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Life history synchronization in a long-lifespan single-cohort *Daphnia* population in a fishless alpine lake

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Abstract One-year data on *Daphnia* and other zooplankton taxa from two neighboring ultra-oligotrophic alpine lakes in the Tatra Mountains, southern Poland, revealed a multi-specific herbivore community of small-bodied cladocerans and rotifers in the lake that has contained fish for millennia, and the large-bodied *Daphnia pulicaria* as the sole herbivore species monopolizing resources in the absence of fish in the other lake, which has never been successfully stocked. *D. pulicaria* co-exists with an abundant *Cyclops* population. In contrast to the non-abundant small-bodied *D. longispina*, which reproduces year-round in the lake with fish, *D. pulicaria* in the fishless lake was found to reproduce only once a year over a short period of time, suggesting strong stabilizing selection for the precise timing of reproductive effort and hatching from diapausing eggs in the clones inhabiting the lake. This is conceivably due to the exceptionally long lifespan of *Daphnia*, which can over-winter either in ephippia or in the form of active adults that have restrained from reproduction until the next year, when, almost 1 year old, they produce eggs. The new-year generation starts from both the ephippial eggs and eggs released by the over-wintering adults. Only a small fraction of the population is recruited as the second new-year generation from eggs released by a few new-generation females that succeed in growing and maturing early. In each of the two generations, reproductive effort is restricted to a short period, evidently the only time window when (1) food levels are high enough to allow juvenile growth and (2) predation by *Cyclops* is low enough for high survival of eggs and neonates. No immediately hatching eggs are produced outside this reproductive window, even when the body lipids of the adult *Daphnia* are as high as at the time of summer reproduction, suggesting a deliberate halt to reproduction and its postponement until the following summer.

Keywords Alpine lakes · Competitive exclusion · *Daphnia* life history · Food limitation · Predation

Introduction

The size efficiency hypothesis of Brooks and Dodson (1965) was an important step toward comprehending the relative importance of fish predation and food limitation in structuring the species composition of zooplankton. Over the decades, the hypothesis has been supported by numerous field and experimental studies showing that small-bodied zooplankton are favored at high fish abundance, and large-bodied zooplankton when fish stocks are low (see Kerfoot and Sih 1987 for review). However, neither as clear a prediction nor as strong evidence have been offered for habitats that are completely free of fish. This is because little attention has been focused on the outcome of the strong interspecific competition expected in the absence of fish predation which would otherwise diminish competition by holding the abundance of all competitors much below carrying capacity level (Gliwicz, 2001). Little evidence has been collected on zooplankton from habitats free of fish to see how many species can peacefully co-exist, sharing resources in the absence of fish predation. The possibility that a superior competitor would monopolize resources, competitively excluding all other species, has not been considered.

Our knowledge about zooplankton released from the impact of fish predation is scarce because habitats free of fish are rare. They were likely commonplace in the time preceding the mid-Mesozoic appearance of fish (Kerfoot and Lynch 1987), but only a few examples can be found because most bodies of water are interconnected, and humans cannot resist stocking every waterhole. Nevertheless, there remain lakes high in the mountains from which fish have always been excluded. The zooplankton community in such fishless lakes is simple, with large-bodied *Daphnia* as the exclusive crustacean herbivore, and one cyclopoid copepod, *Cyclops* or *Tropocyclops*, that preys on *Daphnia* juveniles and eats eggs from

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brood cavities. In some locations, there are also a few non-abundant rotifer species of the genera *Keratella*, *Polyarthra*, or *Asplanchna*. Such zooplankton communities have been reported from fishless lakes in the high Alps, Tatra, and Estrela of Europe (*Daphnia pulicaria*; Gliwicz and Boavida 1996) and the Himalayas (*D. tibetana* Sars; Manca et al. 1994). They are also known from small water bodies in the Canadian high Arctic (*D. middendorffiana*; Haney and Buchanan 1987) and rock pools of the Baltic coast (rock-pool *D. magna* and *D. pulex*; Ranta 1979). The reports have been based on short-term investigations rather than the whole-year studies necessary to see the likely consequences of the permanent absence of predation and intra-specific competition.

Theoretically, the absence of predators and competitors should result in a high population density of the winning species at about the carrying capacity of the habitat. The population should remain in a dynamic equilibrium with its resources that would be held at a very low level. This level should be above the species-specific food threshold concentration, the food level that allows for metabolic maintenance of an animal (Lampert 1977; Gliwicz 1990), and equal to the species-specific equilibrium food concentration, the food level at which a population is maintained through the compensation of death rate by birth rate (Kreutzer and Lampert 1999). The life history bottleneck in such a single population would be the juvenile stage from the time at which the maternal reserves of an egg have been exhausted to the time at which individual body size provides a juvenile with the ability to make effective use of resources comparable to that typical of adults. This is so, because the larger the body size in cladocerans, the lower the food threshold concentration. This has been found experimentally for various daphnids (Gliwicz 1990), and for different instars of the same *Daphnia* species (Kreutzer 1999). Thus, the two major attributes of a species that monopolizes resources in the absence of fish predation would be (1) the lowest possible species-specific food threshold concentration causing other species to be competitively excluded and (2) a life history trait of refraining from reproduction until the appearance of a window with a pulse in food abundance that secures temporary food levels above the threshold concentration typical for juveniles. This should lead to synchronous reproduction, because egg production at any time other than the time window would be maladaptive. The goal of our work was to test whether one of two neighboring natural lakes which differs from the other in being fish free would manifest the attributes of a large-bodied herbivore (1) monopolizing resources or (2) displaying synchronized reproduction over a narrow time window.

Both attributes were expected for a large-bodied *Daphnia* in Czarny Staw pod Rysami (Lake Czarny), one of the deepest ultra-oligotrophic alpine lakes in the Tatra Mountains, a granite rocky formation on the border of Poland and Slovakia, where a single cladoceran, *D. pulicaria* Forbes, also known as *D. wierzejski* (possibly a hybrid with *D. pulex*; Cerny and Weider 1999), co-

exists with a single cyclopoid, *Cyclops abyssorum tatricus* (Kozm.), and a low density of the rotifer *Asplanchna priodonta*, which appears for a short period in early summer. Unlike the neighboring downstream Lake Morskie Oko, fish have been always precluded from Czarny by an outflow that cascades over a moraine edge down to Morskie Oko and is therefore impenetrable to salmonid fish. Salmonids have been present in Morskie Oko for millennia, along with a diverse zooplankton (Gliwicz 1986).

