Copepod life cycle adaptations and success in response to phytoplankton spring bloom phenology

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Abstract

In a seasonal environment, the timing of reproduction is usually scheduled to maximize the survival of offspring. Within deep water bodies, the phytoplankton spring bloom provides a short time window of high food quantity and quality for herbivores. The onset of algal bloom development, however, varies strongly from year to year due to interannual variability in meteorological conditions. Furthermore, the onset is predicted to change with global warming. Here, we use a long-term dataset to study (a) how a cyclopoid copepod, *Cyclops vicinus*, is dealing with the large variability in phytoplankton bloom phenology, and (b) if bloom phenology has an influence on offspring numbers. *C. vicinus* performed a two-phase dormancy, that is, the actual diapause of fourth copepodid stages at the lake bottom is followed by a delay in maturation, that is, a quiescence, within the fifth copepodid stage until the start of the spring bloom. This strategy seems to guarantee a high temporal match of the food requirements for successful offspring development, especially through the highly vulnerable naupliar stages, with the phytoplankton spring bloom. However, despite this match with food availability in all study years, offspring numbers, that is, offspring survival rates were higher in years with an early start of the phytoplankton bloom. In addition, the phenology of copepod development suggested that also within study years, early offspring seems to have lower mortality rates than late produced offspring. We suggest that this is due to a longer predator-free time period and/or reduced time stress for development. Hence, within the present climate variability, the copepod benefited from warmer spring temperatures resulting in an earlier phytoplankton spring bloom. Time will show if the copepod's strategy is flexible enough to cope with future warming.

Keywords: climate change, *Cyclops vicinus*, diapause, match–mismatch, maturation delay, phenological adaptation, photoperiod, reproductive success, time stress, timing

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Introduction

The life cycle of many species incorporates either a dormancy or a migration period to cope with seasonally harsh conditions (Tauber *et al.*, 1986; Alekseev *et al.*, 2007). Such taxa are thought to be especially vulnerable to climate change (Durant *et al.*, 2007) as possible changes in the cues used for breaking diapause or back-migration from overwintering habitats may differ from the phenological changes in the breeding season and habitat. For example, a long-distance migratory passerine, the pied flycatcher *Ficedula hypoleuca*, experienced population declines during the last decades presumably because it was not able to adjust its breeding phenology and arrival from winter grounds, respectively, to an earlier appearance of its food (Both *et al.*, 2006). A mismatch might also occur if a temperature change in the overwintering habitat results in too early remigration into the breeding habitat (Inouye *et al.*, 2000). Likewise, advanced emergence of the marmot *Marmosa flaviventris* from dormancy due to warmer temperatures resulted in a mismatch because the timing of snow melting has not advanced accordingly (Inouye *et al.*, 2000).

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A dormancy period is also widespread within zooplankton (Alekseev *et al.*, 2007). Many species among the cladocerans, copepods, rotifers, and ciliates are known to bridge seasonally unfavourable conditions with a dormancy period. Dormancy in zooplankton is distinguished in quiescence, that is, a phase of retarded development allowing a fast response to changing environmental conditions, and the actual diapause, that is, a period of 'deep sleep', which once initiated is only terminated by certain intrinsic and/or environmental stimuli. Zooplankton use a variety of environmental cues such as temperature (Marcus, 1982), photoperiod (Hairston & Kearns, 1995), density (Kleiven *et al.*, 1992), predator kairomones (Slusarczyk, 1995), and food quality (U. Koch *et al.*, unpublished results) to optimize the timing of dormancy initiation and/or termination. In addition also, maternal effects might be important for the production of diapausing stages (LaMontagne & McCauley, 2001).

Copepods have a complex life cycle with six naupliar stages (N I to N VI), five copepodid stages (C I to C V), and an adult stage with a metamorphosis from the naupliar towards the copepodid stages. Diapause in copepods can occur through the production of resting eggs or due to an interruption of development usually within an advanced copepodid stage (C IV or C V) (Santer, 1998). Despite their global distribution (Humes, 1994) and central position in aquatic food webs, there are no studies, which address the consequences of diapause for copepod fitness in the context of climatic warming or climatic variability.

The freshwater cyclopoid copepod *Cyclops vicinus* is a common member of the zooplankton community in many European lakes (Einsle, 1993). In most of these lakes, the copepod disrupts ontogenetic development within C IV in early summer to perform a diapause. Summer diapause of *C. vicinus* has been suggested to be ultimately due to the dependency of herbivorous nauplii on a high food availability, which is only warranted during the phytoplankton spring bloom, but not during the summer season (Santer & Lampert, 1995). However, high predation pressure during summer has also been discussed as the ultimate cause for the summer diapause of *C. vicinus* (Maier, 1989). The timing of diapause initiation has been suggested to be controlled by photoperiod (Einsle, 1964; Spindler, 1971; George, 1973) and diapause is carried out in the sediment at the bottom of the lake. The strategy to avoid unfavourable conditions thus is a combination of diapause and migration. Especially in deep lakes, this is not without problems as environmental conditions at the bottom of the lake do not provide reliable cues about the conditions at the surface (Straile *et al.*, 2007), that is, on when it is best to terminate diapause. Facing this challenge, individuals

of C IV re-emerge from diapause during winter and complete their development.

In many lakes, the timing of the phytoplankton spring bloom is highly variable and may differ interannually up to 2 months depending on meteorological forcing (Adrian *et al.*, 1999; Peeters *et al.*, 2007a) and nutrient concentration (Thackeray *et al.*, 2008). Given the dependency of successful naupliar development on the algal spring bloom (Santer & Lampert, 1995), this variability poses two major problems for the copepod: (1) diapause termination needs to be well in advance to the onset of the phytoplankton bloom in order to allow for a timely maturation and reproduction to finally ensure that naupliar hatching matches with the phytoplankton bloom, and (2) as diapause induction is primarily controlled by photoperiod, high variability in phytoplankton bloom timing results into a high variability in the time window available for the copepod to develop towards the diapausing stage (C IV). Especially, during years with a late phytoplankton bloom, the shortened time span might result into time stress.

