Seasonal variations in the condition of two *Daphnia* species and their hybrid in a eutrophic lake: evidence for food limitation

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Abstract. As seston and chlorophyll concentrations in eutrophic lakes are usually high all year round, it was hypothesized in the past that food limitation is of major importance for the seasonal dynamics of herbivorous zooplankton in such lakes. Since direct measurements of food are hampered by the lack of knowledge on the exact nature of the food in eutrophic conditions, indirect measurements are necessary to estimate the degree of food limitation in these circumstances. Hence, we used laboratory- and field-derived relationships between the body length and body carbon content of different species of *Daphnia*, which were collected from the highly eutrophic Tjeukemeer, the Netherlands. From a seasonal survey of the carbon content of the daphnids, we concluded that in Tjeukemeer *D.galeata, D.galeata* × *cucullata* and *D.cucullata* are food limited during the largest part of the year. Since the condition of the hybrids was relatively high as compared with the parental species when the food concentration was high, *D.galeata* × *cucullata* is expected to be the more successful taxon during periods of high food availability.

Introduction

According to the PEG model of seasonal succession of planktonic events in fresh waters (Sommer *et al.*, 1986), definite periods of food shortage for herbivorous zooplankton are found in most temperate lakes, as a result of 'clear-water' periods in late spring. In very eutrophic lakes like Tjeukemeer, the Netherlands, however, such clear-water phases are never observed (Moed and Hoogveld, 1982). This has led to the hypothesis that the seasonal dynamics of the herbivorous zooplankton in lakes like Tjeukemeer are more determined by predation, and that food limitation is of minor importance in highly eutrophic lakes (Vijverberg and Richter, 1982; Hanazato and Yasuno, 1985). In order to test this hypothesis, it is essential to obtain either a reliable estimate of the amount of food available for zooplankters in the field or to be able to estimate directly the condition of the zooplankton on a seasonal basis. One of the main problems in eutrophic lakes in particular is, however, that it is difficult to assess the amount of food actually available to herbivorous zooplankton, as the exact food sources for cladoceran zooplankton under these conditions are still unknown. For example, *Daphnia* sp. are reported to feed on detritus (Kerfoot and Kirk, 1991), bacteria (Urabe and Watanabe, 1991), green algae (Horn, 1985) or even cyanobacteria [Bloem and Vijverberg (1984), but see for example Ahlgren *et al.* (1990)]. Apart from the direct food effects, other factors, such as the inhibitory effects of filamentous algae or silt particles (Gliwicz and Lampert, 1990; Kirk, 1992), may also play a role in the uptake of food. In short, as the diet of the daphnids is so mixed and variable, quantifying the amount of food under eutrophic conditions is very difficult.

As direct measurements of food concentrations have been unsuccessful, several alternative methods of estimating food limitation in cladocerans in the

field have been used. Larsson *et al.* (1985) proposed the most direct method, in which animals were cultured under field conditions, and in which measurements of growth and reproduction in these circumstances were made. The workload involved in such experiments is, however, too large to make this method feasible for the estimation of food concentrations on a seasonal basis.

The most widely used method to estimate food limitation in the field is the measurement of fecundity, since it is known that the number of eggs per female is influenced by food concentrations (e.g. Vijverberg, 1976). Unfortunately, no straightforward method is available to quantify the fecundity of a cladoceran population in such a way that the obtained fecundity parameters are comparable in time for the same population, or comparable among populations at the same time. Some authors have used the average number of eggs in the population (Lampert, 1978), but this number is dependent on the average size of the animals, and as within a year the average length of daphnids usually changes, this is not an accurate method. In order to circumvent this length dependence, Hebert (1977) defined the standard egg production (SEP) as the average number of eggs from animals in a given length class. This value was derived from linear regressions with the female length as the independent variable and the number of eggs in the brood pouch as the dependent variable. However, various studies showed (e.g. Duncan, 1989), that both the slope and the intercept of these regression lines were affected by the ambient food levels, and hence that the value of SEP was dependent on the chosen length, yielding for different lengths not only quantitatively but also qualitatively different results. Moreover, Larsson *et al.* (1985) suggested that fish might feed selectively on animals with eggs, thus influencing the length–egg number relationships.

Others have in the past used the measurement of the lipid content of the animals as an estimate of their condition (e.g. Farkas, 1970; Hakanson, 1984). However, although this method seems to provide a good indication of the nutritive status of cladocerans, quantitative methods of lipid microanalysis involve more time and sophistication than most field investigators can provide. Tessier and Goulden (1982) proposed a relatively simple measurement of the lipid content by defining a lipid–ovary index, based on an estimate of the number and size of triglyceride droplets in the animals in relation to their body size. This method does involve a high degree of subjective judgement, however, and in addition is rather crude, since only four separate classes were distinguished. Furthermore, as it is a visual index, results from different species and from different investigators are difficult to compare.

Berberovic (1990) established the ash content of *Daphnia* individuals as a measurement of condition, under the assumption that the absolute ash content is not affected by food concentration, but that the relative content in relation to body weight is. However, Lemcke and Lampert (1975) showed that in starving animals both the relative and the absolute ash content decreased.

Duncan (1985, 1989; Duncan *et al.*, 1985) used the length-dependent carbon content of *Daphnia* individuals to estimate the food limitation of the animals. Duncan found that the slope of the regression lines of the natural logarithm of the carbon content as the dependent variable and the natural logarithm of length

of the animals as the independent variable was constant for different food levels, but that the intercepts of the regression lines differed. Animals reared on higher food concentrations had a higher carbon content, and hence the intercept of the length to carbon regression line was higher. Thus, establishing the intercept of the length to carbon relationship could yield a length-independent measurement of the condition of the animals.