The Lake Czarny *Daphnia* population offers a unique opportunity to investigate how the demography of a cladoceran population and the life history of an individual are shaped in the absence of the impact of planktivorous fish, and of inter-specific competition for resources, which is replaced by intra-specific competition between different individuals of the winner's population. In contrast to other fish-free habitats that are small and shallow, the Lake Czarny offshore habitat is free of interference from littoral and benthic fauna that are well known from mountain ponds (Dodson 1970, 1974) and smaller alpine lakes (McNaught et al. 1999).

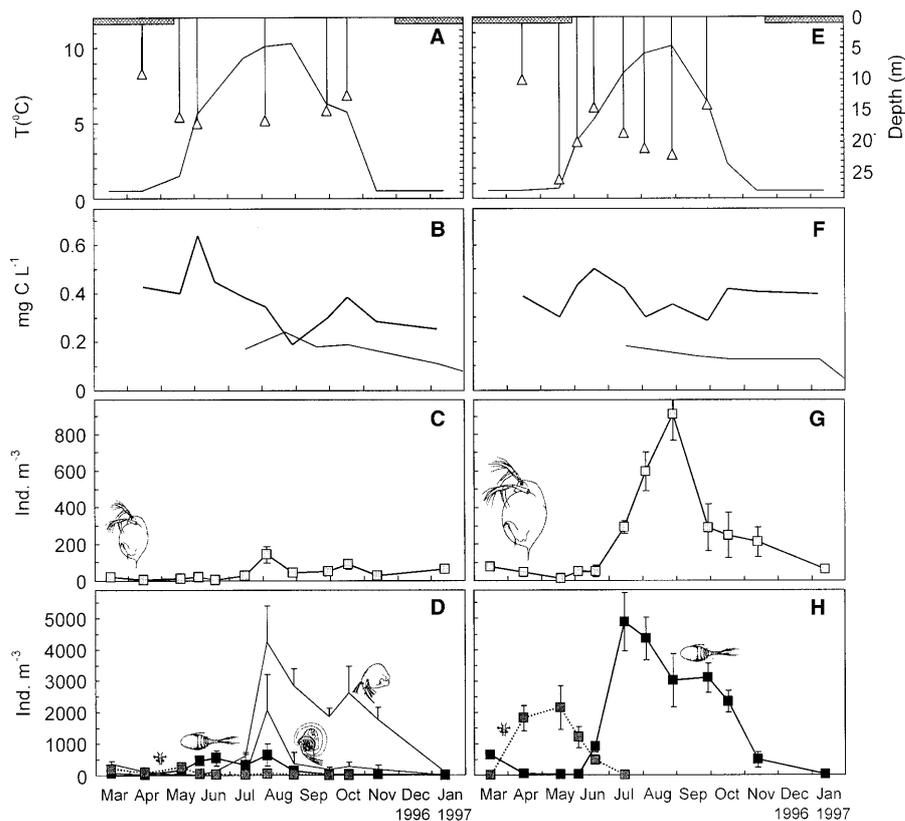
We sampled the two lakes in an attempt to observe differences in the patterns of seasonal change in the density of each *Daphnia* population, and in the life histories of individuals of the two *Daphnia* populations. We were not aware that the two populations were of two different species at the time the sampling was initiated in 1996.

The lakes and their zooplankton

Lakes Czarny and Morskie Oko are located in Dolina Rybiego Potoku, one of the largest valleys cutting into the Tatra ridge from the north. Both lie just above the timberline, at elevations of 1581 and 1395 m above sea level, respectively, and each is known as a classic example of a glacial cirque lake, or tarn, with a regular circular shape, with areas of 21 and 35 ha and maximum depths of 76 and 51 m, respectively. They are both ultra-oligotrophic, with Secchi disc transparency being slightly greater in Czarny (10–24 m) than Morskie Oko (9–18 m), a fact reflecting the extremely low food levels for filter-feeding zooplankton. Earlier samplings in the two lakes showed chlorophyll *a* concentrations below 1 µg l⁻¹ and low densities of phytoplankton composed of small flagellated Chlorophyta (Z.M. Gliwicz, unpublished data).

Both lakes are dimictic, with a long-lasting snow-ice cover, a sharp thermal gradient (to a depth of 6 m) and a temperature of 3.8°C down to the bottom. Both lakes stratify for a short time in summer with epilimnetic temperatures up to 10°C (Fig. 1), a thermocline at a depth of between 5 and 8 m, and hypolimnetic temperatures of 5–6°C. Low summer temperatures of the hypolimnion suggest a very negligible change in the ambient temperature to which the majority of animals are exposed. The hypolimnia remain oxygen saturated to the bottom during summer, but a thin layer of near-bottom anoxia

Fig. 1 Seasonal changes in Lake Morskie Oko (A–D) and Lake Czarny (E–H). **A,E** Mean temperature of the epilimnion (0–5 m depth, T , solid line) and depth (m) of Secchi disc reading (triangles; ice cover represented by horizontal bar). **B,F** Concentration of particulate organic carbon (mg C l^{-1}) at 5–15 m depth estimated with two different methods in 1996 (thick line) and 1997 (thin line). **C,G** Mean (and 90% confidence interval) *Daphnia* population density in 1996. **D,H** Mean (and 90% confidence interval) density of other zooplankton taxa: *Cyclops abyssorum taticus* (thick broken line naupli, thick solid line copepodites+ adults) and the two most abundant cladocerans in Morskie Oko, *Bosmina longirostris* and *Holopedium gibberum* (thin lines). Except for *Cyclops* naupli, the silhouette of each species represents relative body size at first reproduction



can be found in the deepest part of each lake at the end of the long-lasting winter. Littoral vegetation is absent from the barren rocky shores of the lakes and benthic fauna are scarce. In contrast to fishless Lake Czarny, Morskie Oko has always been accessible to migratory fish and is inhabited by several salmonid species, *Salmo trutta morpha fario* L. being the most abundant.