Here, we use a long-term dataset to study the life-cycle strategy of *C. vicinus* in a deep Central European lake with a high variability in phytoplankton bloom phenology (Peeters *et al.*, 2007b). We study the phenology of diapause re-emergence and reproduction of this copepod in response to algal bloom timing. In addition, we analyse the influence of the phenology of reproduction on reproductive success, that is, offspring survival of *C. vicinus*.

Methods

Study site and sampling

The study was done in deep ($z_{max} = 254$ m), large (472 km^2), and warm-monomictic Upper Lake Constance located in southwestern Germany. During the last decades, the lake was influenced by anthropogenic changes in trophic status, that is, a period of eutrophication until the late 1970s and a period of oligotrophication until today (Fig. 1; Bäuerle & Gaedke, 1998; Güde *et al.*, 1998), as well as climate variability (Straile *et al.*, 2003). Both forcing factors were shown to influence zooplankton of Lake Constance in manifold ways resulting in changes of species abundances and phenologies (Straile & Geller, 1998; Straile, 2000; Seebens *et al.*, 2007).

Zooplankton and phytoplankton were sampled fortnightly from 1970 to 1995 in the centre of Upper Lake Constance (main station, depth = 250 m). We excluded 3 years (1970, 1987, and 1995) from further statistical analysis as these years contained large gaps of more than 1 month. The sampling methodology for zooplank-
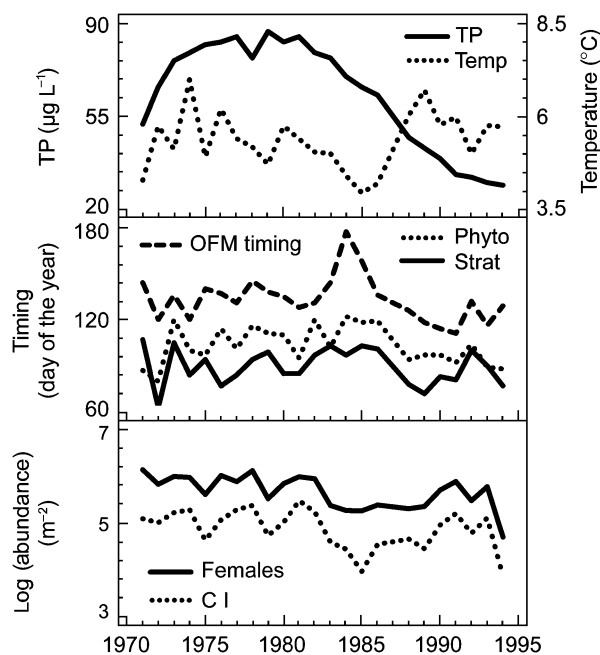
Fig. 1 Time series of April water temperature (Temp), total phosphorus concentration (TP) (upper panel), the timing of the onset of stratification (Strat), start of phytoplankton growth (Phyto), and peak timing of ovigerous females (OFM) (middle panel), as well as abundances of females and offspring (C I) of *Cyclops vicinus* (lower panel).

ton is described in more detail in Seebens *et al.* (2007). All five copepodid stages, males, females, and ovigerous females (OFM) of *C. vicinus* were distinguished. Abundances of *C. vicinus* nauplii were not determined, as they can hardly be distinguished from nauplii of other cyclopoid species. Zooplankton was additionally sampled during 14 years (1972–1985) with the same temporal and ontogenetic resolution within the Bay of Bregenz, at a shallow part of Upper Lake Constance ($z_{max} \approx 60$ m). Phytoplankton was counted from integrated samples of the upper 20 m of the water column (Kümmerlin, 1998) but only at the main station. The trophic status of the lake in a specific year was represented by the maximum total phosphorus (TP) concentration during winter (February, March) mixing. Water temperatures were measured monthly at distinct depths (0, 5, 10, 15, 20, 30, 50, 100, 200, 250 m) during the whole study period. As *C. vicinus* was found at depths from 0 to 20 m during spring, we used the average temperature of the upper 20 m. In order to determine the start of stratification, Schmidt's stability index (Schmidt, 1928) was calculated for all temperature profiles. This index is a measure for the amount of mechanical work necessary to homogenize the water column ($g\,cm\,cm^{-2}$). The index value increases with stratification from a minimum value of zero at isother-

mal conditions. The onset of stratification in spring was defined at the arbitrary threshold of $200\,g\,cm\,cm^{-2}$, which was exceeded on average in early April. The choice of this threshold did not significantly influence the overall results as the timing of exceeding the threshold of, for example, 200 and $100\,g\,cm\,cm^{-2}$ were highly correlated ($r = 0.83$, df $= 25$, $P < 0.0001$).

Statistical analysis

Except for the determination of abundance maxima, all abundances were log-transformed for further statistical analyses in order to reduce the influence of extreme values. The annual peak timing of *C. vicinus* stages and phytoplankton biomass were captured by fitting Weibull distributions to the abundance and biomass curves, respectively, to generate distinct single peaks (Fig. 2). Using Weibull fits revealed similar peak timings as compared with simply choosing the timing of maximum abundance without a fit ($r = 0.93$, df $= 137$, $P < 0.0001$). However, Weibull fits integrate over sampling intervals and therefore produce more realistic peak timings (Fig. 2). The onset of phytoplankton growth was approximated by using the first 20% quantil of the Weibull fit on phytoplankton biomass. The Weibull fits were applied to phytoplankton biovolume from February until June to include all relevant phases of the spring bloom, that is, onset, growth, maximum, and decline. The Weibull fitting procedure is easily applicable to single maximum curves but fails when more then one peak must be fitted within the same year. This was the case only for C IV of *C. vicinus* as two cohorts appear and may even overlap somewhat in specific years. Here, we separated both cohorts (in the following C IV$_1$ and C IV$_2$) at the time between their two peaks when abundance was lowest.

