional threshold below which adults never appear, in this as in all following samples in 1969, were found to correspond to 2350μ. The main part of the exephippial generation reached the reproductive phase during the last ten days of August; the first young were born in early September.

When the collection for 26 August is compared with that for the same date in 1968, it can be seen that, throughout the first part of the cycle of D. middendorffiana, the same growth rhythms occur in both years with the same succession of dynamic events; nonetheless one can detect in 1969, in relation to the preceding year there is some tendency to be ahead by a few days in the completion of the stages of growth in the pre-adult period and hence in the appearance of the first non-exephippial generation.

In the collection of 16 September, 179 daphnids were caught of which 73 came from ephippia and 106 belonged to the generation which developed from subitaneous eggs. Newbirths made up 50% of all the samples; the other non exephippial young were present with fewest individuals and were in the second and third stages respectively (Table 9 and histogram 3 of Fig. 2).

The length of the carapace of the adults ranged from 2470 to 3175μ: such variability, beside the considerations relative to the growth stages of the young, leads one to think that the exephippials are made up of some individuals which have just reached sexual maturity (those less than 2500 to 2600μ) and of others which are respectively in the second, third and possibly fourth adult stages. In each instance, all these daphnids, already before the 16 September, have passed the puberal moult; those of carapace length from 2470 to 2705μ were all ovigerous; among those more conspicuous daphnids, that is longer than 2800μ, 11 individuals carried no eggs.

In all ovigerous individuals, the eggs in process of development have been counted: these were 62 and they were carrying altogether 746 embryos: an average of 12 embryos for each daphnid. One exephippial individual of length 3175μ had laid 22 subitaneous eggs.

The correlation between size of maternal body and number of eggs has been calculated from 43 animals in which it was possible to make individual counts of eggs. The coefficient of correlation was positive and significant at the 5% probability level (Fig. 3).

From this picture there emerge some important differences with respect to the trend of the seasonal cycle of the preceding year. Above all the dimensional increase observed during the succession of moults in the reproductive period always seems larger in 1969. The length of carapace of the longer daphnids of the 16 September 1969 are longer by some 100µ than the corresponding ones caught in the samples of 28 September 1968. In the second place, the exephippial daphnids of 1969 show a reproductive potential greater by far than the daphnids of the same generation of September 1968. The food factor has a prevailing influence on these two aspects of the biology of Daphnia. One should not ignore however that temperature can also exercise a certain role in this phase of the seasonal cycle, accelerating the growth rhythm of individual animals, conserving and prolonging, in regard to the summer-autumn of 1968, those ahead in development time in the decisive phase of the cycle, which was already obvious at the time when sexual maturity was reached.

In the samples for 10 October, 386 daphnids were taken of which 11 were exephippials (Table 9; histogram 4 of Figure 2). Of these latter, four had no eggs and the other seven had laid subitaneous eggs. In the size structure of the population, the exephippials are still quite distinct from the young of the different broods. Of these, the more precocious have already reached sexual maturity and are probably in the first reproductive stage; their size varies within a range which is the same as that shown by the new exephippial adults found on 26 August. The direct correlation between body-size and number of eggs carried by the new adults shown in the two samplings was significant at the 5% probability level. The linear regression was calculated in both instances (Fig. 4): the two lines have almost the same inclination, although the values of the ordinates to the origins are visibly diverse. The ovigerous individuals produce more eggs in October than in August, at parity of growth stage.

Also a direct significant correlation between length of carapace and number of subitaneous eggs has been found for the exephippial ovigerous individuals of 10th October. The line of linear regression is shown in Fig. 3 for comparison with that for the exephippial daphnids collected on the 16th September. A tendency is apparent for egg-production to diminish with advance in instar and increasing age of the individual.