Since the length-dependent carbon content method to investigate food limitation seemed most promising, we studied the role of changing food conditions in eutrophic Tjeukemeer over the seasonal cycle of *Daphnia* using this method. In order to estimate the extent of the food limitation in the field, we compared laboratory-derived length–carbon regressions from individuals of *D.galeata*, *D.cucullata* and their hybrid, which were grown at different food levels, with animals collected in the field. Although hybridization seems to be an ongoing process (Schwenk, 1993), we treated the hybrids here as a separate species. The combination of the laboratory- and the field-derived length–carbon regressions was made in order to quantify the effective food concentrations in the field, by comparing the intercepts of field- and laboratory-derived length–carbon regressions.

In this paper, we test the hypothesis that in a highly eutrophic lake food is not limiting, and address the following questions. (i) Are *Daphnia* individuals food limited during the course of a year, and if so when and to which extent? (ii) Do the different species differ in their response? (iii) What is the significance of the food concentration for the success of the species in the field?

Method

Laboratory

Daphnia galeata, *D.cucullata* and *D.galeata* × *cucullata* were collected from Tjeukemeer, using a 120 μm tow net. The animals were placed individually into 100 ml test tubes, and adapted to a 1:1 food mixture of *Chlamydomonas globosa* and *Scenedesmus obliquus* in 0.45 μm filtered Tjeukemeer water at 17.5°C. The media were changed daily. The newborns produced by these field animals were raised under the same conditions as their mothers. The neonates produced by the second-generation individuals were used for the experiments described below. Four (hybrid) or five (*D.galeata* and *D.cucullata*) food levels using *C.globosa* were made up, i.e. 1500, 5000, 10 000, (20 000) and 100 000 cells ml^{-1}, resulting in algal carbon contents of 0.04, 0.13, 0.25, (0.50) and 2.5 mg C l^{-1}. Since *D.galeata* individuals did not grow well on *C.globosa* (Boersma and Vijverberg, 1994b), we cultured this species on different concentrations of *S.obliquus*. This had consequences for the analysis of the data, since food and species effects did become entangled, and hence a direct comparison of the three species was not possible. The cultures were kept at 17.5°C, and subjected to a day–night rhythm of 16:8 h. Several authors (Carvalho, 1987; Weider and Wolf, 1991) observed differences between different clones of *Daphnia* for several life history characteristics; hence, clones which were randomly collected from Tjeukemeer were evenly distributed over the different food levels. This enabled

us to test for a clonal effect on the carbon content of the animals. At regular intervals during the experiment, adults of different instars and different clones were analysed for carbon.

As the effect of the temperature on the length to carbon relationship is not clear (Duncan, 1985), we carried out a separate experiment with *D.galeata*, and *D.galeata* × *cucullata* cultured at three temperatures (12.5, 17.5 and 22.5°C), at the highest food level.

Field

Tjeukemeer is a shallow eutrophic lake in the northern part of the Netherlands. The lake has a surface area of 21.5 km² and an average depth of 1.5 m. The algal biomass is dominated by filamentous cyanobacteria, mainly *Oscillatoria* species (Moed and Hoogveld, 1982). Average Secchi disc depth in summer is ~40 cm (Vijverberg and Richter, 1982). In eutrophic lakes like Tjeukemeer, the concentration of detritus particles can be 4–6 times higher than the concentration of living algae (Mann, 1988).

The carbon content of daphnids in the field was determined on a weekly basis in the growing season and on a fortnightly basis in winter. Animals were collected using 335 μm tow nets, and the carbon content of on average 30 individuals per species was determined. Quantitative samples were taken simultaneously with a 5 l Friedinger sampler at five stations in the lake, at two depths. These samples were pooled, concentrated by filtration through a 120 μm filter and the animals were preserved in a 4% formaldehyde solution. Total numbers of animals and length–frequency distributions were measured in a one-tenth subsample. The animals were measured from the base of the spine to the top of the eye to the nearest 0.01 mm. Since preserving animals in formaldehyde may cause egg loss from the brood chamber, an additional sample was taken and preserved in 95% ethanol. The length–fecundity relationships were established using these samples.

Carbon analyses

The carbon content of the *Daphnia* species was analysed using a UNICARB carbon analyser (Salonen, 1979). This enabled us to analyse the carbon content of individual animals. All animals were treated alive, as this was found to give the best estimate for carbon content (Boersma and Vijverberg, 1994a). Cultured animals where taken directly from their culture vessels, whereas animals taken from Tjeukemeer were resuspended in lake water and analysed within 5 h after collection.

The carbon content of cladoceran zooplankton is not constant during an intermoult period. Tessier and Goulden (1982) reported a 2-fold increase in weight within one instar, caused by storage of material in the ovaries. To minimize the effect of changes within an instar, we only analysed animals with young eggs. This had several advantages. Firstly, the carbon content of the females was minimized, and hence it provided the best possible estimate of somatic carbon when the carbon content of the eggs was subtracted. Although

the within-instar changes of carbon content are probably more pronounced in adults, the build-up of carbon will also occur in juveniles during an intermoult period. Since it is difficult to estimate the time since the last moult of an instar when no eggs are present, no juveniles or females without eggs were analysed. Secondly, since the eggs were young, it was relatively easy to count them. Thirdly, the young egg stage gave the best estimate of carbon invested in reproduction, because none of the egg membranes were cast yet, and eggs may lose around 20% of their carbon in the course of their development to newborns (Boersma, 1994).