The single-species herbivore community of Czarny contrasts with the diverse herbivore zooplankton of Morskie Oko (with four cladoceran and three rotifer species), which are small-bodied (all cladocerans below 1.5 mm body length), and vary in behavior. Both *Daphnia* and *Cyclops* are known to be non-migratory in the absence of fish in Lake Czarny, but perform long-range diel vertical migrations in Morskie Oko (Gliwicz 1986). In the absence of fish predation, the *Daphnia* and *Cyclops* of Lake Czarny are the only two obviously interacting species, as naupli of the latter compete for scarce phytoplankton with the more effective *Daphnia* (Gliwicz 1985), and *Daphnia* must avoid predation by adult *Cyclops* when juvenile, and predation of eggs in brood cavities by *Cyclops* copepodites when adult (Gliwicz and Boavida 1996). Manifestations of these strong interactions would be expected in the demography of each of the two populations, as well as in the behavior and life histories of individuals of the two species. Demographic and life history responses in *Cyclops*, although showing a more precise synchrony in the single cohort in Czarny than in Morskie Oko (Fig. 1), would seem to be more complex than those in *Daphnia*. Demography and life

history can be studied more easily in *Daphnia* due to (1) its simple ontogeny, (2) the retention of the same food niche as a typical filter-feeding herbivore throughout the lifespan, (3) parthenogenetic reproduction fixing life history traits in the clones inhabiting the lake, and (4) very negligible seasonal change in ambient temperature experienced throughout life, since the overwhelming majority of individuals select depths of 10–20 m, below the summer thermocline, where the temperature amplitude is only 2.2°C (from 3.8°C in winter to 6.0°C during summer).

Materials and methods

Zooplankton were sampled from the two lakes on 13 occasions from 13 March 1996 to 22 January 1997, and from Lake Czarny on an additional 10 occasions from 5 March 1997 to 1 February 1998, from the snow-ice cover or from an inflatable boat anchored in the center of each of the two lakes. The samples (three replicates on each date) were collected by vertical hauls from a depth of 45 m to the surface, using a quantitative closing plankton net of 0.2 mm mesh size, equipped with a conical annex of 6 dm² inflow area, thus of 2,700 l volume. To assess the abundance of small-bodied rotifers and net phytoplankton, a single 45- to 0-m-depth vertical-haul sample was also taken on each date with another quantitative net of 0.05 mm mesh size and 1.5 dm² inflow area. Lake water for organic carbon estimates was taken from depths of 5, 10, and 15 m with a 1-l Patalas water sampler, and pooled into an integrated sample in three replicates. Temperatures and dissolved oxygen concentrations were read with a YSI5740 oxygen probe. The particulate organic carbon (POC) concentration in the fraction <50 µm was assessed from Whatman GF/F filters, each of three replicates retaining particulate material from a 1- or 2-l mixed sample pre-filtered through a 50-µm mesh size net, by the

Szekielda and Krey method (Golterman and Clymo 1969) in 1996 and, in 1997, on a UNDR infrared gas analyzer after combustion in a Pregl-Roth oven (Krambeck et al. 1981).

Animals preserved in 3% sugar-formaldehyde were counted under a Zeiss dissecting microscope, and (in 1996) *Daphnia* individuals (105–995) were assessed for body length (upper edge of head to base of tail spine), clutch size (number of eggs/embryos per clutch), the presence of ephippia, and the presence of *Cyclops* in the brood cavity (a total of 3,770 and 2,800 *Daphnia* individuals were examined from Czarny and Morskie Oko, respectively). From August 1996, before being preserved 4–8 h after sampling from the lake, 100 randomly chosen live *Daphnia* were inspected for body length, visible lipid body reserves, and the ovary size and opaqueness (Tessier and Goulden 1982). All inspected animals were classified according to the amount of visible lipid in the body as 0, 1, 2, 3, 4, or 5, from low to high body lipid, and according to the ovary opaqueness along the scale 0, 1, 2, or 3. The original scale of the visible-body-reserves index (0, 1, 2, and 3) was expanded due to a higher visible lipid content in Lake Czarny *Daphnia* than in the six American *Daphnia* species from the Tessier and Goulden (1982) study. The sum of the two indices was used to compare visible energy reserves in reproducing and non-reproducing animals.

Results

Infrequent sampling, a wide range in the *Daphnia* depth distribution, and an extremely low standing crop of phytoplankton (small flagellated Chlorophyta) precluded both a detailed description of seasonal changes in food levels and precise identification of the time window of increased food levels. Despite quite similar POC concentrations in the two lakes, Secchi disc transparency was markedly higher in Lake Czarny (Fig. 1A, E) where phytoplankton were under the control of large-bodied *Daphnia* at a density unrestricted by fish (Fig. 1C, G). Although the POC concentration was overestimated in 1996 compared to 1997, an apparent maximum was revealed in each of the two lakes in June (Fig. 1B, F). This may suggest a short-lived increase in food levels, likely due to the spring overturn following thawing at the end of May (Fig. 1A, E). In Czarny, this May–June increase in food levels followed by a June–August decline (Fig. 1F) in the time of *Daphnia* population increase (Fig. 1G) could also be seen in Secchi disc transparency (Fig. 1E).

In neither of the two lakes were there any large phytoplankton or small-bodied animals in the 0.05-mm mesh size plankton net other than those retained on the 0.2-mm mesh size net: *D. pulicaria/D. wierzejski* and *C. abyssorum tatricus*, with a brief low density of *A. priodonta* (0.1–0.3 individuals l^{-1} in June and July) in Lake Czarny, and a multi-species community in Lake Morskie Oko with dominant *Bosmina longirostris* O.F. Müller and *Holopedium gibberum* Zaddach, and less abundant *D. longispina*, *Alona* sp. and *Polyphemus pediculus* L., with *C. abyssorum tatricus* being much less abundant (Fig. 1D, H) and the density of *A. priodonta* two orders of magnitude higher than in Czarny (4.5–18.5 individuals l^{-1}) and present year-round. No potential food source for *Asplanchna* could be found in Czarny: with the infrequent sampling we could have missed the brief appearance of other rotifer or ciliate species.

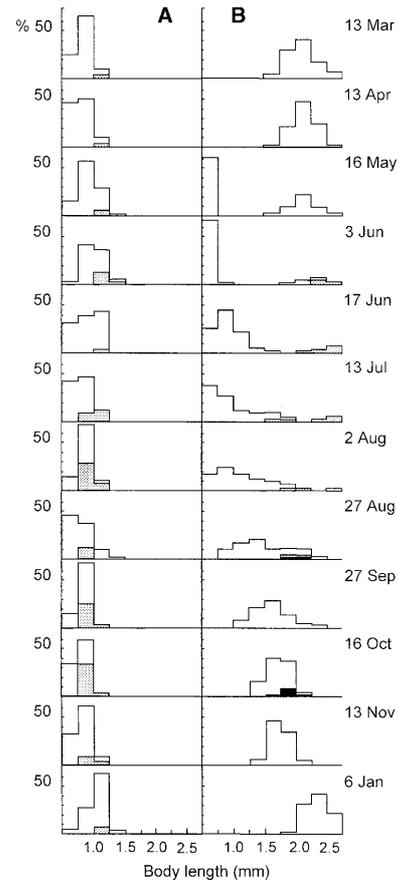


Fig. 2 Body size distribution in the *Daphnia* population in Lake Morskie Oko (*D. longispina*) (A) and Lake Czarny (*D. pulicaria/wierzejski*) (B) as percentage of total population on each of 12 sampling dates from 13 March 1996 to 6 January 1997 (light shading fraction of egg-bearing females, dark shading fraction of ephippia-bearing females). Number of animals used for each diagram, $n \geq 105$; in Lake Czarny from 13 April to 27 August, $n \geq 265$. Two discrete cohorts can clearly be seen in Lake Czarny from 16 May to 2 August 1996, the old generation of adults surviving since summer 1995, and the new 1996 generation hatching from ephippia and released from brood cavities of females of the old and the new generations (up to and after July, respectively)