The daphnids of the first non-exephippial generation to complete the whole preadult development, including the embryonal part, spend about 45 days, that is to say the period from 26 August to 10 October. It can be considered that the ovigerous non-exephippial individuals collected in October are in the first adult stage and that they have developed from the embryos carried by the exephippial individuals at the end of August. All the daphnids on the 10th October less than 2590µ are, in all probability, young of the exephippial individuals. Only in the days after this date will another generation become part of the D. middendorffiana population, the first F_2, in regard to the parental generation of the exephippial individuals.

Continuing the study of the collection of 10 October, it can be seen that all size-classes from 1000 to 2350μ are present and some are characterized by higher frequency. The histogram related to this distribution shows clearly enough the existence of a certain number of quite distinct size groups each of which corresponds probably to a certain growth level. The oldest exephippial individuals in their turn should be already in the sixth reproductive stage: that is to say they have moulted at least twice more in the 24 days elapsed since 16 September.

One other aspect of significance which differentiates this last part of the seasonal cycle of Daphnia compared to 1968 is the complete absence of ephippia in the adult population.

The period of production of the non-exephippial individuals lasted altogether much longer in 1969 than in 1968. It was observed in 1968 that the first ephippial daphnids had appeared already on 28 September in the lake and that in the course of the week following that date all daphnids deposited ephippia.

In this instance entry into diapause by the D. middendorffiana was delayed by at least 15 days compared to the previous year and it continued to add to its numerical abundance by new births. This delay in production of ephippia means that at least some proportion of the exephippial individuals which were early to reach sexual maturity, produced six generations of non-exephippial individuals, probably two more than in 1968.

Some of the 1969 results have been treated statistically. The following statistical parameters have been calculated for each date for the individuals of the exephippial generation: mean, standard deviation, 95% confidence limits of the mean and percentage co-efficient of variation. (Table 10).

The dimensions of the daphnids of this generation are presented on the ordinate axis while on the abscissa are entered the times corresponding to the dates of collection; thus the time 0 corresponds to the 14 July, the time 18 to the first of August and so on for the following collecting dates. The measurements made on the daphnids of 14 July are not taken into consideration because of the characters already discussed in the process of "occupation" of the lacustrine water by the animals which hatch from the ephippia little by little.

While the regression line ($y = 9,324+0,621x$) shown was inadequate to describe the relation between dimension (y) and time (x), the relation between the two variables is better expressed by the following equation of 2 degrees:

$$y = -1,390+1,266x - 0,007x^2$$

The curve represented by the 2° regression can be considered as the growth-line of an average individual of the exephippial generation. Such an analysis has not been made on the complete series of collections for 1968, as it was not possible, at least for those of 28 September and 17 October to separate clearly on the basis of the structural dimensions of the population, the exephippial generation from the filial generation.

CONCLUSIONS

The cycle of alpine Daphnia middendorffiana differs notably from those forms which live in lakes and ponds of the arctic zone (Edmondson 1955; Stross and Kangas 1969). The numerical dimensions of these populations depend in the first place on those of the first exephippial generation. Secondly there is the contribution which comes from the generations which develop from eggs that develop at once since the exephippial animals generally produce an ephippium as first brood. In relation to which the duration of the seasonal cycle of the arctic daphnids is very short. In Lake Imikpuk studied by Edmondson hatching of ephippia began on the 1st July: less than a month later the first females with subitaneous eggs appeared and on 13 August the first ephippia. In a pond in Alaska (Stross and Kangas 1969) the time of the cycle of D. middendorffiana was also very short: mass hatching of ephippia occurred around the 20th June; by the 7 July this generation already contained adults and by 17th July a large number of adults carried ephippia.

The seasonal cycle of arctic daphnids is controlled in particular by water-temperature: the strong daily oscillations in temperature would also be responsible for the reproductive "shift" which intervenes, before a complete generation of non-exephippial individuals has developed, under conditions of continuous light. With such a photoperiod in fact, if the temperature is held constant, the daphnids do not produce resting embryos, as shown by the experiments of Stross (1969).

