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Esteval phenology of an *Acanthocyclops* (Crustacea, Copepoda) in a Colorado tarn with remarks on the *vernalis-robustus* complex

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Abstract

Weekly mean lenghts of adult female *Acanthocyclops robustus* ranged between 1.14 and 1.41 mm in a Colorado alpine lake during the summer of 1966. The smallest animals occurred in late September and the largest in mid-August, corresponding to development in warm and cool water, respectively. The males also declined in mean length during the course of the summer and were about 70% of the length of the females on any date. About 75% of the females possessed a 3.4.4.4 spine formula; 8%, 2.3.3.3 and 17% an anomalous formula. Eight-one percent of the males had a 3.4.4.4 formula; 7%, 2.3.3.3 and 12% were anomalous. Morphological variation, and breeding experiments performed by Price (1958) suggest that *A. robustus* and *A. vernalis* as defined by Kiefer (1976) are likely species complexes, rather than just two species.

Introduction

Morphological variation in *Acanthocyclops vernalis* (Fischer, 1853) and *Acanthocyclops robustus* (G. O. Sars, 1863) has long vexed students of freshwater copepods. Part of the vexation has concerned the armature of the outer margins of the terminal segments of the exopodites of the four pairs of thoracic legs. *Vernalis* was described as typically having 2.3.3.3 spines on these segments and *robustus*, 3.4.4.4. Individuals of both forms have long been known to deviate from these conventional spine formulas. Moreover, asymmetries between left and right members of one pair of legs are frequently noted. These copepods are of wide geographical distribution and abundant occurrence and are commonly met with in many types of waters. Common occurrence combined with morphological variation has resulted in taxonomic confusions with *robustus* variously designated as a subspecies, form, or variety of *vernalis*, although some authors have maintained that they are two distinct, valid species (Kiefer, 1976). Breeding experiments have added fuel to the fire of confusion, for example Lowndes (1928) and Price (1958).

Kiefer (1976) proposed that *vernalis* and *robustus* may be distinguished from each other by criteria other than the exopodite armature; namely the shape of the female genital segment; length-width ratio of the third segment of the P4 endopodite and the relative lengths of the terminal spines on that segment. Animals designated as *vernalis* and *robustus* frequently had spine numbers of 2.3.3.3 and 3.4.4.4 respectively, but much variation was found.

Rüsch (1960) investigated mechanisms that determine sexuality of individuals in several species of freshwater copepods. She found that animals identified by Kiefer as *A. robustus* possessed three pairs of chromosomes (2n = 6). In contrast, animals identified by Brehm as *A. vernalis* possessed four pairs of autosomes and one pair of heterosomes (2n = 10). Einsle (1977) studied an acanthocyclopid which conformed to *A. robustus* in regard to shape of the genital segment and proportions of the terminal segment of endopodite of leg 4 and possessed 2n = 6 chromosomes. Purasjoki and Viljamaa (1984) reported that an acanthocyclopid which they identified as *A. robustus* on morphological characteristics bore physical resemblance to *A. vernalis* and possessed 2n = 10 chromosomes.

The variability in the *vernalis-robustus* group is worthy of study in its own right, apart from taxonomic considerations. No other species or group of species of freshwater copepods is so well known for spine formula variation. In spite of frequent mention by several authors, quantitative descriptions of variability of spinulation in naturally occurring populations are few.

Variation in size among individuals of wild populations of copepods can be striking. Elgmork (1959) remarked (p. 114) that size differences among adult *Cyclops strenuus strenuus* in Norwegian ponds at certain seasons were 'so conspicuous that they can be recognized without making measurements'. Einsle (1977) reported that the mean total length of adult female *A. robustus* in mid-June 1973 samples from Bodensee was about 1.65 mm. Five weeks later the mean length was approximately 1.32 mm and remained so until the end of September, when it began increasing and reached about 1.7 mm toward the close of October.

The aims of this paper are to describe quantitatively the seasonal variations of both spine formula and size, as measured by total length, in an acanthocyclopoid population in a Colorado alpine lake.