Except for its distinctly higher density, the Czarny *Cyclops* population did not differ markedly from that in Morskie Oko, with the same March–April period of intense reproduction, old adults perishing in May, and a new generation of naupli growing up and entering the copepodite stage in May–June (Fig. 1D, H). Due to the much shorter duration of the naupliar than copepodite-adult stages, there was likely rapid renewal of the pool of naupli, so that the density of copepodites and adults could become higher later in July–August than the maximum density of naupli in April–May. This can be seen more clearly in Czarny due to the much higher population density (Fig. 1H) possible in the absence of fish.

Differences were more striking for *Daphnia*, not only in relation to population density (much greater in Czarny than in Morskie Oko; Fig. 1C, G), and not merely in the summer increase that continued for 100 days from mid May to the end of August in Czarny, but was halted

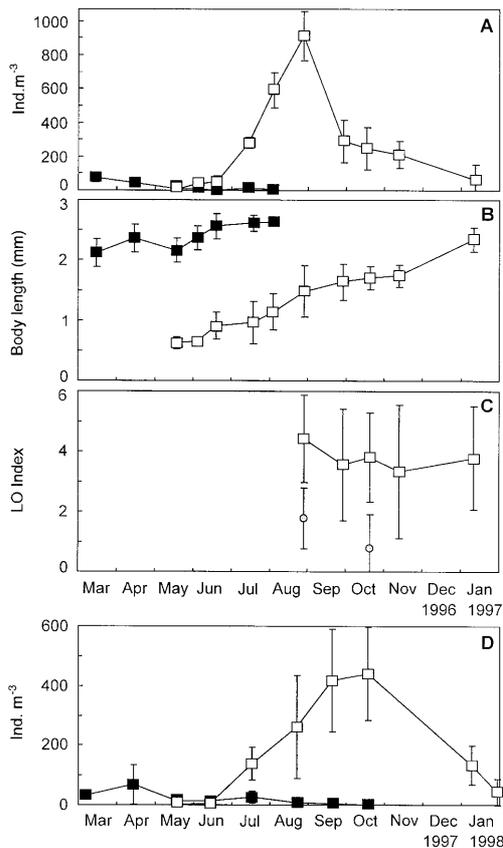


Fig. 3 Seasonal change in Lake Czarny *Daphnia* population density in 1996 (A) and 1997 (D), body size in 1996 (B), and visible body-and-ovary lipid (LO) index in females of more than 2 mm body length in 1996 (C), with the two generations shown separately (filled squares old, open squares new). Bars show 90% (A,D) or 99% (B,C) confidence intervals. The LO index was found to be significantly lower in females with eggs or ephippia (circles) than in females with empty brood cavities (squares), and no difference was detected between the five different dates for non-reproducing females

abruptly in Morskie Oko as early as the beginning of August. This halt to population growth could also be observed in other cladoceran species (Fig. 1D). The two *Daphnia* populations differed dramatically in life histories, the distinction best reflected in the seasonal change in body size distribution (Fig. 2). The *Daphnia* of Morskie Oko grew quickly and start reproduction early, while still of small body size, before the risk of falling prey to fish became too great. Their maximum body length remained below 1.5 mm, the majority of individuals reproducing at below 1 mm body length, reproduction taking place throughout the year (Fig. 2A). In the absence of fish predation, the *Daphnia* of Czarny could continue growing to attain a large body size before starting to reproduce (Fig. 2B). However, at a much higher population density and lower food levels (Fig. 1), individuals born in June and July grew for a long time before they were ready for reproduction. The overwhelming majority of individuals in the new cohort starting in June would either perish before maturity or had to stay alive for an entire year to start reproduction the next summer. As not a single male

was found among the 3,770 or 2,800 *Daphnia* individuals from Czarny or Morskie Oko, respectively, assessed for body length, the two populations could be regarded as asexual ones of a single clone or a mixture of several clones.

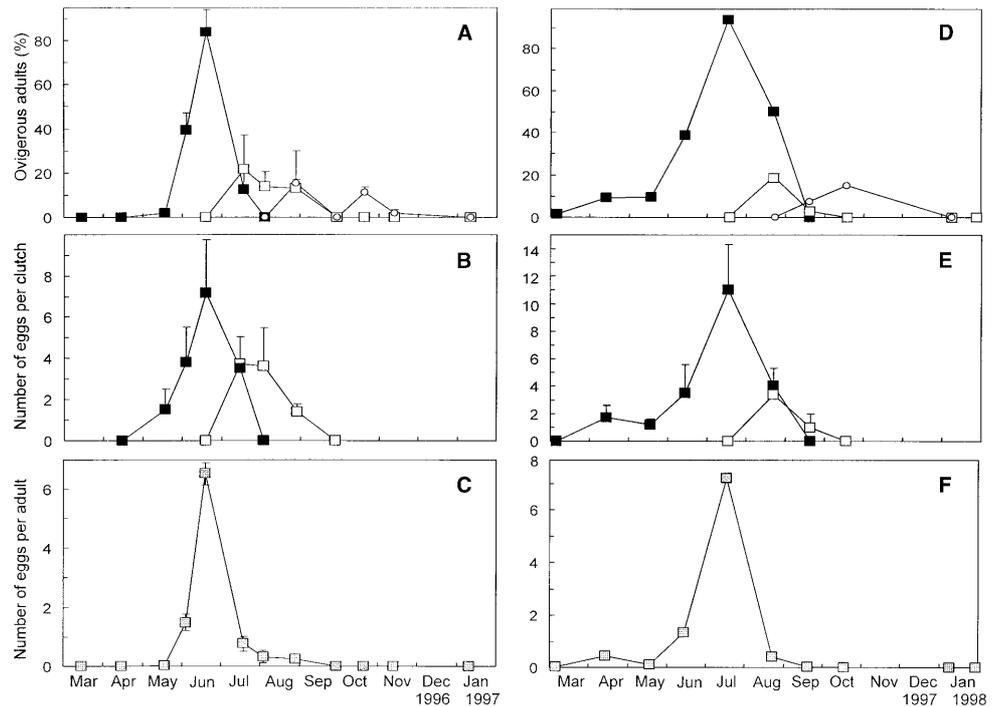
The June–July body size distributions in Fig. 2B show that, unlike the Morskie Oko population with its continuous reproduction, there was a distinct cohort of *Daphnia* in Czarny, a single generation per year, and that the two generations of 2 successive years co-occurred for a short time only when a handful of old-generation survivors co-existed alongside new-generation juveniles. As the two cohorts were easily distinguished, the densities of each could be followed independently in each of the two sampling years, 1996 and 1997 (Fig. 3A, D, respectively). The 1996 body length data could also be used to follow seasonal changes in body size in the old and new generations. The rate of body length increase in the new generation (Fig. 3B) did not reflect the individual growth rate that is expected to be greater at higher food levels in June–July rather than in August, after food levels decreased (Fig. 1E, F) following increased density in the new generation (Fig. 3A) and increased body sizes (Fig. 3B).