Table 9. Number of individuals of <u>Daphnia middendorffiana</u> taken in Lake Campo 4° in the samplings of the summer and autumn of 1969. Dimensions are shown in the first column on the left, as in Table 5. The number of ovigerous individuals (always non-ephippial) are in italics.

Dimensions		14-VII-1969		1-VIII-1969		26-VIII-1969		16-IX-1969		10-X-1969	
u.m.	µ										
14	825	-	-	1	-	-	-	-	-	-	-
15	880	1	-	4	-	-	-	-	-	-	-
16	940	4	-	5	-	-	-	6	-	-	-
17	1.000	3	-	2	-	-	-	26	-	15	-
18	1.060	-	-	9	-	-	-	55	-	35	-
19	1.115	-	-	24	-	-	-	5	-	3	-
20	1.175	-	-	43	-	-	-	-	-	15	-
21	1.235	-	-	2	-	-	-	1	-	15	-
22	1.295	-	-	-	-	-	-	3	-	44	-
23	1.350	-	-	-	-	-	-	1	-	39	-
24	1.410	-	-	-	-	-	-	1	-	14	-
25	1.470	-	-	-	-	-	-	1	-	6	-
26	1.530	-	-	-	-	-	-	1	-	4	-
27	1.590	-	-	-	-	-	-	3	-	11	-
28	2.645	-	-	-	-	-	-	1	-	20	-
29	1.705	-	-	-	-	1	-	1	-	17	-
30	1.765	-	-	-	-	-	-	1	-	18	-
31	1.825	-	-	-	-	1	-	-	-	5	-
32	1.880	-	-	-	-	1	-	-	-	3	-
33	1.940	-	-	-	-	-	-	-	-	2	-
34	2.000	-	-	-	-	-	-	-	-	10	-
35	2.060	-	-	-	-	-	-	-	-	25	-
36	2.115	-	-	-	-	2	-	-	-	32	-
37	2.175	-	-	-	-	1	-	-	-	10	-
38	2.235	-	-	-	-	1	-	-	-	2	-
39	2.295	-	-	-	-	4	-	-	-	2	-
40	2.350	-	-	-	-	6	*1*	-	-	4	*2*
41	2.410	-	-	-	-	6	*2*	-	-	6	*3*
42	2.470	-	-	-	-	10	*6*	1	*1*	7	*6*
43	2.530	-	-	-	-	5	*3*	-	-	6	*6*
44	2.585	-	-	-	-	5	*5*	3	*3*	5	*5*
45	2.645	-	-	-	-	-	-	2	*2*	-	-

Cont'd

Table 9. Continued.

Dimensions u.m.	μ	14-VII-1969		1-VIII-1969		26-VIII-1969		16-IX-1969		10-X-1969	
46	2.705	–	–	–	–	–	–	4	*4*	–	–
47	2.765	–	–	–	–	–	–	–	–	–	–
48	2.820	–	–	–	–	–	–	13	*10*	–	–
49	2.880	–	–	–	–	–	–	6	*4*	–	–
50	2.940	–	–	–	–	–	–	18	*15*	–	–
51	3.000	–	–	–	–	–	–	3	*3*	–	–
52	3.060	–	–	–	–	–	–	13	*13*	1	–
53	3.115	–	–	–	–	–	–	5	*4*	2	*1*
54	3.175	–	–	–	–	–	–	5	*3*	1	*1*
55	3.235	–	–	–	–	–	–	–	–	3	*2*
56	3.295	–	–	–	–	–	–	–	–	1	*1*
57	3.350	–	–	–	–	–	–	–	–	2	*1*
58	3.410	–	–	–	–	–	–	–	–	1	*1*
		8	–	90	–	43	*17*	179	*62*	386	*29*

