

Small effective population sizes in two planktonic freshwater copepod species (*Eudiaptomus*) with apparently large census sizes

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Abstract

In small planktonic organisms, large census sizes (N_c) suggest large effective population sizes (N_e), but reliable estimates are rare. Here, we present N_e/N_c ratios for two freshwater copepod species (*Eudiaptomus* sp.) using temporal samples of multilocus microsatellite genotypes and a pseudo-likelihood approach. N_e/N_c ratios were very small in both *Eudiaptomus* species (10^{-7} – 10^{-8}). Although we hypothesized that the species producing resting eggs (*E. graciloides*) had a larger N_e than the other (*E. gracilis*), estimates were not statistically different (*E. graciloides*: $N_e = 672.7$, CI: 276–1949; *E. gracilis*: $N_e = 1027.4$, CI: 449–2495), suggesting that the propagule bank of *E. graciloides* had no detectable influence on N_e .

Introduction

The effective population size, N_e , summarizes the relative magnitude of random processes (genetic drift) as opposed to deterministic processes (selection) on allele frequencies in a single variable (e.g. Wright, 1931, 1938; Hedrick, 1999). Estimations of N_e from life-history data are difficult if not impossible to obtain in wild populations (Schwartz *et al.*, 1998; Wang & Caballero, 1999; Waples, 2002). Often, estimations from genetic marker data provide the only alternative. From these data, the so-called variance effective size can be obtained by relating the measured variance in allele frequency of a 'real' population to that of an ideal population of infinite population size (Hedrick, 1999).

In various species, there are pronounced discrepancies between the ecological size of a population (census size, N_c) and its population-genetic, effective population size (Frankham, 1995). In planktonic organisms, N_e/N_c ratios have rarely been studied so far. However, even if we expect much lower N_e compared with N_c , effective population sizes should be considerable in planktonic species, as specimens are small and numerous, individuals are passively transported by movements of the water, the combination of mating pairs should be near

random and dispersal should effectively prevent populations from spatial genetic isolation.

Several 'temporal methods' have been developed to estimate N_e from changes in allele frequencies over time (e.g. Krimbas & Tsakas, 1971; Waples, 1989; Williamson & Slatkin, 1999; Wang, 2001). A common assumption of all these approaches is that allele frequencies only change due to genetic drift, whereas the role of migration, mutation and selection is negligible. Temporal methods either follow a moment-based or maximum-likelihood-based approach (ML). The latter was shown to have higher accuracy and precision compared with moment-based approaches (e.g. Williamson & Slatkin, 1999; Berthier *et al.*, 2002; Wang & Whitlock, 2003) and Wang (2001) found that rare alleles are the main cause for the differences in performance. As most natural populations are not completely isolated but open to gene flow, emigration or immigration of alleles by propagules or migrant individuals will change local allele frequencies over time. Recently, Wang & Whitlock (2003) developed a pseudo-likelihood approach to estimate N_e that accounts for both migration and drift, rather than drift alone. Their approach allows us to estimate N_e and the fraction of migrants (m) jointly from temporal samples (Wang & Whitlock, 2003), and will therefore be employed in this study.

Here, we examined N_e of the two planktonic freshwater copepods *Eudiaptomus graciloides* Lilljeborg and *E. gracilis* Sars. We estimated N_e from four temporal

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samples taken from a lake in northern Germany and compared these with the estimates of N_e , which we calculated on the basis of abundances data from earlier investigations (Fußmann, 1996; Hofmann, 1979). Further, we compared N_e between *E. graciloides* and *E. gracilis*. Although both species co-occur in a number of lakes in northern Europe and reveal strong morphological similarities (Kiefer, 1978; Nauwerck, 1980; Einsle, 1993), they exhibit marked differences in their life cycle, namely the presence of a diapausing egg bank in one, but not in the other species (Santer *et al.*, 2000). Long-term diapausing eggs can remain viable over decades and establish resting propagule banks (Hairston *et al.*, 1995; Cáceres, 1998), which can slow down the rate of microevolution (Hairston & DeStasio, 1988). Hence, 'old' genotypes are stored in the sediments and the recruitment of stages that have remained dormant over longer time periods should contribute to the maintenance of genetic diversity of today's populations (reviewed in Bilton *et al.*, 2001). Here, we tested the hypothesis that *E. graciloides* has a larger effective population size compared with its congener *E. gracilis* due to the existence of a resting propagule bank in the former species. Additionally, we explored different scenarios of likely generation times in *Eudiaptomus* species combined with different source populations, from which migrants were received, to investigate how these differences might influence the estimates of N_e and m .