Reproduction in the new 1996 generation started as early as June–July, but only a small fraction of the largest animals were found to have eggs in brood cavities on 13 July and 2 August (Fig. 2B). This proportion did not increase with further increase in body size. On the contrary, it declined on 27 August, most animals producing ephippia rather than subitaneous eggs, despite the fact that body size increased (Fig. 3B) and resources allocated for reproduction stayed at the same level (Fig. 3C). The visible body-and-ovary lipid index in females of more than 2 mm body length, LO, was variable but showed no tendency to decline: no significant difference at $P=0.05$ ($n=358$) was found either between 2 and 27 August or on subsequent dates. The LO index was significantly lower in females with than without eggs or ephippia in brood cavities at $P=0.000013$ and $P<0.00001$ for the August ($U=53$) and September ($U=83$) data, respectively (Mann-Whitney U -test).

The production of subitaneous eggs at the end of August seen as a large fraction of egg-bearing females in the body size categories over 1.75 mm (27 August) did not result in a recruitment into the body size categories below 0.75 mm on subsequent dates (Fig. 2B). All individuals surviving until the late fall were adults. They were all of a body size above that of first reproduction (1.5 mm), yet none were seen to lay subitaneous eggs, even though their visible lipid reserves were as high as in August when many grown individuals had eggs in brood cavities. They were found to refrain from reproduction for a long time, producing eggs in the following summer, 1997, the LO index remaining variable but high (2–4) until reproduction was eventually restarted in April to become intense later in June (Fig. 4).

The data on the Lake Czarny *Daphnia* reproductive effort showed that only a small fraction of the new

Fig. 4 Seasonal change in Lake Czarny *Daphnia* reproductive effort in 1996 (A–C) and 1997 (D–F) (A,C,D,F 90% confidence intervals, B,E 99% confidence intervals). A,D Contribution of egg-bearing (squares: filled old generation, open new generation) and ephippia-bearing (circles) females to the fraction of adults (≥ 1.5 mm body length). B,E Clutch size in each of the two generations (filled old, open new). C,F Average reproductive effort in the entire population (the two generations pooled) shown as the average number of eggs per adult (≥ 1.5 mm body length)



generation produced eggs or ephippia (Fig. 4A, D) and that clutch size was small (Fig. 4B, E) compared to the old generation, the difference being more pronounced in 1997 than 1996 despite less frequent sampling and no replicable data on the proportion of ovigerous adults (Fig. 4D, E compared to A, B, respectively). Despite its lower density, the old generation contributed more to the joint reproductive effort of the population. The overall reproductive effort assessed as the average number of eggs per adult (≥ 1.5 mm body length) in the entire population, pooling the two generations, was concentrated within a short period of time in June (1996) or July (1997), with the average number of eggs per adult found on a single date to be several times higher than on neighboring dates (Fig. 4C, F). In 1996, this maximum was found on 17 June, the only date with a food level increased to 0.5 mg C l^{-1} (Fig. 1F) and Secchi disc transparency reduced to 15 m (Fig. 1E). No significant correlation between the average reproductive effort (Fig. 4C, F) and the food level assessed as either POC concentration or Secchi transparency (Fig. 1F, E, respectively) was found at $P \leq 0.05$ for 1996 or 1997 ($r < 0.53$). However, the reproductive effort was significantly higher on three dates (3 June, 17 June, and 2 August) than on the other nine remaining dates (at $P = 0.0003$, $P = 0.0002$, and $P = 0.0007$, respectively, one-way ANOVA and Tukey HSD post hoc test for three replicate values on each date).

The June maximum in the reproductive effort was apparent in 1996 and 1997 in Lake Czarny (Fig. 4), but not in Morskie Oko, where *Daphnia* with subitaneous eggs in brood cavities were encountered throughout the year (Fig. 2A). Reproduction did not halt in winter despite low temperatures and low light intensity under the more

than a meter of ice-and-snow cover that is typical for both lakes. However, a smaller fraction of adult females had eggs during the winter (Fig. 5A) and they had smaller clutches of eggs than in the summer (Fig. 5B). Distinctly smaller clutch sizes in Morskie Oko were associated with much smaller body size at first reproduction (0.75 mm) compared to Czarny (1.50 mm). In contrast to Lake Czarny (Fig. 4C, F), no tendency for synchronic reproductive effort could be discerned in Morskie Oko (Fig. 5C). The average reproductive effort (Fig. 5C) was significantly correlated with the POC concentration was found to be significant at only $P = 0.089$ ($r = 0.54$) for all the 1996 data points, but the relationship greatly increased when restricted to dates from 14 July to 6 January ($r = 0.79$, $P = 0.033$). No ephippia were ever seen in the *Daphnia* of Morskie Oko.

Even though low food levels were expected to hinder reproduction in both lakes, the patterns of reduced fecundity were different. The distinction could result from different manners of food limitation as well as from different impacts of predation. Under the impact of fish predation in Morskie Oko, large-bodied individuals were exterminated and *Daphnia* could reproduce only at small body size producing small clutches of eggs, so low fecundity was the effect of small clutch size (Fig. 5). In the absence of fish in Lake Czarny, *Daphnia* could reproduce at large body size producing large clutches, but except for a short period in June–July, most females refrained from reproduction (Fig. 4). A few produced subitaneous eggs but the offspring were not recruited into the population (Fig. 2B), suggesting high mortality in newborn neonates. This mortality could either result from insufficient food and/or predation by *Cyclops*.