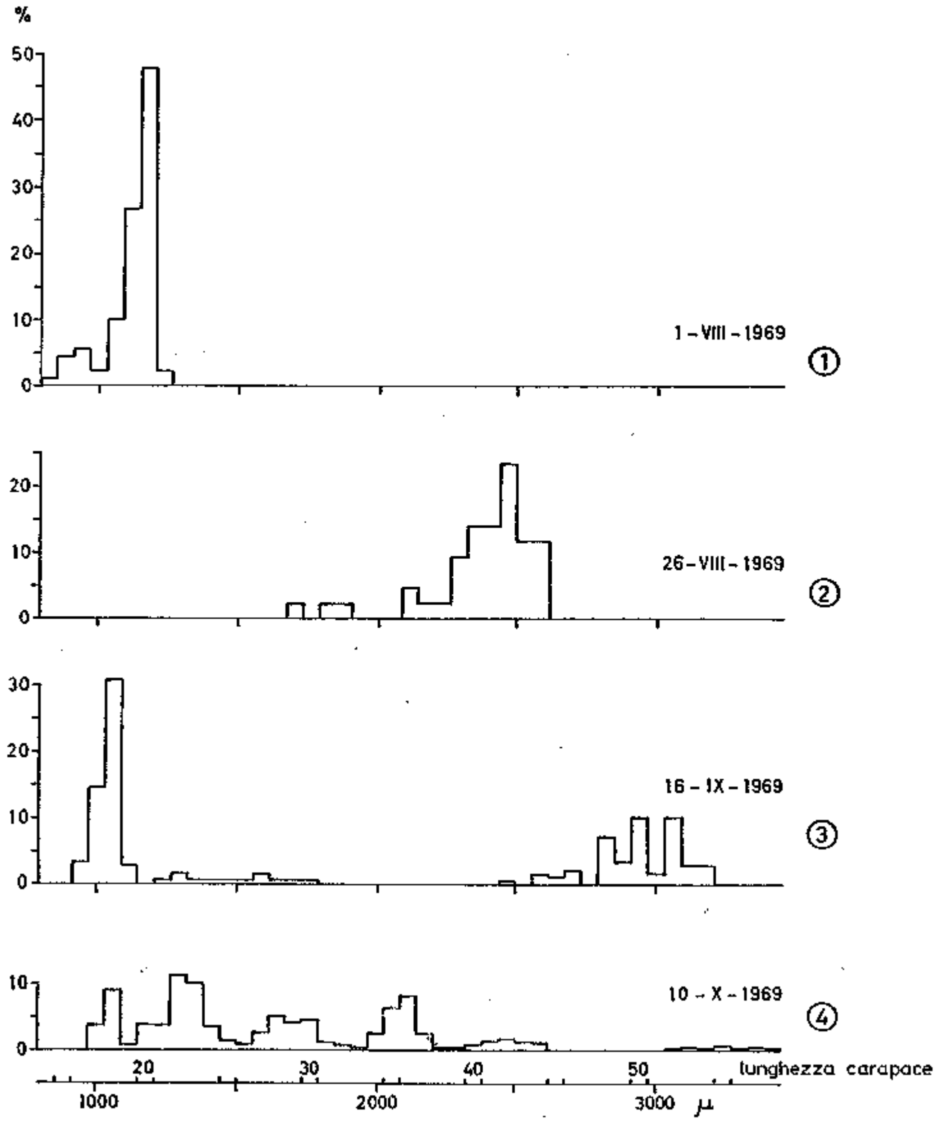

Fig. 2. Percentage composition of size-classes (see text of Fig. 1) of the population of D. middendorffiana. Collections for 1969.

