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STELLA, S. Megacyclops viridis Jurine, abyssal form in Lago Maggiore

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Introduction.

Megacyclops viridis Jurine, noticed in Lago Maggiore in 1912 by De Marchi from the littoral vegetation of Pallanza, is a normal member of the littoral plankton of the lake.

The subgenus Megacyclops, created by Kiefer in his revision of the viridis-vernalis group, contains european and american species some of which are today considered as varieties of the species viridis.

The problem of the morphology and of the distribution of the Italian viridis has been examined in the 1943 paper of Pirocchi, after the report of an abyssal form in Lake Como: in 1947, Pirocchi extended her observations to the littoral viridis of Lake Maggiore in the region of the basin of the Borromee islands: the Cyclopidae showed, on examination, morphological and morphometric diversity of magnitude in the isolated ecological populations, i.e. in biotopes separated from each other by large depths, hardly surmountable by a species which is a poor swimmer and usually remains close to the bottom on which it creeps or frisks about.

To the observations in the coastal zone follow others in deeper regions. In 1942, Pirocchi had already observed, in the same basin of the islands, ovigerous females of large dimensions at 160m. deep. Ravera observed subsequently (1956), in sediment raised from 300m., some stage V copepodids.

In lake Maggiore, the viridis lives then at great depths. In other lakes it has also been noted from deep water: as for references, Zschokke (1911) finds it in the 'lakes of the "Four Cantons" of Geneva,"Jouse", Bernese Oberland and Scotland. Hofer (1899) has found it in Lake Constance; Fehlmann (1912) in Lake Lugano; Gurney (1933) in Ennerdale; Pirocchi (1943) in Lake Como.

The two forms of the lakes of Constance and of Como are especially Interesting: they are examples of remarkable size, without eyes or pigmentation, with 11 segmented antennules ana biramous limbs, to such an extent to be considered forms related to C. gigas in dimensions and to C. clausii in juvenile characters.

Note: batiale describes organisms inhabiting the depth of a lake:
is synonymous with benthic for great depth.

In Oct. 1960, the Istituto Ital. di ldiobiol. kindly put at my disposal, some samples from deep water taken with the Jenkin surface mud sampler at 360m. ; the discovery of C.viridis, as stage V copepodids, confirmed the presence of a profundal population; one may put thus the problem of the phenotype features of the various populations of the lakes and the relations between them.

The viridis of the deep seem to have (Pirocchi 1943) some characters which distinguish them from coastal forms; a larger size, some juvenile characters and, probably in relation to the habitat, sometimes absence of pigment and of eyes.

Are then the profundal populations of C.viridis to be considered as ecological variations of coastal viridis, or an ancient form differentiated by isolation, and related to those northern forms which re-entered the living-space of C. gigas? In this second case the profundal viridis of the cisalpine lakes should be considered a boreoalpine form. Unfortunately the scarcity of evidence, the uncertainty of the specific determination of the various forms of the group, the lack of body measurements and biological data have till now constituted a formidable difficulty for the systematics of the group.

An analysis of the structure and the biology of the populations in the various biotopes in Lake Maggiore might it seemed, contribute to the resolving of some of these problems. Our researches have therefore been directed to the systematic study and the biology of the viridis of the depths of the lake and comparison of. our collections with the littoral form.

METHODS.In this work, the results are given of work done from Jan. 1961-Nov. 1962 and the conclusions which are drawn.

The collecting stations have been fixed in the basin of the Borromee islands, a basin which is considered a separate zone in the layout of the lake and where Pirocchi has collected and studied the littoral viridis. We have determined some levels around 100,200 and300m. deep along the direction Isola Madre-Cerro; the very steep gradient down to about 100m. makes, in that zone, sampling at higher levels problematical.

The samples in the deep were taken with a Jenkin sampler (Mortimer 1942) which proved very effective, permitting one to collect" at the desired depth a column of water of the same height above the mud as that of the sediment. Thus were caught the Cyclopidae which have been in contact with the mud or were swimming just above it. Each collection was made in duplicate.