To analyse the effects of the breeding phenology on offspring performance, we first compared the mean population development within those 8 years ($\approx 1/3$ of all years), which showed the earliest and those 8 years which showed the latest maxima of OFM (in the following early and late OFM years, respectively). To remove a potential influence of TP on seasonal dynamics of *C. vicinus* abundances and phytoplankton biovolumes, we subtracted from the original time series nonlinear regression fits (LOESS) of abundances (biovolume) vs. TP applied to each biweek separately. To restore the typical seasonal dynamics, the grand mean of copepod abundances (phytoplankton biovolume) in the individual biweeks were then added to the residuals.

Tests for trends in state variables were conducted using the nonparametric Mann–Kendall test. The interrelationships between developmental stages and the
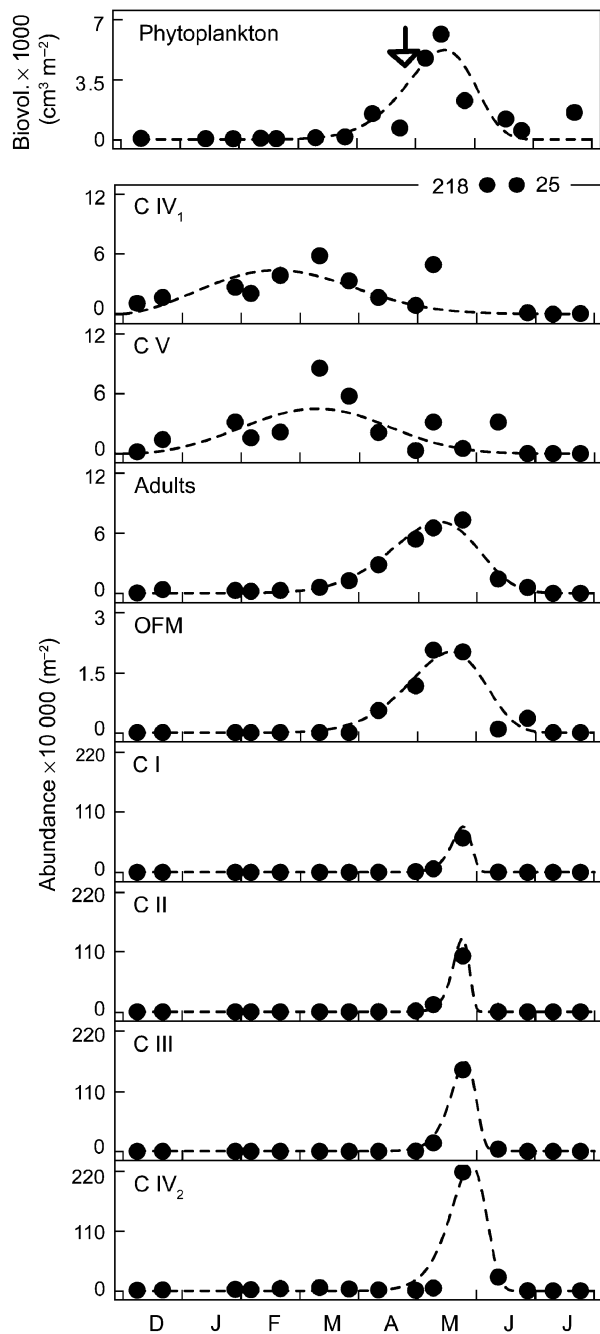
Fig. 2 Phytoplankton development and life cycle of *Cyclops vicinus* in 1976 (December 1975 to July 1976). Dots represent phytoplankton biovolume and abundance of copepodid stages (C IV_1 and C V), adults, ovigerous females (OFM), and offspring copepodid stages (C I, C II, C III, and C IV_2). Dashed lines represent Weibull fits. The arrow shows the 20% quantil of phytoplankton spring development. Numbers in panel C IV_1 indicate high abundances of CIV_2 copepodids beyond the scale of this panel. See panel CIV_2 for a plot of these abundances and note differences in *y*-axes.

copepod responses to environmental factors were analysed with Pearson correlations and Pearson partial correlations. We checked variables for nonnormality and collinearity, that is, near-linear dependencies among variables, using Shapiro–Wilks test and variance inflation factor, respectively (Belsley *et al.*, 1980). A comprehensive model predicting the peak timing of OFM and abundance of offspring was tested with path analysis. Path analysis allows investigating complex multivariate causal models based on path diagrams and tests whether a specific model, represented by this path diagram, is an acceptable description of the underlying causal mechanisms (Grace, 2006). Performing this method, we were able to test a comprehensive model, which could formerly only be assessed for each dependent variable separately. Based on a covariance–variance matrix including all parameters, path analysis calculates path coefficients, which are the same as standardized partial regression coefficients from multiple regression.

The goodness-of-fit of path models was measured by using different tests and indices, in particular χ^2 test, Comparative Fit Index (CFI), root mean square error of approximation (RMSEA), and Hoelter's Critical N (Belsley *et al.*, 1980; Bollen, 1989). The latter represents the sample size, which would result in a significant χ^2 test. Note that a nonsignificant χ^2 test indicates a good fit. Additionally, the total R^2 for the whole model is shown. A model, which results in a nonsignificant χ^2 test, a CFI above 0.95, and RMSEA less than 0.06, is generally regarded as a good description of the data (Hu & Bentler, 1999). Data analysis was performed using SAS software (SAS Institute Inc., 2004) and R (R Development Core Team, 2008).