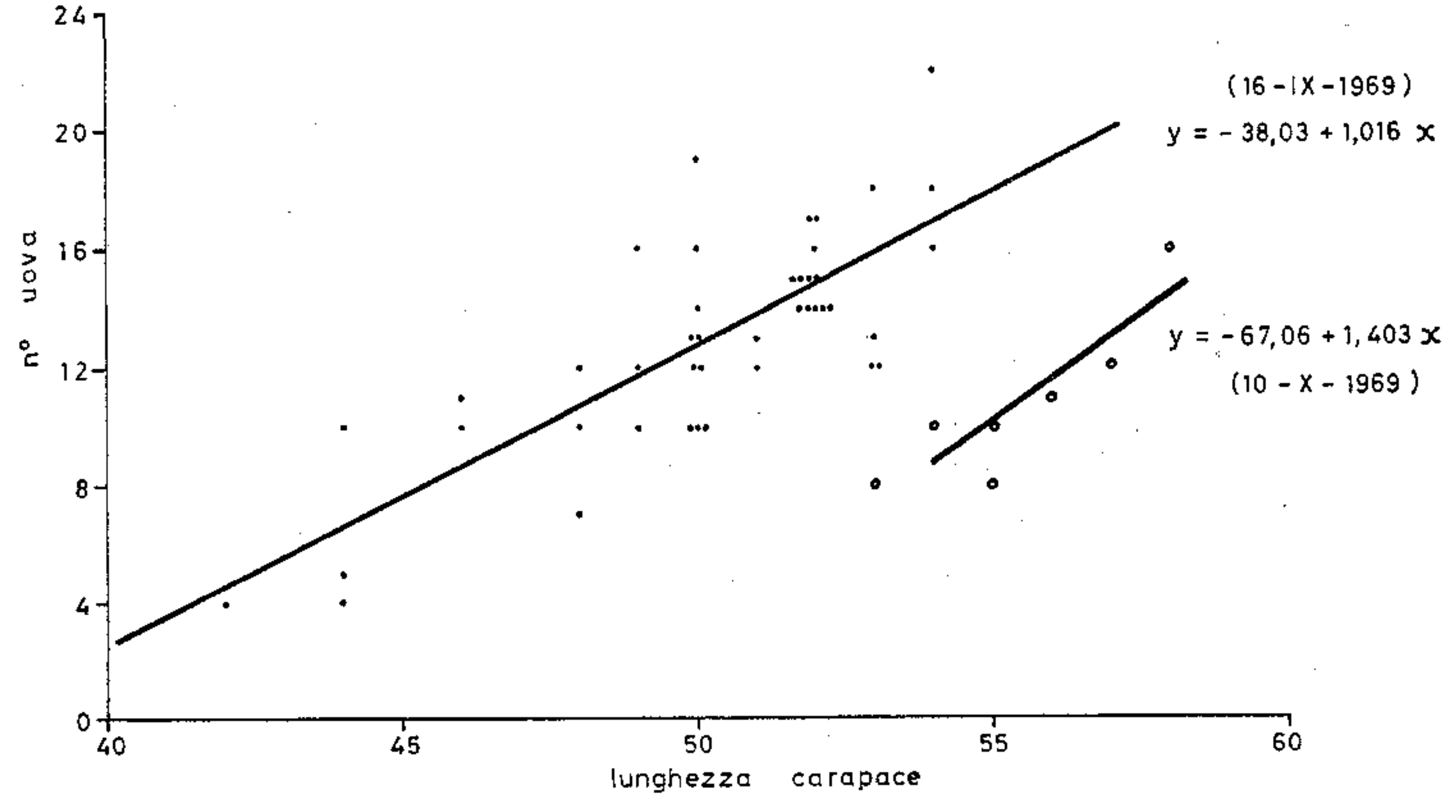

Fig. 3. Number of subitaneous eggs as a function of carapace-length - measured in micrometer units (1 u.m. = 58,8μ) - of the ovigerous individuals of the exephippial generation present in the samples of 16 September (filled circles) and of 10 October 1969 (void circles).