Materials and methods

Sampling

Population samples of each *Eudiaptomus* species were taken from Lake Schöhsee (northern Germany, 54°09'N, 10°26'E) at four sampling dates: March 2002, March/April 2003, August 2003 and March 2004, representing a total of approximately four to six generations (see below). Samples were taken in vertical hauls with a plankton net and stored in 70% ethanol until further processing. As samples were taken from populations apparently much larger than the sample sizes (Waples, 1989; plan 1), we assume that our sampling did not change the available pool of reproductive individuals (Wang & Whitlock, 2003).

DNA extraction and microsatellite analysis

Female copepods ($n = 177$ /sampling date/species) were isolated with dissecting needles and DNA was extracted with the Invisorb DNA Tissue HTS 96-Kit/C from Invitex (Berlin, Germany) following the manufacturer's instructions. For estimating allele frequencies, we used the polymorphism displayed at three and seven microsatellite loci for *E. graciloides* and *E. gracilis* respectively (EGO2, 7 and 10, GenBank accession numbers AY547392–AY547394; EGI1, 3, 8, 12, 13, 17 and 35, GenBank

accession numbers AY547395–AY547401, Zeller & Reusch, 2004). In the present data set, microsatellite loci represented a total of 119 alleles in *E. graciloides* and 292 alleles in *E. gracilis*. Microsatellite procedures were carried out as described in Zeller *et al.* (2006).

Census population size

We made educated guesses on census population sizes (N_c) for both *Eudiaptomus* species using two studies, one by Hofmann (1979) and a more recent one by Fußmann (1996). Hofmann (1979) sampled adult *E. graciloides* and *E. gracilis* from Lake Schöhsee from April to December 1974 biweekly and calculated their abundances. We averaged these abundances (Hofmann, 1979; Fig. 1) for each species (*E. graciloides*: 0.062×10^6 individuals per m^2 ; *E. gracilis*: 0.025×10^6 individuals per m^2). Considering a lake area of 82.3 ha for Lake Schöhsee (Rai, 1982), estimates of census population sizes were 5.1×10^{10} and 2.1×10^{10} for *E. graciloides* and *E. gracilis* respectively. Fußmann (1996) reported abundances for *Eudiaptomus* sp. in Lake Schöhsee of 5–60 copepods per litre in 1993/1994, corresponding to 5.4×10^{10} – 6.5×10^{11} copepods in the entire lake, well within the range of previous census sizes. To accommodate for spatial and temporal patchiness, we conservatively assumed census sizes in the order of magnitude 10^{10} .

Genetic differentiation between sampling dates

In order to quantify temporal genetic differentiation, we calculated pairwise F_{ST} values between sampling dates with GENETIX v. 4.01 (Belkhir *et al.*, 1998), using the estimator θ (Weir & Cockerham, 1984). Allele frequency distributions and changes in allele frequencies between sampling dates are presented in the Supporting Information for all microsatellite loci.

Effective population size (N_e) and fraction of migrating copepods (m)

In order to estimate N_e in *Eudiaptomus*, we used the temporal approach developed by Wang & Whitlock (2003) implemented in MLNE (available at <http://www.zoo.cam.ac.uk/ioz/people/wang.htm>). This method calculates the variance effective population size, N_e , together with m (migration) using a pseudo-likelihood approach, and is based on the assumption that changes in allele frequencies over time are caused by drift and migration, rather than drift only. Therefore, using this method migration sources and gene flow must be known *a priori*. Per definition, temporal samples are taken from a 'focal population', which is receiving migrants from an infinitely large 'source population' (Wang & Whitlock, 2003). We calculated N_e for *E. graciloides* and *E. gracilis* inhabiting Lake Schöhsee in northern Germany (focal populations). To define source populations, we used