Emmaline Lake

Emmaline Lake is a cirque type lake of 1.09 ha and maximum depth of 6.2 m, mean depth 2.5 m (Walters, 1969). The lake is located in the Colorado Rocky Mountains, immediately north of Rocky Mountain National Park (Sec 26 T7N, R74W of 6th principal meridian). The elevation of Emmaline Lake is about 3400 m. It is usually ice covered from October through most of June. Due to shallowness and wind mixing, only small differences were found between surface and bottom temperatures in Emmaline Lake in 1966. Water temperature increased nearly linearly from 5 °C, July 1 to 16 °C, July 21; temperature remained at or above 16 °C to mid-August, with the observed maximum of about 17.5 °C recorded at the end of the first week of August (Walters, 1969). After reaching maximum, temperature declined to 13 °C at the beginning of September, and again reached 5 °C at the end of that month. Plankton samples were acquired by vertical haul with a Birge cone net.

Methods

Adult cyclops were picked from the samples with fine needles and placed on microscope slides in a dilute solution of glycerine and water. Following evaporation of the water, each individual was measured for total length from anterior end of rostrum to the posterior end of the furcal rami. Measurement was done at 100× magnification using an ocular micrometer, read to the nearest 0.01 mm.

After measurement animals were transferred to individual small drops of glycerine for dissection in which the pairs of thoracic legs were removed. To avoid confusion each pair of legs was placed in its own droplet of glycerine. Spines were examined at 200 or if necessary 440×, magnification.

Spines may be broken off in capture or in dissection of animals. The former presence of a broken off spine is clearly revealed by a socket in the exoskeleton that is readily recognized. All adult animals of all collections were both measured and examined for spine formula, but because of damage in collection or other causes, the total of measured individuals was not the same as the total dissected on some dates.

Results

Size

The mean length of adult female copepods decreased from 1.41 mm, August 15 to 1.14 mm, September 22 and then increased to 1.36, Novem-

Table 1. Total length (mm) of adult *Acanthocyclops robustus*, Emmaline Lake, Colorado, 1966.

Date	n	$\bar{x}$	range	standard deviation
Females				
Aug. 15	21	1.41	1.21 – 1.59	.096
Aug. 22	11	1.40	1.26 – 1.58	.103
Sept. 5	12	1.32	1.21 – 1.41	.059
Sept. 22	7	1.14	1.05 – 1.20	.053
Nov. 24	5	1.36	1.25 – 1.64	.166
Males				
Aug. 15	20	.97	.92 – 1.02	.031
Aug. 22	19	.95	.89 – 1.02	.036
Sept. 5	17	.93	.89 – .99	.029
Sept. 22	2	.79	-	-
Nov. 24	4	.95	.85 – 1.05	.082

ber 24 (Table 1). The mean length of male cyclopoids decreased from 0.97 mm, August 15 to 0.79, September 22 and increased to 0.95 mm, November 24. The ranges of total length for each sex exhibited a similar pattern.

The sexes were collected in essentially equal numbers. The mean length of Emmaline females ranged from 142 to 147% of that for males captured on the same date. No adult animals appeared in July collections.

Spinulation

Animals with both of the coventional spine formulas, 3.4.4.4 and 2.3.3.3, occurred in the Emmaline Lake collections, the former being more frequent than the latter. Overall 75% of the females possessed a 3.4.4.4 formula; 8%, 2.3.3.3, and 17%, anomalous (Table 2). Among the males, 81% had a 3.4.4.4 formula; 7%, 2.3.3.3, and 12%, anomalous.

Individuals may show symmetrical or asymmetrical anomalies, for example, 22.33.44.33 or 22.33.34.33. Asymmetrical anomalies were more frequent than symmetrical ones among both sexes (Table 2).

Among symmetrically anomalous animals all of three females and two of three males appeared to have a variation of the 33.44.44.44 spinulation. Among asymmetrically anomalous animals six of eight females and four of five males seemed variants of 33.44.44.44; one male appeared to be a 22.33.33.33 variant. Some anomalous animals resembled one conventional formula as much as the other.

The percentage of anomalous animals did not show a clear seasonal trend. More than half of the females captured September 22 were anomalous in spinulation and none of the males taken September 22 and November 24 were anomalous. Perhaps the most interesting aspect of spinulation is that with the exception of one male caught September 22, all 22.33.33.33 animals were found in the November 24 sample. One anomalous male of August 15 may have been a 22.33.33.33 variant.