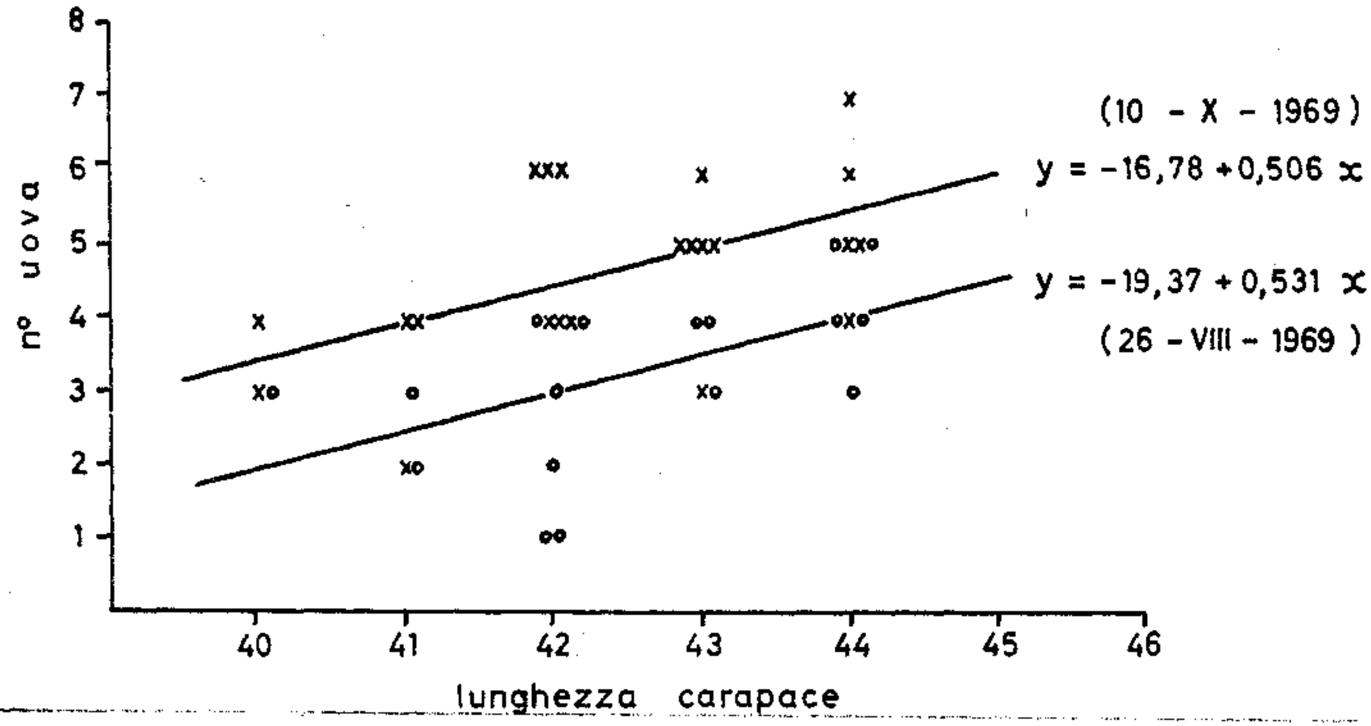

Fig. 4. Number of subitaneous eggs as a function of carapace-length - measured in micrometer units (1 u.m. = 58,8μ) - of the ovigerous neoadults of the exephippial generation (samples of 26 August 1969, circles) and of the first generation non-exephippial (samples of 10 October 1969, crosses).