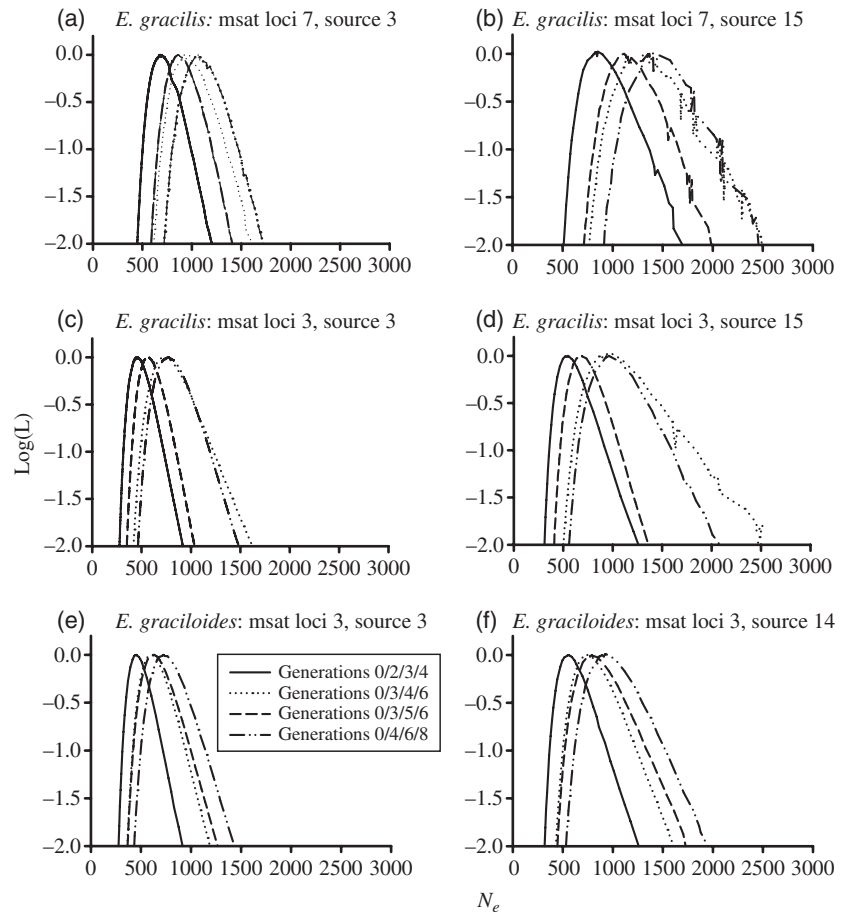


Fig. 1 Log-likelihood curves for effective population sizes (N_e) in *Eudiaptomus graciloides* and *E. gracilis*. Msat, microsatellite; source, numbers of populations that were pooled as source population. The 95% confidence interval for the method implemented here can be calculated as the range of support associated with a drop of two units of log-likelihood (y -axis).

information on allele frequencies, and hence pairwise genetic differentiation, from 14 and 15 populations of *E. graciloides* and *E. gracilis*, respectively, sampled in 2002/2003 from lakes situated on a longitudinal gradient between northern Germany and Lithuania (data from Zeller *et al.*, 2006). Genetic exchange between these populations and the Schöhsee populations differed in intensity. Thus, we rather chose to define two different source populations *a priori* in order to investigate how these differences might influence our estimates of N_e and m . In one source population, we included only three nearby populations from lakes which were interconnected by waterways (Großer Plöner See, lake area: 2910 ha, mean depth: 16 m; Trammer See, lake area:

163 ha, mean depth: 12 m; Kellerssee, lake area: 560 ha, mean depth: 14 m; Muuss *et al.*, 1973) and in a second one we included information from all available populations (Table 1, for further information on source populations see Zeller *et al.*, 2006). Subsequently, we combined the two differently defined source populations with different possible generation times of *Eudiaptomus* sp. in our estimations. As both *Eudiaptomus* species are likely to exhibit two to three generations per year (Bossemann, 1975; Hofmann, 1979; Santer *et al.*, 2000), we calculated N_e for two, three and additionally four generations per year. Accordingly, the number of elapsed generations associated with our four sampling dates (March 2002/March, April 2003/August 2003/March

Table 1 Information on *a priori* defined source populations used in the calculations to estimate N_e and m .