Results

Laboratory

In order to obtain a length-independent analysis of the condition of the *Daphnia* individuals, we first analysed whether the regression lines between the natural logarithm of the length and the natural logarithm of the carbon content only varied in the intercept of the lines or also differed in slope. We found that within species there were no significant differences between the slopes among the different food levels (Table Ia). In addition, the species did not differ significantly. This allowed us to use one common slope for all species–food combinations. Since the computed values for the slopes of the length–carbon regression lines were close, and not significantly different from three—the

Table I. Slopes of the length–carbon relationship, with SE, mean slopes per species, number of animals, F values and P values for the test of homogeneity of slopes for (a) laboratory cultures and (b) field samples

Food concentration	D.galeata	D.galeata × cucullata	D.cucullata
(a)			
0.04	1.01	2.95	3.19
	(1.07)	(0.51)	(0.71)
0.13	2.14	3.25	2.15
	(0.58)	(0.48)	(0.60)
0.25	2.76	2.55	3.30
	(0.58)	(0.48)	(0.76)
0.50	2.73		2.78
	(0.50)		(0.98)
2.50	2.70	2.21	2.94
	(0.33)	0.40	(0.44)
Mean slope	2.61	2.82	2.76
	(0.19)	(0.14)	(0.22)
N	168	114	87
F	1.05	2.37	1.08
P	0.39	0.07	0.37
(b)			
Mean slope	3.02	2.87	2.53
	(0.08)	(0.10)	(0.19)
N	986	713	228
F	5.30	2.96	6.20
P	<0.001	<0.001	<0.001

theoretical value for isometric growth—a slope of three was used for all regression lines. Thus, by comparing the intercepts of the length–carbon relationships we obtained a length-independent measurement for the condition of the animals. The exponent of the value of the intercept represented the carbon content of a 1.00 mm animal, and is hereafter called the standard carbon content (SCC).

Figure 1 shows the SCC values in relation to the different food concentrations for the different species. Since *D.galeata* was cultured on a different food source from the other two species, it was not possible to differentiate between species effects and food effects in a comparison between *D.galeata* and the other two species. Consequently, it was necessary to analyse the species separately. The SCC values of *D.galeata* ($F_{4,171} = 18.48$; $P < 0.001$) and *D.galeata* × *cucullata* ($F_{3,114} = 29.88$; $P < 0.001$) differed significantly between the food levels; *D.cucullata* showed only a marginally significant food concentration effect with regard to the SCC ($F_{4,86} = 2.40$; $P = 0.056$). When *D.cucullata* and the hybrid were analysed together, a significant statistical interaction between the species effect and the food effect was found (Table II). At the lowest food level (0.04 mg C l^{-1}), the SCC of *D.galeata* × *cucullata* was significantly lower than that for *D.cucullata*. At the highest food level (2.50 mg C l^{-1}), the SCC of *D.galeata* × *cucullata* was significantly higher than that of *D.cucullata*. The clonal effect on the carbon content of the animals was small. A significant effect of the clone on the SCC was found only for *D.galeata* (Table III). There were no significant clonal effects for *D.cucullata* and the hybrid.
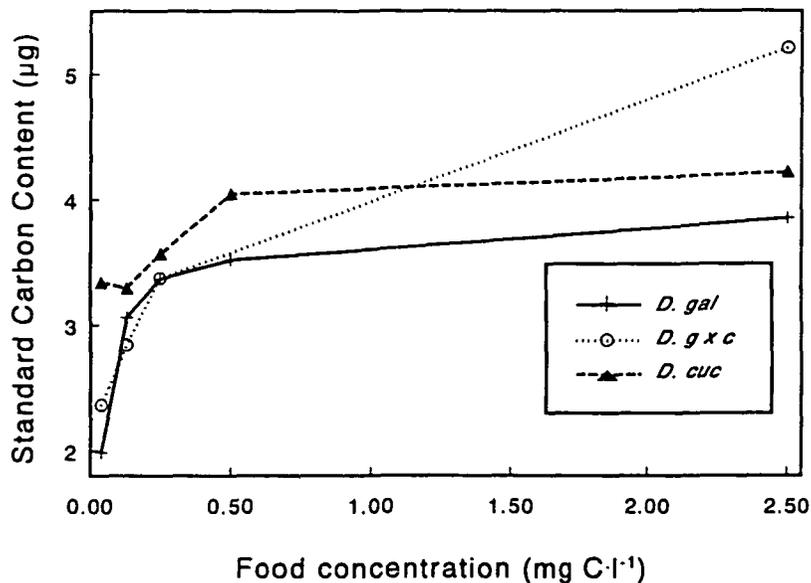

Fig. 1. SCC derived from the first four adult instars of *D.galeata* (*D.gal.* solid line, plus signs), *D.galeata* × *cucullata* (*D.g* × *c.* dotted line, circles) and *D.cucullata* (*D.cuc.* dashed line, triangles) reared in the laboratory on different food concentrations at 17.5°C.