Results

TP concentrations increased from 52 towards 87 μg L^{-1} within the 1970s and declined to 29 μg L^{-1} at the end of the study period. April water temperatures of Lake Constance did not show a significant trend during the study period (Mann–Kendall $\tau = 0.02$, $P > 0.05$). Likewise, the timing of, for example, onset of stratification ($\tau = -0.1$, $P > 0.05$), onset of the phytoplankton bloom ($\tau = -0.11$, $P > 0.05$) and peak timing of OFM ($\tau = -0.26$, $P > 0.05$) showed no long-term trend but high interannual variability. Mean abundances of females ($\tau = -0.43$, $P < 0.01$) but not of CI copepodids ($\tau = -0.27$, $P > 0.05$) decreased during the study period (Fig. 1).

The life cycle of *C. vicinus* is exemplarily shown for 1 year (1976) in Fig. 2. Individuals of the diapausing stage (C IV_1) reappeared in the pelagic zone mainly during winter and reached the adult stage in April shortly after the start of the phytoplankton bloom (Figs 2 and 3). The
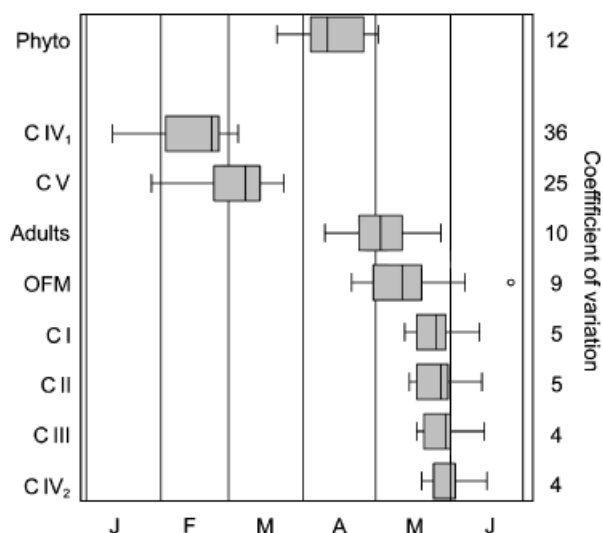
Fig. 3 Timing of the start of the phytoplankton spring bloom and of maximum abundance of *Cyclops vicinus* stages [copepodid stages (C IV$_1$ and C V), adults, ovigerous females (OFM), and offspring copepodid stages (C I, C II, C III, and C IV$_2$)] of all years ($n = 23$) indicated as box-plots. Variability of peak timing is shown as coefficients of variation. 1984 was removed from the calculation of coefficients of variation as the timing of reproduction was determined after occurrence of offspring (outlier of OFM).

Table 1 Pearson correlation coefficients between peak timing of *Cyclops vicinus* stages [copepodid stages (C IV$_1$ and C V), adults, ovigerous females (OFM), and offspring copepodid stages (C I, C II, C III, and C IV$_2$)] within the different study years ($n = 23$)

	C V	Adults	OFM	C I	C II	C III	C IV$_2$
C IV$_1$	0.76****	−0.10	0.19	0.28	0.29	0.27	0.12
C V		−0.02	0.23	0.32	0.30	0.34	0.26
Adults			0.71****	0.60***	0.55**	0.60***	0.73****
OFM				0.78****	0.76****	0.84****	0.82****
C I					0.97****	0.91****	0.81****
C II						0.94****	0.81****
C III							0.91****

*$P < 0.05$
**$P < 0.01$
***$P < 0.005$
****$P < 0.001$.

reproductive period was restricted to the spring bloom in April/May and offspring developed quickly to C IV$_2$, which entered diapause again in late May/early June. There was no change in this general pattern during the study period. On average, the maximum abundance of re-emerging C IV$_1$ was on the 14 February (Fig. 3), soon followed by the maximum abundance of C V (4 March). However, the average timing of the maximum abundance of adults and OFM was more than 2 months delayed (3 May and 13 May, respectively). Maximum abundances of next generation copepodids occurred on average from mid-May onwards with successive ontogenetic stages closely following each other: C I: 25 May, C II: 26 May, C III: 28 May, and C IV$_2$: 31 May. The interannual variability of peak timing decreased with ongoing ontogenetic development as indicated by the declining trend of coefficients of variation (c_v) from stage to stage (Fig. 3) and the negative correlation between c_v and mean peak timing of developmental stages ($r = -0.99$, df $= 7$, $P < 0.0001$). The late winter peak of C IV$_1$ and C V exhibited a variability of peak timing 3.6, respectively, 2.5 times higher as observed for the adult stage and this in turn was twice as variable as offspring (Fig. 3). Thus, within the ontogenetic development there seemed to be two major declines in variability of peak timing: from C V towards adults, and from OFM towards C I (Fig. 3).