	<i>Eudiaptomus graciloides</i>		<i>Eudiaptomus gracilis</i>	
	Source 1	Source 2	Source 1	Source 2
No. of populations (n)	3 (120)	14 (612)	3 (135)	15 (674)
Mean pairwise F_{ST}^* (SD)	0.01 (0.008)	0.02 (0.017)	0.05 (0.004)	0.08 (0.018)

Source 1: copepods were sampled from lakes located a few hundred metres apart from the Schöhsee (focal) population. Source 2: copepods were sampled from lakes located in a range of a few hundred metres to 1000 km to the Schöhsee (focal) population.

*Values from Zeller *et al.* (2006).

2004) was 0/2/3/4, 0/3/4/6 and 0/3/5/6, respectively, and 0/4/6/8. Allele counts were analysed with the programme GENEPOP v. 3.3 (Raymond & Rousset, 1995) and re-arranged for data input in MLNE. For the comparison between species, we also calculated N_e and m for *E. gracilis* with the information from three primer pairs only, which represented a similar number of independent alleles (106; independent alleles = total number of alleles – number of loci, Wang, 2001) as the three primer pairs used for *E. graciloides* (116). The number of independent alleles is an important parameter in the pseudo-likelihood approach, as an increase in independent alleles increases the accuracy and precision of the N_e estimates (Wang, 2001). In our study, we also performed estimations of N_e in *E. gracilis* using a reduced set of alleles in order to evaluate the influence of the number of loci (and alleles) on the estimation procedure.

Additionally, we present estimates of N_e values for both *Eudiatomus* species calculated with a function implemented in the MLNE programme that takes no migration into account (after Wang, 2001; Table 3).

Null alleles

Our previous studies indicated that null alleles might be abundant in some of the microsatellite loci (Zeller *et al.*, 2006). Temporal changes in allele frequencies should be little affected by null alleles as long as their occurrence is not markedly different between sampling dates. We used three parameters (frequencies of nonamplified samples for individual loci, F_{IS} values and calculated null allele frequencies, programme Microchecker 2.2.1, Brookfield 2, Van Oosterhout *et al.*, 2004) as indicators for the abundance of null alleles. Null alleles were equally distributed among sampling dates in both *Eudiatomus* species. In *E. graciloides*, F_{IS} values (one-way ANOVA: $F_{3,8} = 0.24$, $P > 0.5$), calculated null allele frequencies (one-way ANOVA: $F_{3,8} = 0.46$, $P > 0.5$) and frequencies of nonamplified samples (Kruskal–Wallis ANOVA: $H_{3,12} = 1.55$, $P > 0.5$) were not significantly different between sampling dates. The same was true for *E. gracilis* (F_{IS} values: one-way ANOVA: $F_{3,24} = 0.27$, $P > 0.5$; calculated null allele frequencies: one-way ANOVA: $F_{3,24} = 0.13$, $P > 0.5$; frequencies of nonamplified samples: Kruskal–Wallis ANOVA: $H_{3,28} = 0.82$, $P > 0.5$). The highest numerical

differences in calculated null allele frequencies between sampling dates were in a range of 0.00–0.08 in *E. graciloides* and in a range of 0.02–0.06 in *E. gracilis*.

Results

Genetic differentiation between sampling dates

In *E. graciloides*, all pairwise F_{ST} values that included the March 2004 sample differed significantly from zero (Table 2). In *E. gracilis*, all pairwise F_{ST} values differed significantly from zero except for values for the March 2002 vs. March/April 2003 samples and the August 2003 vs. March 2004 samples, independent of the number of microsatellite loci used in the calculations (Table 2).