One male caught September 5 bore five spines on the P4 terminal exopodite. Since this otherwise

Table 2. Spine Formulas of Adult *A. robustus*, Emmaline Lake, Colorado, 1966.

Date	Conventional	N	Anomalous			
			Symmetrical	N	Asymmetrical	N
Females						
Aug. 15	33, 44, 44, 44	19	33, 44, 33, 44	1	23, 44, 43, 33	1
					23, 34, 44, 34	1
					23, 34, 34, 34	1
					33, 44, 44, 43	1
Aug. 22	33, 44, 44, 44	15	0		0	
Sept. 5	33, 44, 44, 44	11	33, 33, 44, 33	1		
Sept. 22	33, 44, 44, 44	3	33, 44, 44, 33	1	33, 44, 34, 44	1
					33, 43, 44, 33	1
					33, 44, 44, 43	1
Nov. 24	33, 44, 44, 44	1			33, 44, 44, 34	1
	22, 33, 33, 33	5				
Total		54		3		8
Males						
Aug. 15	33, 44, 44, 44	20	22, 44, 44, 33	1	23, 33, 33, 33	1
			23, 44, 44, 44	1		
Aug. 22	33, 44, 44, 44	17	0		33, 34, 44, 33	1
					33, 44, 44, 34	1
					33, 44, 43, 44	1
Sept. 5	33, 44, 44, 44	18	33, 44, 44, 43	1	33, 45, 44, 44	1
Sept. 22	33, 44, 44, 44	1	0		0	
	22, 33, 33, 33	1				
Nov. 24	22, 33, 33, 33	4	0		0	
Total		61		3		5

was a 3.4.4.4 animal, this appears to be a case of a supernumary spine. In my experience spines in excess of 3.4.4.4 are infrequently encountered. Coker (1934) found that in rearing the progeny of wild caught *vernalis* from the Paris area at various experimental temperatures spine counts of five occasionally appeared.

Discussion

Size

Students t test for samples of unequal numbers was used to make comparisons of weekly mean lengths of Emmaline Lake copepods (Table 3). The females of August 22 were not significantly smaller than those of August 15, but they were significantly larger than those of September 5, which were significantly larger than September 22 animals. Results of comparisons of males are less conclusive but males of August 22 are significantly smaller than August 15 males at α 0.10 and they are significantly larger than September 5 animals at the same level. Therefore the mean lengths of the September 22 adults of both sexes were approximately 81% of those of the population four to five weeks earlier.

Einsle (1977) reported a similar seasonal change in the mean lengths of female *A. robustus* from Bodensee-Obersee - namely a large late spring form, small mid-summer individuals and large autumnal animals. The Bodensee *robustus* were from 115 to 120% larger than the Emmaline animals. Thus in both Bodensee and Emmaline lakes the smallest adults were associated with the warmest water temperatures and larger adults with cooler water.

The Emmaline Lake acanthocyclopids were within the ranges of total lengths given by various authors (Table 4). Except for the animals collected September 22 the Emmaline copepods were, on the average, about 15% larger than those I collected in Tirol.

Price (1958) performed breeding experiments with the progeny of *Cyclops vernalis* from 30 lakes and ponds. Among the 30 populations he found seven forms, that he called isolates and designated by letter, that behaved as species; i.e., the isolates would not cross in experimental breedings. Some isolates were characteristically larger than others and all grew to a larger size at 10 °C than at 25 °C (Table 4). Price's 10 °C females were appreciably larger than the Emmaline females, while the 25 °C experimental animals were about the size of the wild caught Colorado animals. The Emmaline males for most dates were about the size of Price's isolates F and G grown at 25 °C.

The females caught in Emmaline Lake on September 22 had a mean length similar to that given by Kiefer (1976) for females of both *A. vernalis* and *A. robustus* (Table 4); on other dates the Colorado copepods were from 116 to 125% larger than *vernalis*.

Several authors have noted that copepods grow larger at cool temperatures than at warm. Growth rate may be modified by other factors, notably food supply. Price's 25 °C animals in culture may have received a better food supply than the animals in oligotrophic Emmaline Lake.

Table 3. Comparisons of mean lengths of *A. robustus*, Emmaline Lake, Colorado, 1966.