Assuming a straightforward ontogenetic development, we would expect that more advanced individual stages follow their precursor stages within a regular time schedule, for example, an early occurrence of C IV$_1$ in a specific year will also result in an early occurrence of C V, while a late C IV$_1$ occurrence will also result in a late C V occurrence. That is, there should be a high covariation between the phenologies of different developmental stages. This was indeed observed: the timing of maximum abundances of re-emerging C IV$_1$ copepods was significantly related to the timing of the abundance of the following developmental stage ($r = 0.76$, df $= 22$, $P < 0.0001$; Table 1). Also, the timing of the occurrence of the adult maximum was highly related to the timing of the maximum abundances of the offspring copepodids (Table 1). A notable exception, however, was the relationship between peak timing of C V and adults: it was not possibly to predict the timing of maximum adult abundances from the timing of maximum C V abundances ($r = -0.02$, df $= 22$, $P > 0.1$) even after potential outliers were removed (studentized residuals > 2). Hence, moulting into the adult stage and consequently the timing of reproduction seemed to be specifically triggered by environmental cues. Both, water temperature and the onset of phytoplankton growth were highly correlated with the timing of maximum abundances of OFM and their offspring stages (Table 2). For the latter, however, these relationships were mostly due to the influence of temperature and/or phytoplankton bloom timing on female reproduction: after accounting for the effects of the timing of reproduction in a partial correlation, effects of water temperature and phytoplankton bloom timing were not any more significantly related to the timing of maximum offspring abundances. This is supported by the fact that

Table 2 Pearson correlation coefficients between peak timing of *Cyclops vicinus* stages [copepodid stages (C IV$_1$, and C V), adults, ovigerous females (OFM), and offspring copepodid stages (C I, C II, C III, and C IV$_2$)] and environmental parameters

	T_A	Phyto	T_A (par)	Phyto (par)
C IV$_1$	−0.18	0.33		
C V	−0.31	0.30		
Adults	−0.37°	0.46*		
OFM	−0.71****	0.61***		
C I	−0.65****	0.64***	−0.24	0.34
C II	0.63***	0.62***	−0.23	0.30
C III	−0.75****	0.60***	−0.44*	0.20
C IV$_2$	−0.68****	0.56**	−0.30	0.15

par, Pearson partial correlation coefficients between parameters while holding the influence of the peak timing of OFM constant. For further details see text.
T_A, April water temperatures; Phyto, onset of phytoplankton growth in spring.
°$P < 0.1$
*$P < 0.05$
**$P < 0.01$
***$P < 0.005$
****$P < 0.001$.

the timing of offspring stages were more strongly related to water temperature in April (Table 2) and not to May temperatures (e.g., peak timing of C I: $r = 0.29$, df = 22, $P = 0.17$), the month of their main occurrence (Fig. 3). Correlations with TP – either as single factor or in combination with temperature and onset of phytoplankton growth – revealed no consistent and significant pattern on peak timing (not shown) indicating that the changes in trophic status did not change the phenology of the copepod. At the Bay of Bregenz, adults and OFM reached their maximum abundances 22, respectively, 16 days earlier than at the main station (Table 3), whereas no significant differences were found in the peak timing of copepodid stages.

On average, early and late OFM years differed slightly in vernal warming during March and April (Fig. 4a). A higher algal biovolume during April in early OFM years indicated earlier stratification in these years as compared with late OFM years (Fig. 4b). However, algal biovolume in early and late OFM years did not differ significantly after April. Also, the start of the clear-water phase in early June did not differ between the 2-year groupings. Early OFM years showed higher abundances of OFM in April, whereas late OFM years higher ones in May/June (Fig. 4c). However, the average abundance of OFM from March to June did not differ between early and late OFM years

Table 3 Peak timing (day of the year, mean, and standard deviation) of *Cyclops vicinus* stages [copepodid stages (C IV$_1$, and C V), adults, ovigerous females (OFM), and offspring copepodid stages (C I, C II, C III, and C IV$_2$)] at the main site and the Bay of Bregenz and differences in peak timing between sampling sites (ANOVA)

	Mean peak timing		ANOVA		
	Main station	Bay of Bregenz	F	df	P
C IV$_1$	47 ± 15.0	40 ± 20.7	1.1	25	ns
C V	64 ± 15.1	49 ± 29.5	2.8	25	ns
Adults	131 ± 8.6	109 ± 9.4	39.7	25	<0.0001
OFM	140 ± 14.4	124 ± 7.2	12.3	25	<0.005
C I	147 ± 8.1	145 ± 8.6	0.5	27	ns
C II	149 ± 8.2	147 ± 8.6	0.3	27	ns
C III	151 ± 7.1	151 ± 7.6	0	27	ns
C IV$_2$	153 ± 6.4	153 ± 7.0	0	27	ns

Only those years were included for which measurements at both sites were available ($n = 13$ for C IV$_1$ to OFM and $n = 14$ for C I to C IV$_2$). ns: $P > 0.05$.

($r = -0.19$, df = 22, $P = 0.40$). Abundances of copepodids differed significantly between early and late OFM years but, however, only during the build-up phase of the respective stages (Fig. 4d–g).

As a consequence, average C I abundances during the spring bloom (April–June) were significantly related to OFM timing (Table 4). This was the case for the main station (model I) and when considering both stations together (model II). TP did not contribute significantly to the two models as the average number of females was also included in the models. The number of females also captures the long-term dynamics of the copepod due to changes in lake trophy, but additionally interannual variability of copepod abundances and thus competes successfully for the variability in C I abundances also explained by TP. When excluding the number of females from the model, TP is a significant predictor (models not shown). The abundance of females also accounts for the reproductive potential of the copepods in a specific year. Consequently, the negative effect of OFM timing on C I abundances suggests a reduced realization of this potential, either due to reduced productivity of offspring (smaller clutch sizes or number of clutches) or due to offspring mortality. Model II suggests significant differences in C I abundance (higher abundance at the Bay of Bregenz) even after accounting of female abundances and OFM timing. Note, that the estimates for the effect of OFM timing on C I abundances were similar in both models, suggesting higher abundances of C I at the Bay of Bregenz partially due to earlier OFM timing.