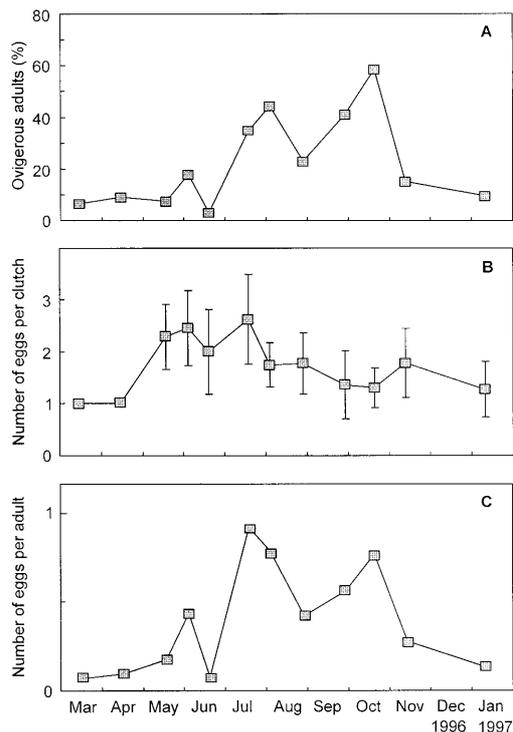
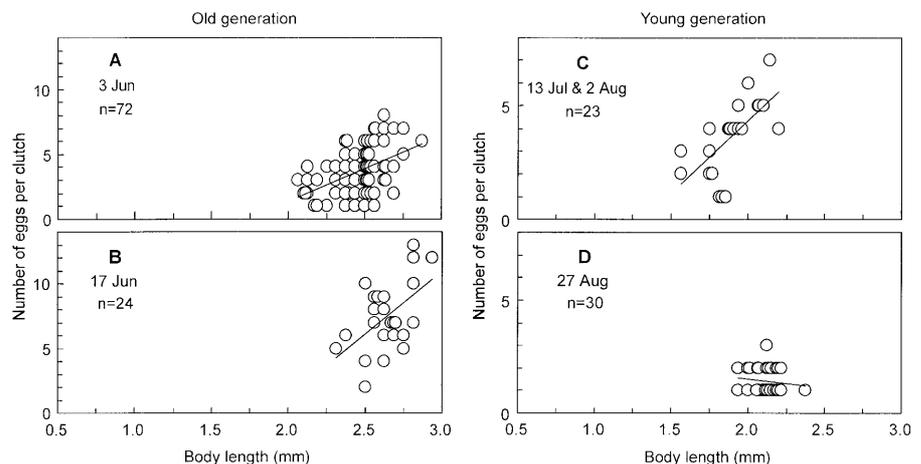


Fig. 5 Seasonal change in Lake Morskie Oko *Daphnia* reproductive effort in 1997: contribution of egg-bearing females to the fraction of adults (≥ 0.75 mm body length) (**A**), their clutch size with the 99% confidence intervals shown as bars (**B**), and the average reproductive effort in the population shown as the average number of eggs per adult (**C**). No ephippia-bearing females were found

Predation on Lake Czarny *Daphnia* newborn neonates and on eggs in brood cavities would seem to be most severe at the end of August. Adult *Daphnia* were already too large to fall prey to *Cyclops*. All *Cyclops* individuals in Czarny were into the first copepodite instars (Fig. 1H) of 0.5–0.6 mm body length, and were thus much too small to be a real threat to the already significantly larger *Daphnia* (1.5–2.2 mm body length; see Fig. 2). However, the newborn neonates and eggs in brood cavities were vulnerable to predation by the early copepodite instars.

Fig. 6 Clutch size on body length in *Daphnia* of Lake Czarny for the old (**A,B**) and the new (**C,D**) generation (note different scales) in the period before (**A–C**) and after (**D**) predation on eggs in brood cavities by *Cyclops* copepodites became intense. Each of the three regressions for **A**, **B**, and **C** is significant at $P < 0.0006$ and their slopes are significantly different from that for **D** (ANCOVA)



Copepods in the copepodite stage were still small enough to be able to squeeze into brood cavities, and their abundance was much higher than that of *Daphnia*, at a ratio of about 5 to 1 (Fig. 1H, G, respectively). Despite the short time a copepodite needs to be in the brood cavity to eat an egg, *Cyclops* copepodites were found inside *Daphnia* brood cavities in fixed samples of 27 August (5 of the 265 adult *Daphnia* from that date contained a single copepodite each), when the number of eggs in the brood cavity was reduced to one or two even in the largest females (Fig. 6D) with a potential of generating seven eggs per clutch a month earlier (Fig. 6C). Each of the regressions in Fig. 6A–C was significant at $P < 0.0006$ (the 18 May data with mean clutch of 1.5 eggs were excluded because $n=4$). In the old generation, clutch size was lower on 3 than on 17 June (Fig. 6A, B, respectively) with means (± 1 SD) of 3.78 (± 1.75) and 7.45 (± 2.73). In the young generation (Fig. 6C, D), however, it differed between earlier dates and 27 August. While the slope of the regression for clutches C on body length L ($C=a+bL$) on 13 July–2 August reflected an expected increase in clutch size with body length ($n=23$, $a=-8.41$, $b=6.38$, $r^2=0.432$, $F=15.99$, $P < 0.0006$), the slope of the regression on 27 August was reversed but non-significant ($n=31$, $a=3.03$, $b=-0.76$, $r^2=0.01$, $F=0.42$, $P < 0.52$), suggesting higher vulnerability of larger *Daphnia* to *Cyclops* predation on eggs. The two regressions differed significantly (ANCOVA) in their slopes ($df=1,50$, $F=5.39$, $P < 0.024$) and their adjusted means ($df=1,51$, $F=65.72$, $P < 0.00001$).