In the experiment concerning the effect of temperature on the condition of
D.galeata and *D.galeata* × *cucullata*, we found a significant effect of tempera-
ture on the SCC values, with the lowest SCC value for the animals reared at
17.5°C (Table IV). The regression lines between length and carbon content
among the different food concentrations and species differed significantly in
slope ($F_{5,266} = 3.36$; $P < 0.01$). The effect of temperature, however, was small.
When we computed the explained variance of a regression analysis between the
log-transformed length of the animals and the log-transformed carbon content,
93% of the total variance was explained. Including the temperature as an

Table II. ANOVA table of SCC values derived from the laboratory cultures at 17.5°C, with a
common slope in the length–carbon relationship of three for *D.galeata* × *cucullata* and *D.cucullata*

Effect	Mean squares	d.f.	F	P
Species	0.18	1	2.08	0.151
Food	1.75	3	20.43	<0.001
Species × food	0.58	3	6.74	<0.001
Error	0.09	185		

Table III. ANOVA tables of clonal effects on the carbon content of (a) *D.galeata*, (b) *D.galeata* ×
cucullata and (c) *D.cucullata*, reared under laboratory conditions at different food concentrations at
17.5°C. Some clones were not represented in all food concentrations and hence were left out of this
analysis

Effect	Mean squares	d.f.	F	P
(a)				
Food	1.33	4	14.26	<0.001
Clone	1.53	4	5.62	<0.001
Food × clone	0.08	16	0.86	0.614
Error	0.09	68		
(b)				
Food	1.67	3	18.15	<0.001
Clone	0.10	3	1.05	0.375
Food × clone	0.09	9	0.93	0.501
Error	0.09	80		
(c)				
Food	0.07	4	1.07	0.384
Clone	0.01	2	0.08	0.925
Food × clone	0.09	8	1.45	0.203
Error	0.06	45		

Table IV. ANOVA table of the experiment on the effect of temperature on the carbon content of
D.galeata and *D.galeata* × *cucullata* at a food level of 2.5 mg C l^{-1}, and three different temperatures
(12.5, 17.5 and 22.5°C), with average SCC values for the two species of 4.85, 4.06 and 5.00 μg,
respectively

Effect	Mean squares	d.f.	F	P
Species	0.04	1	0.42	0.519
Temperature	1.82	2	20.01	<0.001
Species × temperature	0.05	2	0.57	0.569
Error	0.09	271		

independent factor increased the explained variance by only 1%, meaning that although temperature explained a substantial proportion of the residual variance, the increase in explained variance was negligible.

Field

Daphnia galeata was dominant in the spring of 1991, with the other two species only showing small spring peaks (Figure 2). In contrast to 1991, the spring of 1992 was dominated by *D.galeata* × *cucullata*. In total, 986 individuals of *D.galeata*, 713 *D.galeata* × *cucullata* and 228 *D.cucullata* were analysed for carbon over 31 separate sampling dates. Contrary to the length–carbon regressions made for the animals reared in the laboratory, the regression lines for the animals from the field differed significantly in slope between dates (*D.galeata*: $F_{31,922}$ = 5.3; $P < 0.001$; *D.galeata* × *cucullata*: $F_{28,655}$ = 2.96; $P < 0.001$; *D.cucullata*: $F_{12,173}$ = 6.2; $P < 0.001$) (Table Ib). This poses a problem with regard to the further analysis of the data. Three approaches are possible. (i) Ignoring the seasonal differences in the slope of the regression lines and analysing the data assuming a fixed slope. This approach could be justified by the observation that the increase in explained variance when both slopes and intercepts were estimated was small. For *D.galeata*, estimating both slopes and intercepts separately for every day yielded an explained variance of 87%. When the slope of the different regression lines was fixed to a common slope, the explained variance decreased less than 2%. For *D.galeata* × *cucullata* and *D.cucullata*, the differences were even smaller. (ii) Establishing separate length–
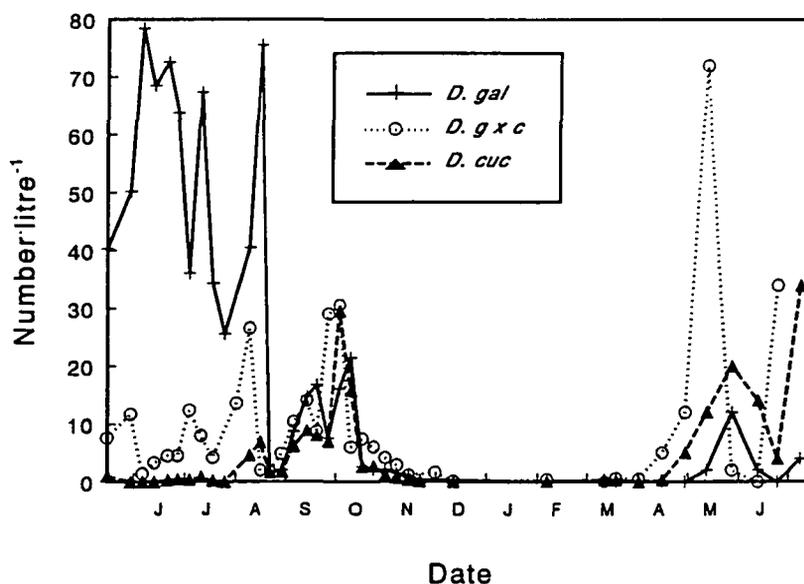

Fig. 2. Seasonal dynamics of *D.galeata* (solid line, plus signs), *D.galeata* × *cucullata* (dotted line, circles) and *D.cucullata* (dashed line, triangles) in Tjeukemeer 1991–1992.