In the laboratory the water was siphoned out and the individuals counted and sorted according to sex and instar. The material was then in part fixed in formalin and preserved in alcohol and glycerine, and in part kept alive for breeding.

We have made the first observations on the behaviour of the animals directly in the Jenkin tube which we have been careful not to shake to avoid muddying the water with results always fatal to Cyclopidae: left undisturbed they live for some weeks feeding on the organic detritus and on the small organisms present in the mud. The adults swim very actively rising above the bottom: young copepodids swim more slowly and generally remain close to the mud-surface.

The superficial layer of the mud appears soft, has a brown colour and is rich in organic substances, contains vegetable fragments, diatoms, protozoa, dendrocchi, oligochaetes (always in small numbers), a few examples of *Cyclops serrulatus* Fischer, *Paracyclops fimbricatus* (Fischer) and exceptionally some young copepodid stages of *Cyclops strenuus abyssorum praealpinus* Kiefer.

Breeding has been studied in crystallizing dishes with lake water and a layer of mud on the bottom: the vessels are placed at 6-8°C, mean temperatures of the water of the lake at 100m.

The animals, prevalently carnivores, are fed with small Daphnia or Artemia: observations in the laboratory were made on the cycle of development and tentative experiments on the influence of temperature, of starving, and of light on Cyclopidae.

In quantitative estimates, we have met some difficulties: at each station all individuals have been counted and. sex and stage of development noted in the two samples. The volume of water taken in the Jenkin, whose cylinder has a base area of 38.5cm^2, is about 1½l. with some variation according to the depth of the mud. The difficulty of collecting has not however permitted numerous collections: for this reason the counts are not able to furnish an exact representation of the bottom populations but only an indication of their structure and stability.

Quantitatively, *C.viridis* is scarce at all depths. Table1 gives the collecting dates and the number of individuals of various stages represented in the samples (always duplicated) between 100 and 300m. in the two years.

ANALYSIS OF THE MORPHOLOGY AND BODY MEASUREMENTS.

Materials examined: 22 females (12+eggs) and 10 adult males collected on 18 Jan. 1961 at 120 and 300m. depth. After a first examination and after some preliminary measurements we have observed the presence at 120m. of females with eggs of two distinct sizes, of 2.3-2.6mm. with 20-30 eggs/sac and females of 3.0-3.5mm. with 40-50 eggs/sac. A dualism in size has been described for other Cyclopidae: in *C. scutifer* of northern Sweden (Lindstrom 1952-58: Ascelson 1961) the two types, called respectively A the smallest and B the Greatest are morphologically alike and differ instead in the annual cycle. We have examined the morphology of the adults of two types and of the two sexes.

Ovigerous female type A. Fig 1A. Body 1t. 2310-2650 . Colour off-white with green shade. Eye red fuchsia well formed. Body massive. Cephalothorax elliptical with maximum width at the level of the distal third of the first somite. Width of prosoma 55% of the length. First somite bell-shaped with lateral angles rounded, succeeding , segments with flattened blunt angles turned back towards the bottom. Sixth somite fused with the first abdominal to form the genital somite, flattened lateral protuberances and rounded, posterior margins denticulate.

Metasoma (Fig 2A) slender. First abdominal somite dilated; posterior margins denticuiate on all abdominal somites, with denticles more pronounced at the insertion of the furcal rami.

Furcal rami (Fig3A) slender. Ratio lt: width, 4. 3:1.

Inside margins of the rami with long and thin hairs: inner furcal setae shorter than the ramus and shorter than twice the length of the outer seta. Ratio of the middle furcal setae inner to outer=1.29:1. Lateral seta hairless, inserted a little below the distal third: and outer inside setae with hairs on their whole length, median setae hairless on the proximal ends. Dorsal setae smooth.

Seminal receptaculum of the form characteristic of the species with strong concavity on the anterior margin.