Discussion

When contrasted with the persistent and irregular reproductive episodes of *Daphnia* in Morskie Oko, the synchronized reproductive effort of *Daphnia* in Lake Czarny seems a clear example of the work of stabilizing selection, an example resembling that in the Canada goose (Cooke and Findlay 1982) which is one of the most widely discussed in ecology handbooks (e.g., Krebs 1985). As in other examples, the forces of natural selection

in Lake Czarny *Daphnia* also seem quite obvious – neither before nor after the optimum time window could the reproductive effort be more effective because of the amplified severity of food limitation or increased predation risk. Neither of the two factors has to be more important. On the contrary, both may work side by side complementing and reinforcing each other, individual fitness stemming from a tradeoff solution in terms of apprehending the right time for reproduction. The *Daphnia* clones that have succeeded in taking over the offshore habitat of Lake Czarny had to be well suited to a situation with a brief once-a-year period of reasonable chances for juvenile survival. We do not know whether it is a fixed or inducible life history trait that has made them more successful here than other cladoceran species. In the Canada goose, excessive earliness means increased predation risk in the very few nests with eggs laid somewhat too early, while too late means not enough food to feed the large clutch of goslings, with the resources already being over-exploited by superior optimizers. The sequence may be the reverse in Lake Czarny *Daphnia*, with too early meaning severe food limitation and food levels not high enough for juveniles to survive, and too late meaning a high risk of predation by *Cyclops* copepodites for newborn juveniles.

Neither the timing of the mass hatching from diapause nor the timing of peak *Daphnia* reproductive effort at the time when the density of conspecific competitors and predators are highest would seem to be very adaptive at first glance. The synchrony in June–July hatching from ephippia, responsible for a part of the population density increase, shows, however, that this is just the time window when neonate survival is the highest possible, a conclusion reinforced by the synchrony in the reproductive effort of the old and the new generation (Fig. 4) and its overlap with the only time when food levels increased to 0.5 mg C l^{-1} . The young-generation animals had barely enough time to mature. The old-generation animals had restrained from reproduction, awaiting a food level increase above a threshold food concentration necessary for newborn neonates to equal respiration by assimilation, which is lower for small neonates than for large-bodied adults (Gliwicz 1990; Kreutzer 1999). The lack of a significant correlation between food levels (Fig. 1F) and the average reproductive effort in the two generations (Fig. 4C, F) should thus not be surprising. A switch response should be expected here rather than the linear relationship which might be expected when food limitation is behind the phenomenon of adjusting clutch size to food levels, as shown experimentally elsewhere (Gliwicz and Giusande 1992) and found also in Morskie Oko (Fig. 5), where *Daphnia* could reproduce all year round, facing the problem of how many eggs to produce rather than whether the eggs should be produced at all and risk being wasted.

The synchronized emergence of *Daphnia* neonates from diapause by hatching from ephippia may be the main reason for the June–August population increase in Lake Czarny. Even though no successful assessment

could be made of the relative importance of the two modes of reproduction toward the density increase of the new-year generation, the share of ephippial eggs in the spring recruitment may be significant. For the entire June–August population increase ($900 \text{ individuals m}^{-3}$; Fig. 1G) to be explained by subitaneous-egg reproduction alone, at least three consecutive clutches of a minimum of ten eggs per clutch would be necessary in each of the 30 over-wintering females m^{-3} , as well as 100% juvenile survival, a rather unlikely prospect. On the other hand, however, reproduction through ephippia cannot explain the entire June–August population increase of $900 \text{ individuals m}^{-3}$ either. To be explained by neonates hatching from ephippia alone, at least 450 females m^{-3} would have to produce viable ephippial eggs (two per ephippium) during the preceding autumn, a number equal to the number of females vanishing in September–October 1996 (Fig. 3A), when ephippia are produced (Fig. 4A).

The high rate of offspring recruitment in June–July is clearly reflected in a rapid population increase (Fig. 3A, D) and the large proportion of juveniles in the population (Fig. 2B), showing that this is the only time at which reproductive investment is not wasted. It seems to be a complete waste somewhat earlier for the old generation and somewhat later for the new generation, which might explain why none of the old-generation females produced eggs earlier, and also why only a small fraction of the new-generation females took the risk of investing in subitaneous eggs later. The majority produced ephippia (Fig. 4) or refrained from reproduction to wait for the time window in the following year. We cannot envisage any alternative strategy for the new-generation *Daphnia*. Competition for resources rather than predation would seem to be the major force of selection. The same seems to be the case for *Cyclops*, with its own single cohort per year and synchronized life history precisely tuned to the seasonal change in food resources: the May–June pulse in the phytoplankton for nauplii, and *Daphnia* eggs and juveniles throughout the short summer. This is so only in the fish-free habitat of Lake Czarny, however, not in Morskie Oko, where *Cyclops* demography and life history are subordinated to predation by fish (Fig. 1).

There is no way to judge whether the life history of the Lake Czarny *Daphnia* is an adjustment to the time window free of harsh food limitation for the juveniles alone, or the time window with higher food levels combined with relaxed predation by copepods on defenseless offspring at the stage of eggs in brood cavities or the stage of small and defenseless neonates released from brood cavities. The role of temperature cannot be excluded either, although there are two reasons why the summer warming does not seem crucially important. First, it is negligible at the 10–20 m depths selected by the majority of animals. Second, there is no halting effect of lower late-fall temperatures on the intensity of reproduction in *Daphnia* of Morskie Oko where reproduction, albeit slowed down, continues throughout the winter (Fig. 5). The data on depth distribution showed

that *Daphnia* had to avoid the daytime fish predation by descending to the deep strata of the lake, thus having reproduction reduced at the end of winter when fish of Tatra lakes are known to shift to planktonic food resources (Gliwicz and Rowan 1984).

The synchrony of reproductive effort was most evident in the over-wintering animals which are able to produce very large clutches of eggs (Fig. 4). Experimental studies on *D. pulicaria* and *D. hyalina* showed that a reduced number of eggs per clutch at low food levels allows increased per-offspring investment to raise the chances of juvenile survival, and that the number of eggs per clutch can also be increased substantially at high food levels because reduced per-offspring investment maximizes fitness by optimizing the tradeoff between per-egg investment and number of eggs (Gliwicz and Guisande 1992). In Lake Czarny, the large number of eggs per clutch observed in late June among the similarly large-bodied *Daphnia* of the old generation suggests that the resources allocated to reproduction could be partitioned into a larger number of viable offspring, thus imparting greater fitness to individuals that reproduce within this short period, as opposed to somewhat earlier or later. Earlier, lower food levels would require more per-offspring investment, and clutch size would have to be reduced – as it actually was on the earlier dates of 1996, 16 May (1.5 eggs per clutch) and 3 June (3.78 eggs per clutch; Fig. 6). Later in July, food levels would either be reduced again by the increasing number of *Daphnia* growing rapidly in size (Fig. 3), or the risk of having most eggs destroyed by copepods entering brood cavities would be greatly increased due to the increasing numbers of copepodites in the lake at that time (Fig. 1H).