carbon regression lines for every sampling date and species. The advantage of this approach is that no *a priori* values are assumed for the parameters of the length–carbon regression lines. The main disadvantage, however, is that the comparison between the species and between the sampling dates becomes more difficult, since two, non-independent variables have to be compared. One way to bypass this would be to choose a particular length, and to compare the carbon contents derived from the regression lines for this length. This approach would be equivalent to the computation of SEP, with the same disadvantage, i.e. the length dependence. (iii) The most direct way of analysing the data would be to select a certain size class of animals and to compute the average carbon content of these animals. Apart from the bias introduced by size differences within such a size class, the main disadvantage of such a method is the same as that of the second approach, i.e. this method is dependent on the size class taken; the conclusions drawn from these measurements are valid only for the size class which was analysed. Moreover, since in this study emphasis was put on the analysis of a wide length range of animals, the number of animals falling within a given size class was limited and therefore the standard errors were high. The three methods were compared by comparing the carbon content of 1.00 mm *D.galeata* individuals, computed using the above-mentioned approaches (Figure 3). The size class taken for the direct analysis (Method 3) was 1.00–1.25 mm, so obviously the carbon values derived from this method were higher than those derived from Methods 1 and 2. Nonetheless, the correlation coefficients
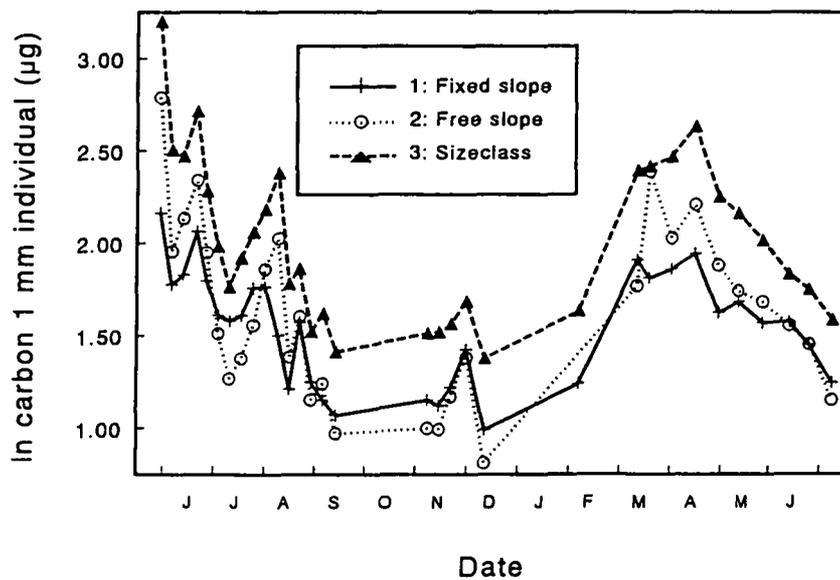

Fig. 3. A comparison of three different methods to determine the carbon content of 1.00 mm individuals of *D.galeata*. Method 1: slope fixed to 3, intercepts estimated (solid line, plus signs); Method 2: both slopes and intercepts estimated (dotted line, circles); Method 3: average carbon content of all animals between 1.00 and 1.25 mm (dashed line, triangles).

between the values for the three different approaches were high; all values were >0.9 and significantly different from zero. We observed a significant serial correlation coefficient (time lag 1) between the slopes of the length–carbon regression lines ($r = 0.53$; $P = 0.003$) as computed with Method 2. The serial correlation coefficients with longer time lags were non-significant. Moreover, we observed significant negative correlations between the carbon values computed for 1.00 mm *D.galeata* individuals and the slope of the regression lines at the time (Method 1: $r = -0.45$; $P = 0.011$; Method 2: $r = -0.67$; $P < 0.001$; Method 3: $r = -0.64$; $P < 0.001$). This means that when the condition of *D.galeata* increased, the slope of the length–carbon regression line decreased, indicating that when feeding conditions were good the smaller (= younger) animals benefited more compared with the larger animals. Since the correlation coefficients between the different approaches were high, we decided to only use the approach of the fixed slopes to analyse the field data further. Most slopes of *D.galeata, D.galeata × cucullata* and *D.cullata* did not differ significantly from three. Consequently, this slope was also used for the field data, and SCC values were calculated.

A distinct seasonal variation in the carbon content of 1.00 mm animals was found, implying drastic seasonal changes in food conditions, with good food conditions in winter and spring and low food availability in summer (Figure 4). The seasonal patterns of SCC of the three species were similar. The absolute SCC values were also close, leading to a non-significant species effect on SCC, and a non-significant interaction between sampling date and species. In other
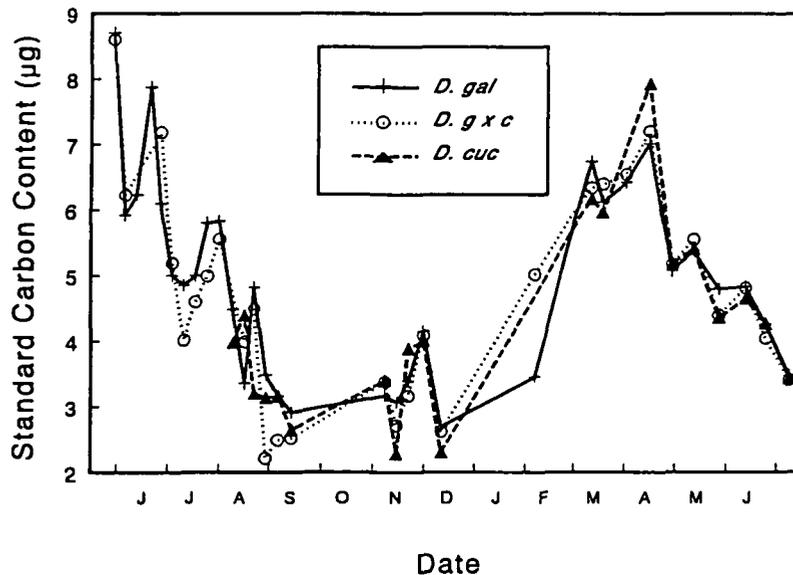