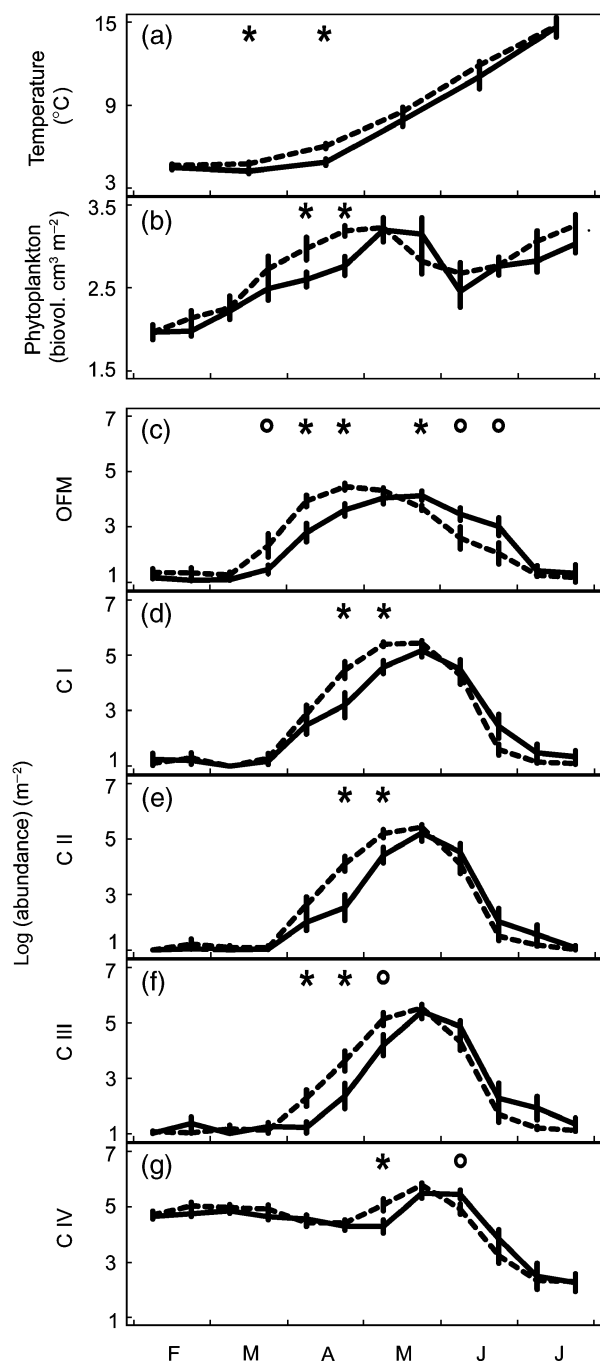The potential mechanisms underlying the timing of the maximum of OFM and abundance of offspring at

Fig. 4 Mean spring development of temperature (a), biovolume of phytoplankton (b), abundance of *Cyclops vicinus* stages (c–g) [(c) ovigerous females (OFM), and (d–g) offspring copepodid stages (C I, C II, C III, and C IV)] in years associated with an early peak timing of OFM (dashed line) ($n = 8$) and years of late peak timing (solid line) ($n = 8$). Bars indicate one standard deviation of the mean. The potential influence of total phosphorus (TP) on the dynamics of algae and *C. vicinus* stages within the individual biweeks was removed by subtracting LOESS fits. For further details see text. Significant differences of state variables between early vs. late OFM years are indicated by 'o' ($P < 0.1$) and '*' ($P < 0.05$), respectively.

Table 4 Linear models relating average abundance of *Cyclops vicinus* C I in spring (April–June) to female abundance ($A_{females}$), timing of ovigerous females (T_{OFM}), and total phosphorus (TP) at the main station (Model I) and at both stations (Model II)

	Estimate	*t*-value	*P*	R^2
Model I				
$A_{females}$	1.030	9.33	<0.0001	0.90
T_{OFM}	−0.100	−2.33	<0.05	
TP	0.001	0.69	ns	
Model II				
$A_{females}$	1.150	11.8	<0.0001	0.89
T_{OFM}	−0.100	−2.51	<0.05	
TP	0.001	0.67	ns	
Site	0.160	2.29	<0.05	

Model II additionally includes sampling site (Site) as a categorical variable to account for site differences. R^2 is given for each model. ns: $P > 0.05$.
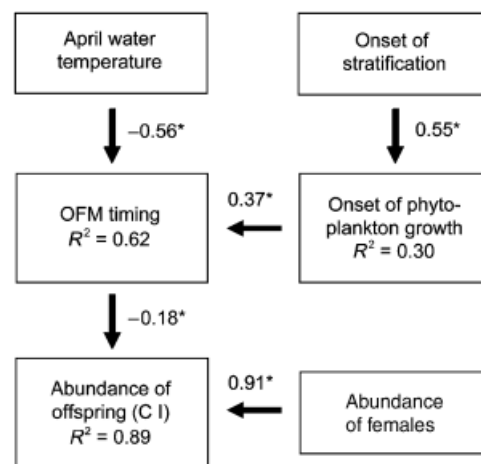

Fig. 5 Path diagram summarizing *a priori* assumptions of the potential underlying mechanisms determining the timing of reproduction of *Cyclops vicinus* and the abundance of offspring. Arrows indicate unidirectional relationships whereas their strength is shown as path coefficients (*$P < 0.05$) estimated by path analysis. For dependent variables, R^2 is given. Several goodness-of-fit statistics calculated for the overall model [e.g., $\chi^2 = 2.0$, df $= 7$, $P = 0.96$, $R^2 = 0.95$, Comparative Fit Index (CFI) $= 1.0$, root mean square error of approximation (RMSEA) < 0.001, Hoelter's $N = 159$] indicate that this model is an adequate description of the data. For further details see text. OFM, ovigerous females.

the main station were summarized in a path diagram (Fig. 5). All goodness-of-fit indices (see legend to Fig. 5) indicated a good fit of the path analysis model. Moreover, despite its simplicity the model was able to describe a large amount of the variability of the data ($R^2 = 0.95$) and all estimated path coefficients signifi-

cantly differed from zero. Sixty-two percent of the variability of OFM timing could be explained by temperature and onset of phytoplankton growth. The latter was significantly related to the onset of stratification. Finally, OFM timing and abundance of females determined C I abundances ($R^2 = 0.89$), that is, the path analysis suggests a strong effect of timing of reproduction on abundance of offspring even after including the overall abundance of females.