Means	Calculated t*	Table t (level) (degrees freedom)
Females		
Aug. 15 vs Aug. 22	.273	(.05) (30) 2.042
Aug. 22 vs Sept. 5	2.327	(.05) (21) 2.080
Sept. 5 vs Sept. 22	6.555	(.001) (17) 3.965
Sept. 5 vs Nov. 24	.757	(.05) (15) 2.131
Males		
Aug. 15 vs Aug. 22	1.660	(.10) (37) 1.687
Aug. 22 vs Sept. 5	1.601	(.10) (34) 1.691
Nov. 24 vs Sept. 5	.448	(.05) (19) 2.093

* Calculated according to Simpson et al 1960 for samples of unequal sizes

Table 4. Total lengths (mm) of *Acanthocyclops robustus* and *A. vernalis.*

Species					Author
C. vernalis	7♀	1.37 – 2.17 England			Gurney 1933
C. robustus	4♀	1.10 – 1.80 England			Gurney 1933
A. vernalis	103♀	.8 – 1.5, mode 1.2	Tirol		Reed 1971
A. vernalis	68♂	.54 – 1.17, mode .8	Tirol		Reed 1971
A. robustus	18♀	1.03 – 1.22, mean 1.14	lecto types	Norway	Kiefer 1976
A. vernalis	♀	1.0 – 1.29, mean 1.17		Norway	Kiefer 1976
A. robustus	3♀	1.2 – 1.27 U.S.N.M.	#62637	Kansas	Kiefer 1976
A. robustus	1♀	1.5 U.S.N.M.	#62638	Kansas	Kiefer 1976
A. vernalis	5♀	1.04 – 1.3 U.S.N.M.	#62638	Kansas	Kiefer 1976
A. vernalis	12♀	1.4 – 1.5 U.S.N.M.	64515	Alaska	Kiefer 1976
C. vernalis	23♀	1.24, mean 25 °C	4♀ 1.55 10 °C	isolate A	Price 1958
C. vernalis	19♀	1.33, mean 25 °C	9♀ 1.71 10 °C	isolate B	Price 1958
C. vernalis	18♀	1.24, mean 25 °C		isolate C	Price 1958
C. vernalis	6♀	1.40, mean 25 °C	1♀ 1.53 10 °C	isolate D	Price 1958
C. vernalis	4♀	1.35	8♀ 1.66 10 °C	isolate E	Price 1958
C. vernalis	16♀	1.36	3♀ 1.73 19 °C	isolate F	Price 1958
C. vernalis	7♀	1.29, mean 25 °C	6♀ 1.58 10 °C	isolate G	Price 1958
C. vernalis	40♂	.80, mean 25 °C	4♂ 1.00 10 °C	isolate A	Price 1958
C. vernalis	29♂	.88, mean 25 °C	14♂ 1.19 10 °C	isolate B	Price 1958
C. vernalis	20♂	.83, mean 25 °C	- - 10 °C	isolate C	Price 1958
C. vernalis	11♂	.88, mean 25 °C	3♂ .95 10 °C	isolate D	Price 1958
C. vernalis	-	-	4♂ 1.12 10 °C	isolate E	Price 1958
C. vernalis	7♂	.95, mean 25 °C	- -	isolate F	Price 1958
C. vernalis	16♂	.99, mean 26 °C	4♂ 1.14 10 °C	isolate G	Price 1958

Spinulation

Lowndes (1927) performed various breeding experiments with the progeny of wild caught *C. americanus* which would now be considered as *A. vernalis* or *A. robustus* and obtained an assortment of spine formulas. Even though animals were carried through back crosses, Lowndes was unable to detect any simple Mendelian ratio. Interestingly, Lowndes wrote 'no individuals were obtained with a spine formula that could be considered a mixture', i.e., no anomalies. Such was not the case of Emmaline Lake animals nor in *Acanthocyclops* from Tirol (Reed, 1970 and 1971). As a 'control' Lowndes subjected representatives of *Cyclops (Macrocyclops) albidus* and *C. (M.) fuscus* to the manipulation used on the acanthocyclopids and obtained no deviations in spine formulas.

Elgmork (1959: p. 37) examined 50 individuals of *Cyclops strenuus strenuus,* a species of 3.4.3.3 formula, and found two (4%) with asymmetrical anomalous counts: 23.44.33.33 and 33.54.33.33; the latter appearing to be an instance of supernumary spinulation.