Fig. 4. Seasonal variation in SCC of *D.galeata* (solid line, plus signs), *D.galeata × cucullata* (dotted line, circles) and *D.cucullata* (dashed line, triangles) in Tjeukemeer 1991–1992.

words, on a given date a *Daphnia* individual of 1.00 mm length had a certain carbon content irrespective of the species it belonged to.

SCC values include all body tissue of the females and the eggs in the brood pouch. Since the mean number of eggs per female changes seasonally, changes in SCC may have been caused entirely by changes in egg number, and/ or by changes in individual egg weight. Figure 5 shows the seasonal changes of the carbon content of individual eggs, showing distinct changes in carbon content. The lowest values were found in the summer periods. Individual egg weight was positively correlated with SCC in *D.galeata* and in the hybrid (Table V). Owing to the low densities of this species in the field, no positive correlation could be found in *D.cucullata*. Total carbon content data, which were combined with the individual egg carbon contents, yielded the average somatic carbon content per sampling date for *D.galeata* (Figure 6a) and *D.galeata* × *cucullata* (Figure 6b). The absolute contribution of the eggs to the total carbon was relatively constant for both species. The reproductive carbon was high only in the spring/winter situations. This means that changes in SCC were mainly caused by changes in somatic carbon, and less by changes in reproductive carbon, which was confirmed by the higher correlation coefficients of somatic carbon content with SCC, compared with the correlation coefficients of reproductive carbon with SCC (*D.galeata* $r = 0.80$ and 0.41, respectively; *D.galeata* × *cucullata* 0.75 and 0.64; *D.cucullata* 0.51 and -0.02). All correlation coefficients were significant, with the exception of the last one. The SCC values were also closely related with the average length of the animals in the field (Table V). No significant correlations were found between the natural
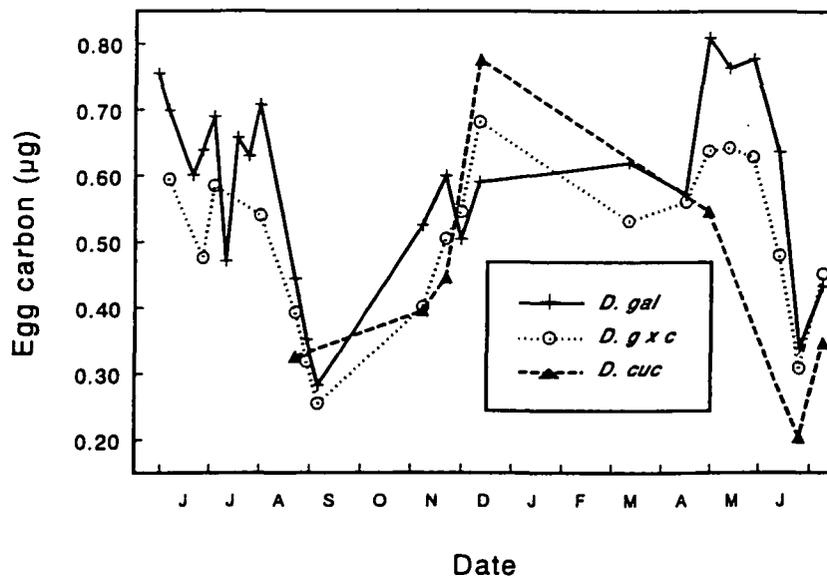