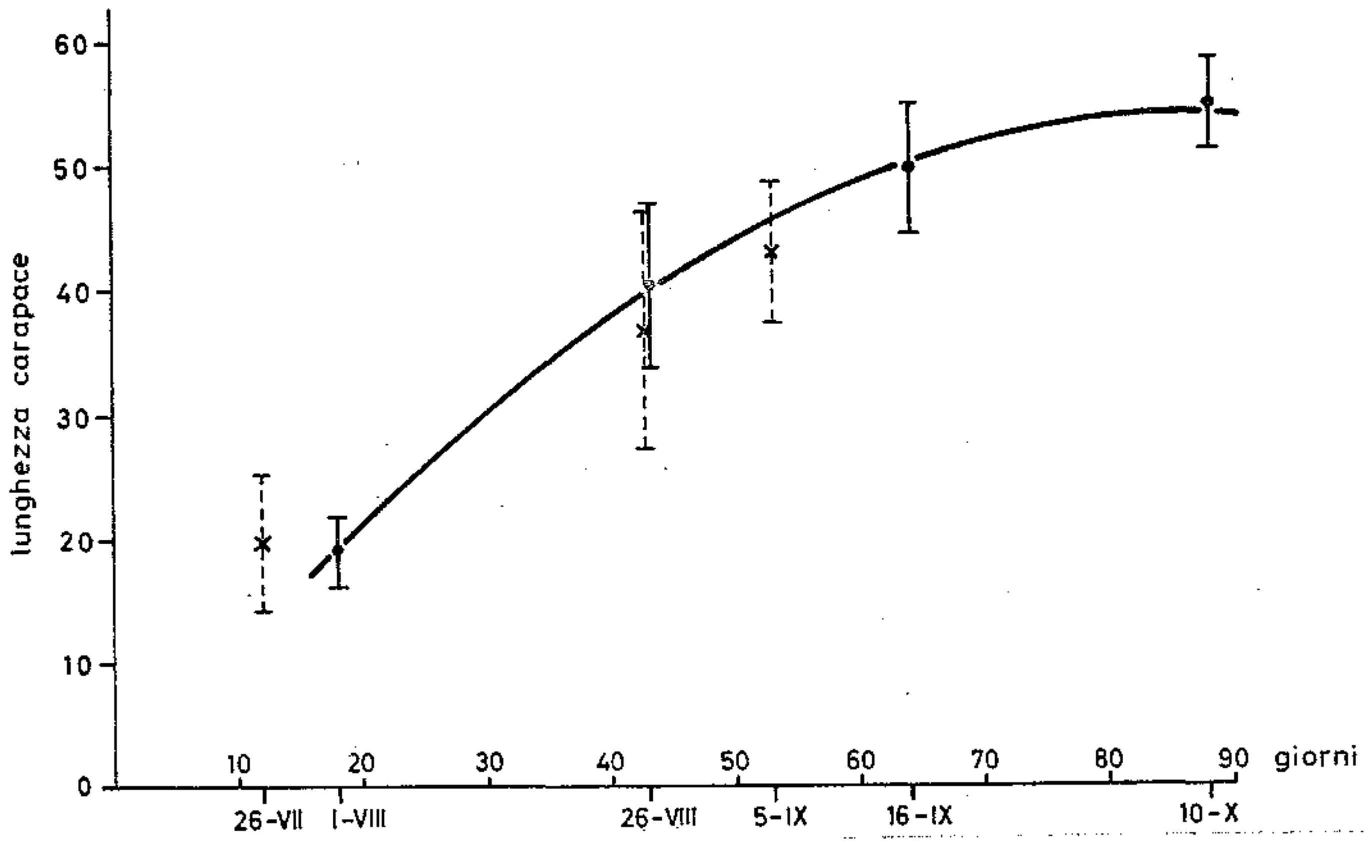

Fig. 5. Regression line of the length - in micrometer units (1 u.m. = 58,8μ) - of individuals of the exephippial generation in 1969, against time. The mean values and relative fiducial limits are shown for each date of collection. For comparison, means (shown by a cross) and their fiducial limits (dashed lines) for collections in 1968 are shown.

Table 10. Statistical parameters (see text Table 6) calculated on the length of carapace (in μ) of the individuals (m) of Daphnia middendorffiana belonging to the exephippial generation taken in the samples of summer - autumn 1969.

	1-VIII-1969	26-VIII-1969	16-IX-1969	10-X-1969
n	90	43	73	11
$\bar{x}$	1.116	2.374	2.924	3.234
σ	89	119	158	112
C.V. %	7,9	8,4	5,4	3,4
l.f.i. 95%	942	1.984	2.615	3.015
l.f.s. 95%	1.289	2.763	3.233	3.453

In Lago di Campo 4° the ambient conditions become limiting with respect to numerical growth of the population only after three to four months from the hatching of the ephippia. In the course of this period the daphnids produce a certain number of broods (up to six, for certain, in 1969). In their turn, the more precocious of these non-exephippial individuals reach maturity and give rise to a new generation of young. In 1968 this last generation did not appear and the cycle was arrested with entrance of all adults into diapause which began at the end of September.