In an examination of 171 copepods collected from 27 ponds in the Austrian alps roughly one-quarter were 2.3.3.3 animals; one-half, 3.4.4.4, and one-quarter of anomalous formulas. However 'pure' populations of either of the conventional formulas alone made up a small percentage of the total copepods collected (Table 5). About 30% of the total came from populations with 3.4.4.4 and anomalous animals and over one-half came from populations showing both of the conventional formulas mixed with anomalous animals.

Price (1958) reported that the isolates found in breeding experiments varied in their spine formulas (Table 6). Isolate C was the most consistent in its spinulation, 97% showing 3.4.4.4. Isolate G had the greatest percentage of 2.3.3.3 animals, 71%. Isolates D, E, and G each were over one-fourth anomalous. Of the more than 1 100 animals examined by Price, about 60% were of the 3.4.4.4 formula; 21%, 2.3.3.3 and 19%, anomalous.

Of 44 populations examined by Kiefer (1976) about 57% had the 3.4.4.4 formula, 18%, 2.3.3.3 and 25% contained animals of both formulas. Kiefer did not give numbers for anomalies.

Table 5. Percentages of Acanthocyclopid adults and populations with various spine formulas, Ötztal Alps Tirol. Data from Reed (1971).

Spine formula	Percentage of animals	Percentage of populations
2333 only	6.4	18.5
2333 & anom.	2.3	7.4
3444 only	7.0	18.5
3444 & anom.	29.2	22.2
2333, 3444 & anom.	53.8	25.9
anom. only	1.1	7.4

Table 6. Percentages of Acanthocyclopid isolates and spine formulas. From Price 1958.

Isolate	Spine formula		
	3444	2333	Anomalous
A	61	23	16
B	73	9	18
C	97	0	3
D	49	23	28
E	52	16	31
F	59	22	19
G	3	71	26

Concluding remarks

In addition to the characteristics already mentioned, Kiefer (1976) proposed that an indentation on the inner margin of the first segment of the endopod of the fourth leg of *A. robustus* was useful in distinguishing it from *A. vernalis.*

Forbes (1897) wrote regarding *Cyclops insectus,* since considered a synonym of *A. vernalis:* 'There is a very peculiar semicircular indentation on the outer side of the basal segment of the inner ramus of the fourth foot often present in this variety, but it may be entirely absent or only present in a slight degree of development'.

Price (1958) examined the first endoped segment of leg four of the isolates and found that the outer margin ranged from straight (no indentation) for isolate C through various degrees to a marked depression in isolate E. Price noticed other small differences in this segment among the isolates. Both Price and Kiefer found the indentation lacking in males.

Price pointed out that isolate C, a 3.4.4.4 spine formula animal with pronounced spines on the outer and terminal margins of the terminal segment of all exopods and spine-like outer terminal seta of the furcal rami, seemed to correspond to *Cyclops brevispinous* Herrick and he favored reinstating that specific name. Forbes (1897) wrote of the armature of the thoracic legs: 'the spines of these appendages are extravagantly long and heavy'. Price (1958) also called attention to the fact that *brevispinous* is nearly always found limnetically, the only isolate occupying a clearly defined habitat.

The shapes of the genital segments of Price's isolates were not described or figured so it is unknown if they were rounded as Kiefer described for *A. robustus* or angular as in *A. vernalis.* Price provided tracings of photomicrographs of the terminal segment of the endopods of leg four of isolates A, B, C, and D. All appear to be more reminiscent of the attentuated proportions that Kiefer stated characterized *A. robustus* than of the relatively wider endopod of *A. vernalis.*

Price (1958) was unable to give morphological characteristics that discriminated among the isolate males. Similarly, Kiefer (1976) could not distinguish between males of *vernalis* and *robustus* on the basis of the features that he used to identify females of the two forms.

Kiefer's criteria permit the recognition of two groups in the *vernalis-robustus* complex, but the situation may be more complex than just two species. There is direct evidence (Price's isolates) and circumstantial evidence (the variations found by Kiefer and other researchers) that one or both *A. vernalis* and *A. robustus* as defined by Kiefer may in fact be a group of sibling species. Aided by the criteria set forth by Kiefer (1976) it may now be possible for copepodists to move forward in untangling the ecological and morphological relationships of the common copepods known as *A. robustus* and *A. vernalis.*

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