Effective population size (N_e) and fraction of migrating copepods (m)

For *E. graciloides*, we estimated N_e values in the hundreds (mean \pm SD N_e for all scenarios: 672.7 ± 141.7) with confidence intervals ranging from 276 to 1949 (Table 3). For *E. gracilis*, mean estimates of N_e calculated with information from all seven microsatellite loci were $1027.4 (\pm 239.1)$ with confidence intervals between 449 and 2495 (Table 3). Mean estimates of N_e for *E. gracilis* calculated with three primer pairs were $696.4 (\pm 171.2)$ with confidence intervals ranging from 277 to 2084 (Table 3). Mean migration rate was 0.03 (± 0.01) for *E. graciloides*, 0.02 (± 0.01) and 0.01 (± 0.004) for *E. gracilis* calculated with information from three and seven microsatellite loci respectively (data for single scenarios, see Table 3). For likelihood curves on which ML estimates and confidence intervals were based, see Figs 1 and 2. The occurrence of a lot of private rare alleles can lead to uneven log-likelihood curves (J. Wang personal communication), as they tend to cause difficulties in the computational process. Estimates of N_e increased consistently with increasing generation times for all scenarios within both *Eudiatomus* species (Table 3). N_e estimates and the associated confidence intervals were shifted upwards considering more populations in the *a priori* defined source population (Table 3). In *E. gracilis*, values of N_e calculated with seven microsatellite loci were higher compared with that in *E. graciloides*, but confi-

	<i>Eudiatomus graciloides</i>	<i>Eudiatomus gracilis</i> (7)	<i>E. gracilis</i> (3)
March 2002–March/April 2003	–0.0002	0.0002	0.0005
March 2002–August 2003	0.0004	0.0015	0.0026
March 2002–March 2004	0.0027	0.0013	0.0030
March/April 2003–August 2003	0.0006	0.0058	0.0103
March/April 2003–March 2004	0.0035	0.0046	0.0097
August 2003–March 2004	0.0046	0.0002	–0.0010

Table 2 Pairwise genetic differentiation between sampling dates.

The number of used microsatellite loci is given in brackets. F_{ST} values that differed significantly from zero are given in bold.

Table 3 Effective population sizes (N_e) and fraction of migrating copepods (m) for *Eudiatomus graciloides* and *E. gracilis* estimated by MLNE according to a pseudo-likelihood approach.

	GS	Migration taken into account (Wang & Whitlock, 2003)								No migration assumed (Wang, 2001)	
		Source 1				Source 2				N_e	95% CI
		N_e	95% CI	m	95% CI	N_e	95% CI	m	95% CI		
<i>E. graciloides</i>	0, 2, 3, 4	452.9	276.3–917.0	0.045	0.020–0.083	551.8	317.4–1258.7	0.025	0.010–0.048	1182.2	644.5–3861.8
	0, 3, 4, 6	601.8	364.1–1190.0	0.032	0.015–0.058	745.5	431.4–1609.0	0.018	0.008–0.033	1593.4	870.6–4977.3
	0, 3, 5, 6	621.9	367.0–1268.0	0.031	0.014–0.057	789.4	445.5–1729.1	0.016	0.007–0.032	1701.1	924.5–5338.5
	0, 4, 6, 8	726.0	433.6–1435.6	0.025	0.012–0.046	892.5	531.4–1949.1	0.014	0.006–0.026	2056.1	1125.7–6178.2
<i>E. gracilis</i> (7)	0, 2, 3, 4	677.3	449.4–1201.5	0.022	0.011–0.035	837.3	510.8–1697.2	0.017	0.007–0.029	2269.8	1241.9–8411.5
	0, 3, 4, 6	968.6	617.8–1613.5	0.015	0.008–0.024	1322.1	765.6–2495.3	0.010	0.008–0.018	3434.6	1798.5–16 746.2
	0, 3, 5, 6	867.3	589.9–1412.6	0.016	0.010–0.026	1114.8	712.5–1987.6	0.012	0.006–0.019	2912.1	1677.2–8152.0
	0, 4, 6, 8	1062.9	718.6–1724.6	0.013	0.008–0.020	1368.8	913.2–2459.6	0.009	0.007–0.015	3915.0	2183.4–12 597.8
<i>E. gracilis</i> (3)	0, 2, 3, 4	455.5	276.9–919.4	0.032	0.015–0.060	548.6	309.9–1260.6	0.021	0.008–0.042	1258.2	658.8–4860.8
	0, 3, 4, 6	728.3	418.6–1624.3	0.019	0.008–0.037	871.2	502.1–2465.4	0.013	0.004–0.024	2189.5	1033.1–20 262.0
	0, 3, 5, 6	564.8	351.6–1037.6	0.025	0.013–0.045	672.9	407.4–1361.9	0.016	0.008–0.030	1505.5	848.4–4010.2
	0, 4, 6, 8	767.3	463.8–1486.0	0.018	0.009–0.032	962.3	558.7–2083.7	0.011	0.005–0.021	2198.9	1164.2–7552.9