Fig. 5. Seasonal variation in egg carbon content of *D.galeata* (solid line, plus signs), *D.galeata* × *cucullata* (dotted line, circles) and *D.cucullata* (dashed line, triangles) in Tjeukemeer 1991–1992.
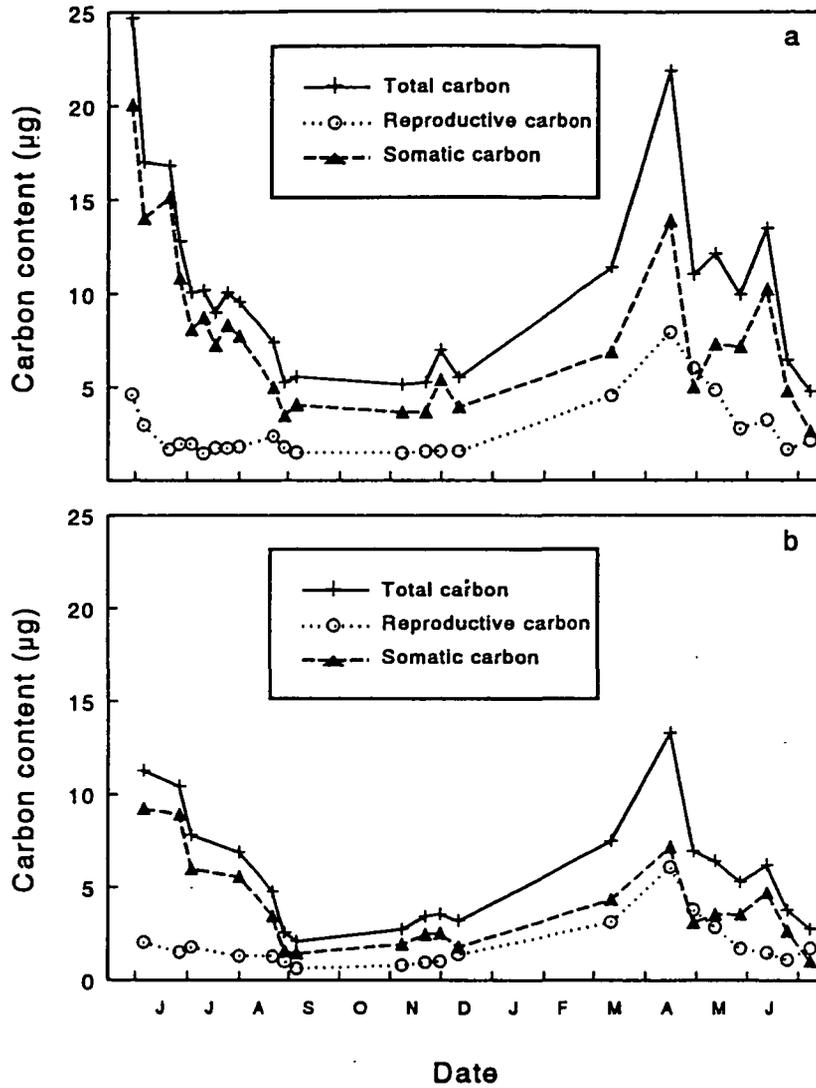
Fig. 6. Seasonal variation in total carbon (solid line, plus signs), reproductive carbon (dotted line, circles) and somatic carbon (dashed line, triangles) in Tjeukemeer 1991–1992 of (a) *D.galeata* and (b) *D.galeata* × *cucullata*.

rate of increase, *r*, as computed from the changes in numbers in the field, and the values of SCC for any of the species under consideration. In addition, neither chlorophyll content of the water nor the temperature correlated significantly with the SCC (Table V).

Field and laboratory

A comparison of the observations from the field with those from the laboratory showed that a lot of the SCC values in the field were higher than the highest

Table V. Correlation coefficients between the natural logarithm of the SCC and life history parameters in the field. Correlations with temperature and chlorophyll content of the water (*P* value)

	D.galeata	*D.galeata* × *cucullata*	*D.cucullata*
Density	0.35	0.28	−0.01
	(0.060)	(0.158)	(0.964)
Length	0.48	0.58	0.60
	(0.007)	(0.001)	(0.005)
Number of eggs	0.38	0.43	0.77
	(0.037)	(0.021)	(0.001)
Egg carbon	0.55	0.68	−0.59
	(0.005)	(0.001)	(0.160)
τ	0.18	0.05	0.34
	(0.495)	(0.843)	(0.214)
Temperature	0.11	−0.06	−0.03
	(0.557)	(0.749)	(0.917)
Chlorophyll *a*	0.41	0.28	0.49
	(0.163)	(0.357)	(0.176)

values observed in the laboratory experiments. Moreover, we found that, although for *D.cucullata* the variation in SCC values in the laboratory was small, the variation found in the field was larger (Figure 3). At first glance, our finding from the laboratory, i.e. the hybrids showing the highest variance in SCC values, was not discernible in the field data. However, when we computed the correlation coefficient between the average value of SCC of the three species observed in the field as a measurement of food concentration, and the difference in SCC values between *D.galeata* and *D.galeata* × *cucullata*, we found a significantly negative correlation coefficient ($r = -0.47$; $N = 28$; $P < 0.013$). Thus, although in the field the SCC values of *D.galeata* and *D. galeata* × *cucullata* were similar, and not significantly different, it can be concluded that at high food concentrations the SCC of *D.galeata* × *cucullata* was higher than that of *D.galeata*.

Discussion

This paper shows that the method for determining food limitation by estimating the intercepts from the length–carbon regression lines, as was proposed by Duncan (1985, 1989), has its flaws. Regression lines for animals grown at different food concentrations may be parallel under controlled laboratory circumstances with only one food source given in different concentrations, or even for animals taken from the field at different depths at one moment in time (Duncan *et al.*, 1993), but our analysis of seasonal data yielded different results. As yet, not many authors have considered changes in length–weight relationships over time. We, however, have found significant differences in the slopes of the length–carbon regression lines throughout the year, as did Geller and Müller (1985) and Hessen (1989). We observed a serial correlation between the slopes of the regression lines, and a negative correlation between the carbon contents of 1.00 mm animals and the slope of the regression lines. This indicates that the