Antennules of 17 segments which do not extend beyond the first segment, of the cephalothorax. Swimming appendages with endopite and exopodite 3 segmented (Fig 14&15): spine formula 2:3:3:3. Terminal segments of the endopodite of leg 4 (Fig 3A; slender with ratio lt:width= 2.49:1 End spines of the segments unequal:ratio inner spine length to lt. of terminal segment=0.935:1. Leg 5 (Fig. 6) with first segment broad, inner spine of the second segment short and segmented. Setae smooth: the seta of the second* segment shorter than that of the second. (See footnote) Egg-sacs adhering to the abdomen, with 20-40 eggs of about 120 micron in diameter.

Type B. (Fig 1 B) Body length 2800-3290µ. Prosoma massive, with width 57% of the length. Metasoma large (Fig 2B.) Furcal rami slender (Fig 3B) with ratio length:width = 4.34:1. Ratio of the length:width of the second segment of the . ' endopodite of leg 4 (Fig 5B) = 2.56:1. Ratio spine (inner) length to the length of last segment of endopodite = 0.953:1. Lateral furcal setae inserted distally. Ratio of inner median furcal seta to outer ditto = 1.24:1

Egg-sacs large, with 40-60 eggs of 140-150 microns diameter.

Adult male. (Fig 4) The morphological differences between males of different size are practically insignificant. Here then only type A is described and the two types are examined separately under "body measurements".

Type A. Body length 2047-2106µ. Type B. . Body length 2366-2377µ. Body slender. Antennule of 17 segments. Furcal rami (Fig 7) slender with ratio length:width=3.6-3.9:1 Terminal inner furcal setae shorter than ramus. Genital somite (Fig 20) well developed. 5th pair as in the female. 6th pair (Fig 3) with robust inner spine as long as the median setae.

Body measurements.

20 ovigerous females have been measured for 27 characters and 12 adult males for 12 by the methods given by Baldi and Pirocchi (1943)

For each type the mean value (M) in microns has been computed and the standard error (e) for each character. The results are listed in Table 2. In the table a difference is evident in the two types in the characters which are related to the body dimensions: the differences are more pronounced in the females than in the males. The different characters are those of length and breadth of prosoma, the metasoma and subordinately of the furcal setae. One observes next some difference in the measurements of the relative characters of leg 4, less in that of leg 5.

The percentages, calculated for the two types, show a relative isometry for the values of the 4th and 5th pairs of legs and a greater variation in the lengths

*(Footnote-:The Italian word "secondo" is used here in each case but presumably the second "second", should read "first")

of the metasoma, of the abdomen and of the furcal rami, less in the measurements of the width.

In males, differences are evident in characters 1,2,3, and 11 relative to the body dimensions, yet in a way less obvious than in the female and less evident in the dimensions of the furcal rami and of the furcal setae.

The morphological physiognomy of the two types appears very similar and on the basis of the analysis of the body measurements one is not able to conclude that two distinct populations are present: individuals of different size are mixed in the same samples and show a series of values from minimal to maximal, with increase of the same about two means which characterise precisely the two types A and B which we have separated the popuations.

In Table 3 are set out for comparison, with reference to the characters more significant in ovigerous females, the two types of the profundal form with the littoral form of the, lake and some related forms. One deduces from it that the type A corresponds most to the coastal viridis of the lake (Pirocchi 1947) while the type B approaches C.gigas for large size and in some morphological characters, especially in the relative lengths of the median furcal setae and of the spines of the last segment of the endopodite of leg 4 in the females and in the male in the structure of leg 6.

C. gigas is not yet accepted by all authors as a good species, being considered for example by Schmeil(l892) Lilljiborg (1901) Sara (1903) a giant form of C.viridis. Kiefer (1928) and Gurney (1933) on the contrary consider it a species in its own right through the large body size, the shortness of the antenna, the slenderness of the endopodite of leg 4 and the position of insertion of the lateral seta on the furcal ramir There are still some characters less well defined and subject however to variation according to the individual and in particular to the age.