GS, generation sequence assumed in the calculations. Source 1: formed from three nearby populations ($n = 120$ and 135 for *E. graciloides* and *E. gracilis* respectively). Source 2: formed from 14 populations in *E. graciloides* ($n = 612$) and 15 populations ($n = 674$) in *E. gracilis*. The number of microsatellite loci used is given in parantheses. Values in bold belong to nonsmoothed log-likelihood curves (see Figs 1 and 2).

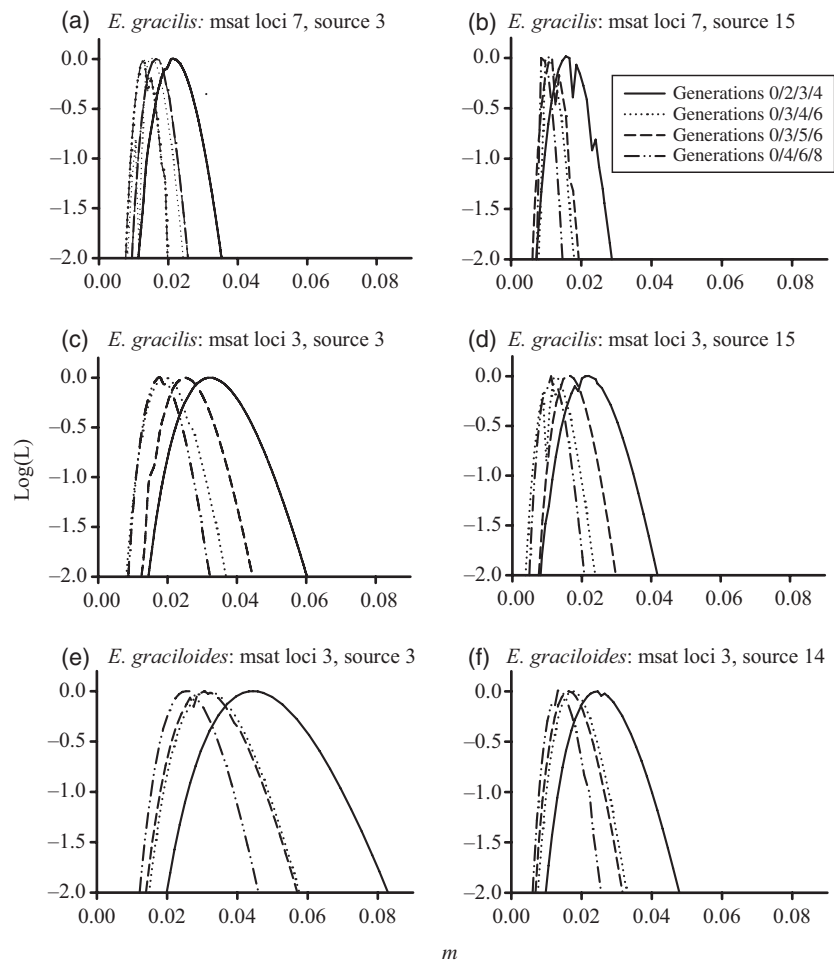


Fig. 2 Log-likelihood curves for the proportion of migrating copepods (m) in *Eudiatomus graciloides* and *E. gracilis*. Msat, microsatellite; source, numbers of populations that were pooled as source population. The 95% confidence interval for the method implemented here can be calculated as the range of support associated with a drop of two units of log-likelihood (y-axis).

dence intervals were overlapping (Table 3). Estimates of effective population sizes were biased upwards approximately two- to threefold when no gene flow was assumed (Wang, 2001), Table 3.