Discussion

The study period was characterized by a long-term change in nutrient concentrations and a high interannual climatic variability. The latter resulted in, for example, a $3\,^{\circ}C$ range in mean April temperatures and a range in the timing of the onset of stratification and algal growth of 44, respectively, 42 days. However, there was neither a trend in April temperatures nor in stratification or bloom timing during the study period. While the latter holds also when considering a longer time period (Peeters *et al.*, 2007a), spring water temperatures in Lake Constance showed an increasing trend from the 1960s towards recent years (Straile *et al.*, 2003). Because of the absence of any water temperature or stratification timing trend during our study period, changes in meteorological forcing are unlikely the cause for the long-term decrease in *C. vicinus* abundances. Rather, this decrease seems to be caused by an increase of predation pressure on copepods during spring, apparently affecting all copepod species present during this time of the year (Seebens *et al.*, in preparation).

The focus of this study is the response of *C. vicinus* phenology and reproductive success to phenological variability of its food availability. In several studies of copepod populations, the naupliar stage has been considered as a population bottleneck (e.g., Santer & Lampert, 1995; Eiane & Ohman, 2004). Consequently, the seasonal timing of reproduction in copepods should be scheduled to minimize naupliar mortality due to starvation and predation. The risk of mistimed reproduction seems to be especially high for a copepod species such as *C. vicinus*, which nauplii do have rather high food requirements (Santer & Van den Bosch, 1994). In contrast, the freshwater calanoid copepod *Eudiaptomus gracilis* reproduces throughout the year and nauplii can develop also during periods of food scarcity, for example, in winter (Seebens *et al.*, 2007).

In order not to miss the phytoplankton spring bloom, C IV_1 of *C. vicinus* re-emerge well in advance of the bloom and subsequently develop in C V. Development through the C V stage, that is, maturation at $5\,^{\circ}C$ should take 13.1 and 8.3 days for females and males, respec-

tively (Munro, 1974). The average time lag between the maxima of C V and adults is, however, almost 2 months suggesting that maturation of *C. vicinus* was indeed retarded. The temperature-dependency of copepod development also suggests that increased food availability due to the onset of the phytoplankton bloom and not increasing temperature is responsible for maturation although we were not able to distinguish statistically between the effects of both factors. This interruption of development is, however, unlikely due to food limitation of C V *per se* as copepodids re-emerging from diapause usually possess energy reserves, which should allow them to finish development and even reproduce (Santer & Boldt, 1998). The timing of maturation also seems not to be triggered by photoperiod as the average timing of maturation was 22 days earlier in the Bay of Bregenz than at the main station. This corresponds to an earlier onset of the phytoplankton bloom in the Bay (approximately 2 weeks according to chlorophyll measurements from 2006 and 2007; Kümmerlin, personal communication) and suggests that increased food availability due to the start of the phytoplankton bloom acts as a cue for the continuation of development, that is, for maturation. A role of food availability for maturation rates and egg production was also reported for *Calanus finmarchicus* in the Labrador Sea (Head *et al.*, 2000). However, maturation delay in *C. finmarchicus* was suggested to be due to food limitation *per se*.

Independent of the cues involved in diapause termination and finally maturation, our data suggest that *C. vicinus* uses a two-phase dormancy, that is, diapause as a C IV and developmental retardation or quiescence as a C V, to ensure a match of their offspring food requirements with phytoplankton spring bloom phenology. This complex strategy has not been shown before for this species, possibly because it might be difficult to detect without long-term data on ontogenetic development. Alternatively, this two-phase dormancy might be a special adaptation to deep lakes due to the absence of any reliable cues indicating phytoplankton bloom onset at the bottom of these lakes. Likewise, *C. vicinus* is able to adapt its diapause behaviour to the environmental conditions in eutrophic lakes allowing for reproduction in summer also (Santer & Hansen, 2006).

Earlier bloom timing seems to cause higher offspring numbers, that is, increased survival of nauplii. This may be due to various reasons ranging from food availability, seasonal predator avoidance, maternal aging, and the costs of time constraints. Nauplii developing after May probably face food shortage as the clear-water phase on average takes place around June independently whether there was an early or late onset of the phytoplankton bloom (see Fig. 4). However, a number