Stenothermic in cold water, C. gigas is described as a northern form in small basins (Scandinavia, Bohemia, Hungary, Switzerland) and on the bottom of large lakes, as we have said above and as confirmed also in recent reports; the lake of Plön (Herbst 1951) and the lake of Constance (Kiefer 1963)

Cycle of development.

On the basis of the observations made in 1961 the annual cycle of the profundal C.viridis is as follows:-

In May they are found on the bottom in the first copepodid stages: III and IV; we have not found younger stages or nauplii. It is probable that the spring copepodids come from winter eggs: the stage III copepodids are about 1mm long (Fig9) and they have seven somites of which two are abdominal and the antennules have 9 segments.

The copepodids of stage IV (Fig 10) are a little bigger and have 8 somites of which 3 are abdominal: they show the outline of the 5th pair of legs and antennules of 9 segments in both sexes, in the males shorter and more robust. The last segment of the cephalothorax has a finely toothed posterior margin.

These first postlarval stages can be distinguished from those of C. strenuus abyssorum, which are found on the bottom in the period of circulation, on the basis of the description of the larval development of this last species illustrated by Gurney (1933). The first copepodid stages of C .viridis are more advanced in organisation in comparison with those of C. strenuus, indeed at stage IV the two sexes are already identifiable.

The spring catch is made up almost exclusively of young copepodids of the 5th stage: they measure from 1.6-1.8mm: the females (Fig 11} are larger than the males (Fig 12).The males form 40% in respect to the females. The body has 9 somites of which 4 are abdominal, the swimming legs are biramous, the antennule has 11 segments in both sexes ana are more slender in the female (Fig 17) more thick-set in the male(Figl8) The 4th and 5th somites of the cephalothorax have the posterior margin toothed.

On 1st July larger copepodids are found: the females about 2mm long and the males a little smaller. The first two pairs of limbs(appendages) have biramous exo- and endopodites, the third and fourth however show a hint of division, consisting of a transverse line of setules on the segment of the two arms. Males and females are now sexually mature: under the binocular the ovaries and oviducts can be seen with eggs in various stages of maturation and the testes and spermatic ducts. In this stage the copepodites remain all the summer without moulting: they become covered with epibionts but remain active. Up to the second half of September the character of the population does not change: this is made up almost exclusively of mature stage V copepodids and only a few immature stages. The transformation comes towards the end of October: in the. collection of 23 Oct. 1961, taken at 115m.depth we found several stage V females with the spermatophores attached to the genital segment (Fig. 21) and some recently moulted young females accompanied by males with antennules not yet geniculate (preadult stage?)(Fig. 19)

Some stage V copepodids have also been observed pairing. Egg-bearing females which have about twenty eggs per sac measure at most 2.8mm and belong therefore to the smaller group (type A). During succeeding weeks one can observe a progressive increase in the number of adults and a gradual disappearance of the postlarval stages: by January one finds at last only adults, females for the most part with eggs and males in the proportion of 40% in respect of the females. Dualism in size is evident in the winter population.

Fig 22 shows the annual cycle of Cyclopidae at depths between 100 and 200m. The curves show the % of adults, mature stage V copepodids and immature stages collected in the sampling seasons. It is evident that the adults (males and females, egg-bearing and not) have a maximum first in Jan-Feb, a gradual full in spring and a second maximum in Sept- Nov. The copepodids show a different cycle with an absolute maximum in summer and autumn; the juveniles are restricted to the spring-time with a brief appearance in winter. The different rhythm of the annual cycle at different depths is able, in our opinion, to explain the variability of size of the <u>viridis</u> in the depths. The adults of type A, that is the smaller individuals, which represent the more abundant fraction, are derived from the stage V copepodids precociously fecundated or from the young autumn adults: those of the typeB, the larger, come from the older copepodids or are older adults.