Discussion

In this study, we present estimates of N_e for two species of copepods (*Eudiaptomus* sp.) using high-resolution microsatellite markers together with a novel temporal approach that takes migration into account. We found effective population sizes that were several orders of magnitude smaller than their likely census sizes. Although census sizes are often larger than effective population sizes as reported from a number of species (Frankham, 1995), such small N_e/N_c ratios are nevertheless surprising for plankton organisms. One would assume considerable within-lake exchange of individuals due to passive transport within a relatively small water body. Earlier studies had already pointed to small effective population sizes in *Eudiaptomus* spp. due to the occurrence of high F_{IS} values and departures from Hardy–Weinberg equilibrium in a number of *E. graciloides* and *E. gracilis* populations (Zeller *et al.*, 2006). However, it was difficult to decide whether this was caused by a real biological process or, for example, by the presence of null alleles. It is likely that null alleles occurred in the present study, but their frequencies were equally distributed between sampling dates. Therefore, the calculation of N_e and m should not have been seriously affected (see Jehle *et al.*, 2001; Wang & Whitlock, 2003).

In aquatic environments, temporal methods to investigate N_e have mostly been applied to teleost species (e.g. Hauser *et al.*, 2002; Heath *et al.*, 2002; Hoarau *et al.*, 2005). Consistent with our findings in copepods, in these studies, N_e was often surprisingly low, although restrictions to genetic exchange among fish individuals are difficult to imagine, and census sizes are large.

Census sizes (N_c) were not known for populations of *E. graciloides* and *E. gracilis*, but it is likely that they are at least in a range of 10^{10} for each species in the entire lake. Thus, N_e/N_c ratios in *E. graciloides* and *E. gracilis* were in a range of approximately 10^{-7} – 10^{-8} . In planktonic organisms, N_e/N_c ratios have rarely been studied so far. Only a few investigations have been made in order to estimate N_e from single genetic samples as $N\mu$ (N = effective population size, μ = mutation rate) or Nm (m = migration) using the polymorphism of microsatellite loci or the variation of mtDNA sequences (Bucklin & Wiebe, 1998; Pálsson, 2000; Bohonak *et al.*, 2006). Bucklin & Wiebe (1998) measured the long-term effective population sizes for the marine copepods *Calanus finmarchicus* and *Nannocalanus minor*, using the variation in mitochondrial DNA sequences. This method relies on the scaling of mutation rates with time, and may be biased due to several historical processes

such as vicariance and recolonization history. Their results of much larger N_e values in a range of approximately 10^8 , and effective female population sizes (from nucleotide diversities) of $\sim 10^5$ are therefore not necessarily at odds with our results. Bohonak *et al.* (2006) calculated relative population sizes ($N_e\mu$) for *E. graciloides* and *E. gracilis* from three lakes in northern Germany with information from mitochondrial DNA sequences. Phylogeographic analyses and Bayesian skyline plots resulted in $N_e\mu$ being rather greater in *E. gracilis* compared with that in *E. graciloides*, although the contrast was statistically not significant for most time points, including the most recent one. Thus, these authors found a pattern congruent with our results despite the fact that the markers and methods used to estimate $N_e\mu$ differed in both studies and that Bohonak *et al.* (2006) calculated $N_e\mu$ together for populations from three lakes, only including the Schöhsee. Therefore, it is likely that a more general phenomenon was found in *Eudiaptomus*.

Small effective population sizes despite much larger census sizes can be caused by substructuring of populations, a skewed reproduction success, unequal sex ratios, nonrandom mating or fluctuating population sizes (e.g. Franklin, 1980; Caballero, 1994; Frankham, 1995; Hedrick, 2005). Earlier studies showed that abundances of both *Eudiaptomus* species in Lake Schöhsee had fluctuated over the years, what might contribute to low N_e (Hofmann, 1979; Santer *et al.*, 2000). More life-history and demographic information for *E. graciloides* and *E. gracilis* would be needed to identify factors that are likely to reduce effective population sizes seven to eight orders of magnitude below their census sizes.