On the whole, the duration of the seasonal cycle depends on the moment at which the first daphnids are born from ephippia (and this event is related to the prevailing general meteorological conditions which influence the thaw in early summer), but especially on the time at which the ephippia of the adult population appear in the autumn. With the production in mass of ephippia, the population no longer increases numerically but tends to die out, resting meantime in the biological promise of renewal of the cycle in the summer of the following year. The autumnal restriction on numerical growth of the population is perhaps conditioned in the first place by the conditions which sustain the production of resting eggs.

In this context, there should be taken into consideration the hypothesis of Stross on the importance of photoperiod, associated with the influence of the thermal regime of the lacustrine water on the initiation of diapause; at the same time, this author has experimented on arctic populations of Daphnia middendorffiana which have selected, through latitudinal adaptation, an extremely long critical photoperiod corresponding to a relation between hours of light and hours of dark of 22:2.

We do not possess the results for a definition on a quantitative basis of the thermal regime of Campo 4° nor for a comparison between the two years: but it is somewhat improbable that from one year to the other the thermal "pattern" (referring especially to the amplitude of the daily variations) could undergo such important modifications. The retarding in the start of the phenomenon of diapause which was observed in 1969 compared with the preceding year is indeed very considerable being more than 15 days.

The decisive factor in the initiation of the production of resistant eggs seems to be linked somewhat with the availability of food. The exephippial individuals of 1969 are clearly more prolific than those of 1968; also the high number of eggs produced by the neoadults in October 1969 (Fig. 4) is evidence that the animals could feed abundantly in a period very advanced in the seasonal cycle. In the main, better feeding conditions characterized the course of the seasonal cycle of summer and autumn of 1969 and to this is due the greater resistance of the population to the reproductive "shift". This does not exclude the importance of other factors, including that of photoperiod; one can suppose rather that the level of food available for the daphnids, within the ambit of values of photoperiod compatible with the production of ephippia, becomes the true limiting factor.

It is quite unlikely that in the two years of research the trophic state of the lake, in general and from the point of view of the nutrients necessary for D. middendorffiana, has altered appreciably. The difference found between the dates for the start of the phenomenon of diapause can be accounted for most realistically by the processes in the formation of the population, in particular the loss of individuals from the water of the lake by the action of the outflow, at the time of more rapid exchange of water and namely during the first phase of development of the exephippial generation.

The factors which control the numerical density of the daphnid population in early summer have a decisive importance on the development of the more advanced phases of the seasonal cycle of the species. These determine directly the numerical dimensions of the population which reaches sexual maturity.

When the cycle of broods with subitaneous eggs commences, the values of crowding tend to rise with sharp increases correlated to successive waves of births until there will be reached critical levels incompatible with further numerical expansion of the population, or with new depositions of subitaneous eggs. Each increase in the values of numerical density of the daphnid population, in a lake as typically oligotrophic as that of Campo 4°, brings about immediately an impoverishment of the available food, taking into account also the liveliest metabolic activity of the younger individuals. Entry into diapause would thus be the response of the population to the beginning of a critical relationship between the degree of crowding and quantity of nutrients available for grazing by the existing population. The fact that the reproductive "shift" in the autumn of 1969 was appreciably later than in 1968 can also be explained allowing that at sexual maturity a more modest total of daphnids was reached: in such a way the process of maximisation of the values of crowding , relative to the availability of food, has been able to occupy more time and effect, as well, a larger number of non-exephippial broods.

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Notice

Please note that these translations were produced to assist the scientific staff of the FBA (Freshwater Biological Association) in their research. These translations were done by scientific staff with relevant language skills and not by professional translators.