In nature, there is then the possibility of a precocious fecundation. To verify whether this phenomenon is rare or common, we have reared some stage V copepodids: 15 males and 15 females matured were isolated in Oct 1962 from a collection taken from a depth of 115m. Fed in the usual way and kept at a constant temperature of 8°C.the Cyclopidae were watched each week: only one female was fecundated at once as a 5th stage copepodid but died without moulting: the other females moulted after a few days: two died, eight were mated by newly moulted males (which retained the juvenile character of a non-geniculate antennule) They produced egg-sacs and many eggs which hatched into nauplii which reached finally the sixth stage. The other moulted females were not fecundated at once and grew in size:(p.72) the males increased only in length. These adults copulated and the females formed egg-sacs. We have also subjected the autumn copepodid V to different temperatures from the optimal temperature of 6-8°C. Some individuals were held in dishes at 0°C

These gradually slowed down and ceased to feed but put back at 6°C they rapidly became active again: some moulted but did not reproduce. Individuals held at 14°C died within a few days. Other individuals were starved: they were watched for two weeks without increase in size: the copepodids did not moult and the fecundated females did not lay eggs. From these few experiments, one cannot draw definite conclusions; one may however state that very high or low temperatures slow down the metabolism giving rise to a state of quiescence in all stages and one can conclude also that the last moult is delayed by low temperarures but also by lack of food.

Variation in size.

for a more exact evaluation of individual variability we decided to measure all the examples collected in 1961, adult females with and without eggs, adult males and stage V copepodids. The measurements were made on specimens preserved in formalin at weak concentrations and refer to body length excluding the furcal setae.

The results are given in Figs 23and 24. In spite of the small number of individuals, some remarks can be made. The lengths of females can always be grouped around the values 2.3 and 2.8 mm., while the males do not show a bimodality in the curve of distribution so obviously: the mean values are 2.0 & 2.2mm The mature female stage V copepodids have values that tend to lie between 2.0 and 2.3 mm. with maximum of 2.7 and minimum of 1.7mm. The spring stage IV copepodids do not show any noticeable Variation in length: the increment in length of the stage V copepodid is apparent only after the summer inactivity, which coincides with the rising of the metabolism when the characteristic number of body segments and appendages have been acquired. The autumn adults are little bigger than the copepodids.

Conclusions.

The profundal populations of *G. viridis* are found down to 300m. with greater frequency around 100m. and they are distinguished from the littoral populations in size and cycle of development.

The littoral forms, on the basis of published accounts and the observations completed in 1963 on material collected through the kindness of the Istituto Italiano di Idiobiologia from 2-7m. depth, breed from December to March: copepodid stage V, with gonads still undeveloped are present throughout the summer and autumn months: the final moult takes place at the beginning of the winter and the gonads mature only in the adult instar. Thus reproduction is always only in the cold months.

The profundal viridis develops most rapidly in the larval stages and in the first postlarval: the nauplii and stage I and II copepodids occur certainly in less deep water. In spring stage III and IV copepodids appear on the bottom, then at the start of the summer the stage V copepodids which are then sexually mature: they remain quiescent without however entering a torpid state until in October when they make the first moult. Males moult before the females that is to say they have developed more rapidly and the evidence shows that they are less numerous than the females in the proportion of about 30%. A fraction of copepodids undergo the last moult in October; the females are immediately fecundated by young males which retain the juvenile character of non-geniculate antenna (preadult stage). The females form egg-sacs and like the males are small (type A) A proportion of copepodids do not moult at once but increase in size and moult only in November: then the older females are fecundated by larger males possessing a geniculate antenna. The adults of this period are the type B.

In the samples collected in Jan- Feb. the types of different size are found together because the large individuals which represent the older adults, join themselves with the individuals first moulted from copepodids in the late autumn, evidently providing some late nauplii: it is evident that the eggs do not all hatch contemporaneously. The fact then that the small individuals predominate at higher levels and the large at greater depths can be explained by the different length of the life-cycle; the increase in the last postlarval stages, especially stageV and of the adults, occurs more slowly in the profundal depths in response probably to the lower trophic level of the environment.

In the profundal form one notices also a tendency towards precocious maturation in contrast to the littoral form.