The pseudo-likelihood approach we used in our study accounts for migration and drift, rather than drift alone, and therefore considers a more realistic scenario for estimating N_e in natural populations (Wang & Whitlock, 2003). Nevertheless, source populations must be defined *a priori* and the reliability of N_e estimates might depend on information available on population structure. Consequently, it can be difficult to decide, which migration model provides the most reliable N_e estimates. Reassuringly for our case study, we can utilize extensive data on *Eudiaptomus* population structure from the area. Moreover, for both migrant pool scenarios assumed, we observe essentially identical estimates. Under the absence of migration, estimates of N_e were two- to threefold higher for both *Eudiaptomus* species. Ignoring migration should lead to an overestimation of N_e in cases where constant migration and genetic drift cause populations to approach an equilibrium level of genetic differentiation, and therefore migration slows down the rate of change of allele frequencies (Wang & Whitlock, 2003). However, even if the no migration case was true in our study, N_e/N_c ratios for both study species remain within the same order of magnitude regardless of which migration model was applied.

Estimates of N_e and confidence intervals were both shifted upwards when we included more populations in the *a priori* defined source populations and when using more generations per year in the calculations. In the short term, drift would generally be overestimated, and hence N_e underestimated, if migration was not taken into account for calculations of N_e (Wang & Whitlock, 2003). In our study, migration from only three surrounding populations seemed not to have reflected all migration events properly, leading to smaller N_e values compared with when larger source populations were taken into account. However, migration was low in both species and even lower in the case of the larger source population, which consisted of 612 individuals of *E. graciloides* and 674 individuals of *E. gracilis*. Thus, the impact of gene flow on changes in allele frequencies seemed higher compared with when only three populations are functioning as source population. On the other hand, more alleles seemed to be present in the source population that differed from alleles occurring in the focal population, and thus migration occurred to be lower compared with calculations where smaller source populations were used. Nevertheless, N_e was small and within the same order of magnitude for any of the applied scenarios.

Despite a tendency of N_e estimates to be higher in *E. gracilis* compared with that in *E. graciloides*, this difference was statistically not significant. This suggested that genotypes stored in the resting propagule banks of Lake Schöhsee did not affect the size of the water column population of *E. graciloides* as we initially hypothesized. N_e estimates and confidence intervals were even more congruent when comparable amounts of independent alleles were used in the calculations. Nevertheless, in *E. graciloides*, a prolonged generation time should be considered due to the hatching of long-term diapausing eggs from the sediments. Bohonak *et al.* (2006) estimated a mean generation time of 0.48 per year for *E. graciloides* in Lake Schöhsee from the average of copepods originating from subitaneous eggs and animals that hatched from the egg bank. This generation time approximately corresponded to the generation sequence 0, 2, 3, 4 in our calculations. *Eudiaptomus gracilis* is most likely to exhibit two to three generations per year (Hofmann, 1979; Santer *et al.*, 2000; B. Santer, unpublished data). Therefore, for *E. gracilis*, in the present investigation, N_e probably equals an intermediate value of the estimates made for generation sequences 0, 2, 3, 4 and 0, 3, 5, 6/0, 3, 4, 6. Taking prolonged generation times for *E. graciloides* into account, differences in N_e estimates between both *Eudiaptomus* species were more pronounced, but still not statistically significant. Thus, within our model system of *Eudiaptomus* sp. inhabiting Lake Schöhsee, we could not confirm the general assumption of effective population sizes being greater in a species with a resting propagule bank compared with a closely related species that lacks diapause.

Our study is a further data point for an emerging consensus that, in small planktonic species, effective population sizes can be much smaller than expected. It will be interesting to see whether this pattern holds once more species have been rigorously studied using high-resolution genetic markers and temporal variation in allele frequencies.

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Supporting information

Additional supporting information may be found in the online version of this article:

Table S1 Allele frequencies at each locus for *Eudiaptomus graciloides* and *E. gracilis* at four sampling dates.

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