The danger from *Cyclops* seems more relevant for the reproductive effort in the new generation, whose individuals are too young to reproduce at the best time, in June. The earliest time some, most likely the survivors of the earliest new-generation clutches, could reach maturity, is July (Fig. 4B, E). On 13 July 1996, when 20% of adults bore eggs in clutches of on average four eggs (Fig. 6C), the population was already in its phase of fast increase (Fig. 3A), and food levels must have been declining due to increasing grazing, a fact reflected in both the POC decrease and transparency increase (Fig. 1F, E, respectively). A further increase in population density must limit food still further in August, when the contributions of egg-bearing adults and the clutch size both decrease (Figs. 4A, D, 6D), and when the first ephippial eggs are produced by some adults (Fig. 4B, E).

However, a drop in food levels does not explain why the decline in fecundity is greater in individuals of greater body size, as was evident on 27 August in the much lower contribution of ovigerous females from the class of the largest individuals, as opposed to two classes of smaller adults (Fig. 2), and in a greater reduction in clutch size in large-bodied than in small-bodied adults (Fig. 6D). In her review, Duncan (1989) argues that, with declining food levels, “the slope of the regression flattens as the

food level is lower until, at extreme food limitation, no significant regression can be calculated as all sizes of female are able to produce no more than one or two eggs in a brood”. This reasoning would also have nicely explained the situation observed on 27 August (Fig. 6D) if the food levels were substantially reduced after the earlier dates of 13 July and 2 August, when the mean clutch sizes were 3.69 and 3.62, respectively, and the regression slope was highly significant (Fig. 6C). However, no decline in POC concentration and only a slight increase in Secchi disc transparency (from 21.5 to 23.0 m) could be detected between 2 and 27 August 1996 (Fig. 1E), and nearly 50% ovigerous females were found with 2 or 3 eggs in a clutch, 1.43 in average, on 27 August.

Contrary to the data from the earlier dates when the clutch size on body length regression is typical for a situation of food limitation (Fig. 6A–C, significant regression slopes and adjusted means reflecting the food level as suggested by Duncan 1985, 1989), the regression for the 27 August data is reversed (slope non-significant), suggesting that clutch size is more strongly restricted at greater body size. According to our experimental data on several *Daphnia* species (Gliwicz and Lampert 1994), and field data on *D. pulicaria* itself (Gliwicz and Boavida 1996), such an inversion in the slope of the clutch size on body size regression is typical for strong size-selective predation. A decline in clutch size is more evident in *Daphnia* of greater body size because planktivorous fish selectively remove females with large clutches, and copepods reduce clutch size by removing eggs and embryos from brood cavities of large-bodied *Daphnia*.

Despite much lower numbers of copepodites earlier in June, the clutch size of the old-generation *Daphnia* could also be affected, as seen especially on 17 June (Fig. 6B) when the density of *Cyclops* copepodites had grown from 0 to 900 individuals m^{-3} . At a low *Daphnia* density of 30 individuals m^{-3} , the *Cyclops:Daphnia* ratio increased to about 30:1 (Fig. 1H, G, respectively), and large-bodied *Daphnia* had larger clutches of eggs due to the food level increase of 3–17 June (Fig. 1F). Whether by removal of eggs from brood cavities or by preying on freshly hatched offspring, the abundant *Cyclops*, deprived of any alternate pelagic food source in Lake Czarny, could possibly crop all the offspring produced in August, which were thus not recruited to the *Daphnia* population on any subsequent date (Fig. 2).

Cyclops predation of *Daphnia* juveniles might be an important phenomenon in Lake Czarny, where these become the sole, if difficult, prey, as the rotifers, including *A. priodonta*, have all been exterminated by the end of June, and the naupli gradually moving into the copepodite stage are no longer able to feed on the scarce algae. *Cyclops* copepodites persist at high densities until Lake Czarny freezes in November (Fig. 1), and then likely withdraw into sediments where they form resting stages, as known from other alpine lakes in the Tatra Mountains (Gliwicz and Rowan 1984; Papinska 1988). This withdrawal, which may, in other lakes, be induced by the

disappearance of over-exploited rotifer prey, may, in Lake Czarny, be caused by the lack of *Daphnia* juveniles and *Daphnia* eggs in brood cavities. The prospect of losing eggs from brood cavities may combine with the low chances of neonate survival under the markedly higher risk of *Cyclops* predation in August and September to ensure the replacement of reproduction by immediately hatching eggs with the production of diapausing eggs in ephippia. This shift may reflect either direct encounters with late *Cyclops* copepodites, or information from chemical cues on the danger of predation risk. It may also be an effect of a seasonal change in photoperiod, which can be conceived as an easy cue to the changing predation risk and food level (Slusarczyk 1999).

The extraordinarily long lifespan of *Daphnia* from Lake Czarny and the phenomenon of a single cohort with a few individuals surviving the long winter to reproduce a year later would seem to be an expression of the forces, one selecting for the most efficient utilization of resources, and another selecting for the most effective avoidance of predation risk in the lake. Each seems achievable through proper timing of reproduction and hatching from the dormant stage. This is so because it is the first instars, the neonates, that are most vulnerable to both hunger and copepod predation. Both low food levels and high predation risk are body size related, as demonstrated for *Daphnia* (Gliwicz 1990, 1994, respectively). The life history synchronization in the Lake Czarny *Daphnia* population can therefore be regarded as an example of strong intra-specific competition for scarce resources which is won by those individuals born at the proper time coinciding with a short-lived time window of increased food level and relaxed danger of predation by copepods. Such strong synchrony in the life history may only happen when a single planktonic herbivore species monopolizes resources in the absence of fish predation, such that the density of its population is equal to the carrying capacity of the habitat.

Intuitively, one might presume that, in accordance with Pearl's (1928) hypothesis of "longevity as a consequence of rate of living," the exceptionally long lifespan of *Daphnia* in Lake Czarny might be related to the low temperatures typical for an alpine lake. Living at a slow pace might simply take longer. However, harsh food limitation seems to be a more convincing explanation, for longevity has been already shown to be enhanced by a low feeding rate. In nicely designed laboratory experiments with a single clone of *D. pulex*, Lynch and Ennis (1983) found that lifespan increased gradually with decreasing food availability up to a critical food concentration at which longevity declined rapidly. The low food levels in Lake Czarny are apparently being maintained by *Daphnia* grazing at a level that is just a bit above that critical food concentration. This may allow animals to live for a year, a phenomenon not expected in the majority of aquatic habitats where food levels are higher because planktonic herbivores are controlled by fish.

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