In the last postlarval stage the ovary is indeed well developed with large diverticula and the eggs fill up, the oviducts, so that immediately after the last moult, females are fecundated rapidly. There is also the possibility of pairing in the fifth copepodids stage, as shown in the results of October 1962 in which stage V females wore found with spermatophores attached to the genital segment. Can one regard this as a phenomen of neoteny? In other copepods a precocious maturation has been observed: Marshall and On (1955) found in some populations of Calanus finmarchius (Gunn) female stage V copepodids with mature gonads and sometimes with spermatophores. In Cylopidae of brackish water belonging to the species Diacyclops bicuspidatus Claus and D.bisetosus Rehberg some neotenous characters are evident in particular the reduced number of the segment of the antennule: 14 rather than 17. Margalef (1949) attributes to the neoteny of these forms selective value and thinks that they should be considered, because they live in particular environments, as subspecies or ecological races.

The ancient forms of Copepoda would have been much larger than at present and would have had more segments in the antennae and a larger number of larval stages, probably 13. The smaller size and number of stages in present day copepods are attributed (Margalef 1949) (Serban 1960) to the effect of advantageous selection: the elimination of a larval stage leads to the shortening of the life-cycle with preservation of small size: this condition one can verify in some Cyclopidae of underground and interstitial waters.

Some other profundal forms of G.viridis living in great depths in lakes show; a tendency to suppress the last stage: the form clausii in the profundal of Lake Constance described by Hofer(l899) and the abyss form of C.viridis in Lake Como (Pirocchi 1943) are in effect able to be considered as neotenous forms.

In a C. gigas, living in small collections of water in Bohemia, Mrazek (1913) describes a cycle which is very like that of our profundal viridis: accelerated larval development, the summer passed as stage V copepodids, reproduction in the cold months ana a similar cycle is reported for other large northern forms, attributed always to C. gigas e.g. Gurney (l929 and 1933) Herbst(l95l) and Røen (1957) Mrazek besides describes individuals of stage V in copulation and considers that fecundation can happen before the last moult; and. observes that there are two breeding times, one in autumn of the young adults which have just moulted, the other in winter when the adults have acquired the typical size of the species. Also according to Røen C. gigas has two deposition of eggs in egg-sacs, but the females are fecundated twice.

In the profundal C.viridis of Lago Maggiore, there may also be two depositions of eggs in egg-sacs in the same female at different times, I am in the course of making observations to find out whether fecundation comes after the last moult and if a second fecundation occurs later among older adults.

If, as we think, variation in size of the profundal populations is
[ovatuna = defoliation of eggs in egg-sacs.]

related to the different periods of the cycle of development, one cannot say that the C. viridis in lakes belong to different species or different subspecies but rather that there are ecological races living in diverse biotopes. The validity of the species C. gigas, with which C. viridis of the profundal has clear morphological and biological affinities, would therefore be questionable. Also if biometrical researches apply strictly to results determined only from numerous populations, there the results at our disposal lead us to exclude the presence of two species.

Various systems have been proposed recently to settle the position of species, subspecies and races in the sphere of the so-called "difficult groups" of the Cyclopidae.

Einsle (1963) put forward a cytological criterion in populations of Cyclops furcifer Glaus changing with size: according to the quantity of chromatin eliminated during meiosis of the chromosomes there would result differences in size and from this there would result differences in size of individuals.

Dussart (1959) reintroduced the biometrical methods as criteria of specific and subspecific differences, employing a system of circular diagrams on which are indicated the relations of a large number of values. In such a way one can compare the polygons obtained for the various populations with those of the typical species. It is necessary to have available numerous populations and to possess a large number of body measurements.

Kiefer (1963) has published an account of a study on the variability and population cycles of G. viridis and C. gigas recently found at different depths in Lake Constance: the results of his researches on the two forms permit a most interesting comparison with our observations on Cyclops viridis in Lake Maggiore, which will be continued further.

Footnote.

Dr Stella tells me that:-

batiale is used for organisms inhabiting the depth of a basin.

ovatura means deposition of eggs in egg-sacs. e.g. Cyclopidae may have one, two, three ovatura in the course of a year.