slope differences were not created by chance alone. Our results suggest that when food was plentiful, and hence the condition of the animals good, the condition of larger animals was relatively bad as compared with smaller animals. When the condition of the animals was bad, the regression lines were steeper. Hence, when food levels were lower large animals had relatively good conditions. Geller and Müller (1985) and Hessen (1989) reported similar slope patterns. Although fixing the slope of the length–carbon regression lines to a value of three inevitably caused information loss, the explained variance decreased only slightly. The benefits of this fixing of the slope were high, because it permitted a direct comparison independent of the length of the animals. Moreover, unlike the lipid index (Sterner *et al.*, 1992), we observed significant positive correlations between the intrinsic rate of increase, *r*, and the SCC in the laboratory cultures for *D.galeata* ($r = 0.97$; $N = 5$; $P = 0.007$), and for *D.galeata* × *cucullata* ($r = 0.98$; $N = 4$; $P = 0.021$). The correlation between SCC and *r* in *D.cucullata* was not significant ($r = 0.75$; $N = 5$; $P = 0.14$), but also positive. Hence, under laboratory conditions the SCC is a good indicator of the potential growth rate of a particular species. The average length of the adult animals in the population was also closely correlated with the SCC on a given date, which suggests that the reduction in mean adult length was caused by food limitations rather than by other factors, such as positive size-selective predation by fish (e.g. Tessier *et al.*, 1992). Moreover, we found a positive correlation between egg number and SCC, as did Duncan (1985) and Hessen (1989). Although the correlation of somatic carbon content with SCC was higher than the correlation of reproductive carbon content with SCC, animals had more eggs when the SCC was high.

The carbon content of *Daphnia* individuals varied substantially in the field. The average carbon content of a 1.00 mm individual of both *D.galeata* and *D.galeata* × *cucullata* varied by a factor of 2–3 within a 3-month period. Hessen (1989) also found seasonal variations in carbon content equivalent to a factor of two and Duncan (1985) reported a factor of three. The large changes in carbon content suggest that the food conditions for *Daphnia* deteriorate severely during certain parts of the year. Alternatively, the changes in carbon content could also have been caused by changes in the clonal structure of the population, since in laboratory experiments we showed clonal differences in carbon content for *D.galeata* (see also Glazier and Calow, 1992). These differences were smaller than the differences in SCC values found in the field (Table III). It is, however, not likely that a change in clonal composition of the *Daphnia* population caused the change in carbon content of the animals, since a similar pattern in the SCC values was found in all three species, and neither the hybrid nor *D.cucullata* showed differences in SCC between clones in the laboratory. Moreover, judging by the enzyme markers, the clonal composition of *D.galeata* was fairly constant during the season (Spaak, 1994).

In poikilotherms, the efficiency of the food assimilated to body tissue usually decreases with increasing temperature. One would therefore expect animals with lower SCC values at higher temperatures (Duncan, 1985). In our laboratory cultures, however, we observed the lowest SCC values at intermediate (17.5°C)

temperatures. Moreover, the correlation between the SCC and the ambient water temperature in the field was not significant. It is unlikely that no relationship exists at all between the temperature and the SCC, but rather that the absence of a significant correlation between SCC and water temperature in the field suggests that this relationship is not a simple linear one. We do, however, not expect the direct effect of temperature on SCC to be large since under laboratory conditions the added explained variance by temperature was small.

In our field observations, the maximum SCC values found were higher than those observed in the laboratory cultures. This means that one of the original goals of this study, i.e. to estimate the effective food concentration for the animals in the field, has become difficult to achieve, as apparently feeding conditions in Tjeukemeer are at times so good that these conditions are impossible to reach under laboratory circumstances with mono-algal diets. It is unlikely that an increase in the food concentration in our experiments would have yielded higher values for SCC, since between 0.25 and 2.5 mg C l^{-1} the SCC hardly increased for *D.cucullata* and *D.galeata*. We must conclude, therefore, that although the lake seston was dominated by the unfavoured cyanobacteria for the largest part of the year, during the winter months the food quality was apparently very high. This could possibly be caused by a high density of the more favoured diatoms and Cryptophyceae (Moed and Hoogveld, 1982). From the data on the seasonal changes in the SCC values, we also conclude that if it is assumed that an SCC value >7 µg C means that the animals are not food limited, then the *Daphnia* individuals are food limited during the largest part of the year, even in highly eutrophic Tjeukemeer. Particulate carbon in Tjeukemeer is known to vary between 6 and 10 mg C l^{-1} (Gulati, 1975). If we assume a daily ration for *Daphnia* of 125% (Gulati *et al.*, 1982), an average carbon content of the animals of 10 µg C and 80 daphnids l^{-1}, the amount of carbon consumed per day would be 1.0 mg. This suggests that indeed a substantial amount of the carbon present is not available as food for the *Daphnia* species.

Although variable over time, the field SCC values for the three *Daphnia* species were remarkably similar, suggesting that the food for these three species was similar under natural conditions, or that the food sources for the different species were positively correlated. Duncan (1985) also found small differences between *D.pulicaria* and *D.thorata* in seasonal changes in carbon content in Lake Washington, as did Berberovic (1990) for *D.hyalina* and *D.galeata* in Lake Constance. Thus, it is difficult to attribute the changes in relative abundance of the three species in Tjeukemeer in the years under study to fluctuations in food concentration, although changes in food concentration seem to be important for growth and reproduction in the field. In the years 1991–1992, the differences in timing of peak densities in the field were small compared with other years (Spaak and Hoekstra, 1994) and, hence, one could speculate that differences in SCC values between the species would have been larger in years with distinctly different seasonal peaks. The hybrids seem to have a higher SCC value when food concentrations are high, and hence one would expect a dominance of hybrids under high food conditions, i.e. if food were the only determining factor.

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In conclusion, individuals of *D.galeata*, *D.galeata* × *cucullata* and *D.cucullata* show distinct seasonal patterns in condition. This reveals food limitations for daphnids in the field during the largest part of the year, even in a very eutrophic lake.

Acknowledgements

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