of copepodids declined already in early June probably due to a decrease of nauplii in May even in late OFM years although abundance of OFM and food availability in these years was at its maximum during May. This suggests that food limitation seems not to be responsible for the early decline of copepodid numbers especially in late OFM years. Another possibility for this decline might be reduced clutch size and offspring survival due to maternal aging as has been observed for *Cyclops kolensis* (Jamieson & Santer, 2003). However, as *C. vicinus* seems to postpone maturation until the start of the algal bloom the role of maternal aging might not be the crucial factor for the reduced success in late OFM years. A third possibility might be a seasonal increase of predation pressure during May: this increase might be caused by, for example, the hatching of perch larvae around May (Wang & Appenzeller, 1998), cannibalistic feeding of, for example, early C IV copepods (Brandl & Fernando, 1981), or predation from *Mesocyclops* (Kawabata, 1991). Both C IV$_2$ of *C. vicinus* and adult stages of *Mesocyclops leuckarti* (unpublished data) have their peak timing in mid-May with relatively little interannual variability. Peak timing of adult *M. leuckarti* may be used as an indicator of the interannual variability of diapause termination and hence start of feeding activity of this species, as in this species the C V performs an active diapause (Elgmork, 1980) during winter in Lake Constance. Although we cannot quantify the predation pressure of individual predator species, it is probably safe to assume that the nauplii of *C. vicinus* will be exposed to strongly increasing predation pressure from May onwards. Finally, reduced survival of nauplii might indirectly result from time stress on development. As diapause migration is controlled by photoperiod (Einsle, 1964; Spindler, 1971; George, 1973) and usually takes place in early June, nauplii hatching late do have considerably less time to complete development towards the C IV stage than early nauplii.

The role of time stress for development has received considerable attention in, for example, insects and amphibians (e.g., Newman, 1992; Gotthard *et al.*, 1999). These studies have shown that larvae without time stress do not develop with maximum rates as this involves costs. To speed up development under time stress, larvae aim either for an increased uptake of resources or a change in habitat. Both might increase their susceptibility towards predation as predators might be attracted by increased feeding activity or habitat overlap might be larger within the new habitat. For example, copepod nauplii might migrate under time stress towards warmer surface waters, which should allow for faster growth, but may also increase their encounter rate with predators. In addition, larvae may change resource allocation towards increased in-

vestment into growth and development. Costs of changes in resource allocation might be immunity suppression (Rolff *et al.*, 2004), increased oxidative stress (De Block & Stoks, 2008), or increased fluctuating asymmetry (De Block *et al.*, 2008). Unfortunately, the role of time stress for copepod development has hardly received any attention up to now in studies of copepod ecology. However, a recent modelling study has considered the role of time stress for a marine copepod (Varpe *et al.*, 2007). In addition, there is experimental evidence showing that photoperiod can indeed influence the development of *C. vicinus* nauplii (Einsle, 1964).

The timing of the C I maximum followed OFM after 7 days at the main station. Given a developmental time of nauplii of 37 days at 5 °C and 15 days at 10 °C, the short interval suggests that the C I peak was not the consequence of the development of the majority of clutches produced but rather corresponds to the successful development of early clutches, whereas the later ones faced increasing mortality. A seasonal minimum in predation pressure on copepod nauplii at or even before the timing of the phytoplankton spring bloom has also been suggested for the calanoid copepod *E. gracilis* in Lake Constance (Seebens *et al.*, 2007). In addition, a disproportionately large contribution of early eggs to reproductive success was predicted by a modelling study of Varpe *et al.* (2007), which shows that eggs of the subantarctic copepod *Calanoides acutus* have the best prospects when laid even about 1 month before the start of the phytoplankton bloom. This prediction was based on the assumptions of seasonally increasing predation pressure and time constraints in order to reach a state capable for diapause. Differences in the biology between *C. vicinus* and *C. acutus* and the modelling approach used by Varpe *et al.* (2007) might explain the presumably differing strategies of the two species. In contrast to *C. vicinus* (Santer & Van den Bosch, 1994), the first nauplii stages of *C. acutus* develop without feeding allowing them to bridge a certain prebloom period without starvation. However, the results regarding the optimal strategy of *C. acutus* reproduction might also depend on the modelling approach as the optimization model used cannot predict optimal life histories in environments that vary interannually, for example, in algal bloom phenology (Varpe *et al.*, 2007).

Seasonally increasing mortality and/or differences in developmental rates due to time constraints can also explain the increasing interannual synchronization of phenological events with life-cycle development, for example, from maturation and egg production towards offspring (see Fig. 3). Comparatively early C I peaks within late OFM years, that is, a small time lag between the peaks of C I and OFM might be expected when there

is a higher mortality for the late nauplii, and/or when late nauplii develop faster due to time constraints. This synchronization also occurs when comparing the two sampling stations as there is a large and significant difference in phenology of OFM timing, whereas only a small and nonsignificant one in, for example, C I phenology. Again, differences in naupliar developmental rate and/or nauplii mortality might contribute to this synchronization. Peak timing of adult *M. leuckarti* did not differ between the two sampling stations (main station: 26 May ± 10.0 days, Bregenz: 25 May ± 9.4 days). That is, predation by *M. leuckarti* will increase at both stations rather simultaneously whereas maturation and reproduction of *C. vicinus* was significantly earlier at the Bay of Bregenz. This should give nauplii of *C. vicinus* at the Bay a longer time window for successful development and consequently contribute to the higher numbers of C I at this site.

To conclude, we have shown that *C. vicinus* copes with the problem of high interannual variability of his reproductive period, that is, the timing of the phytoplankton bloom, by returning early from diapause but postponing maturation until the bloom starts. This suggests that the diapause of the copepod is actually composed of two phases: a dormancy period of the C IV stage and a subsequent developmental retardation, or quiescence, of the C V stage until the start of the spring bloom. The copepod benefited from early phytoplankton blooms as this seemed to give the nauplii a longer time window for successful development into C I possibly as a result of reduced predation pressure early in the season and/or reduced time constraints. Simulations with a coupled hydrodynamical–biological model predict that in Lake Constance, algal bloom timing will advance up to 1 month with global warming (Peeters *et al.*, 2007a). This might suggest that *C. vicinus* will benefit from further warming. However, the life cycle of the species, for example, diapause induction and termination, is adapted to the current climate and the predicted large shift in phytoplankton phenology might nevertheless result either into a mismatch of the copepods life cycle with the new environmental phenology or microevolutionary adaptations to the new situation.

Acknowledgements

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