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## ABSTRACT

To investigate the extent to which anthropogenic perturbations such as eutrophication and trace metal pollution (i.e., environmental stress sensu Odum 1985) influence the reproductive modes of cladoceran populations, we analyzed the abundance of subfossils of the chydorids *Alonella nana* (Baird, 1850) and *Alona affinis* (Leydig, 1860) in sediment cores from three Finnish lakes. Reconstruction of lakes' pollutant history and the biological response of chydorids indicate that in two of the lakes the proportion of individuals reproducing sexually increased with environmental stressors. More specifically, *A. nana* responded to eutrophication in Lake Hampträsk with greater production of ephippia, while *A. affinis* responded to aluminum pollution or acidification in Lake Pieni Majaslampi. In contrast, the reference lake, Lake Iso Lehmälampi, showed no radical changes in sexual reproduction over the two-millennium long sediment record. We conclude that chydorids may use sexual reproduction as a strategy for overcoming unexpected environmental stresses.

Key words: aquatic environments, Crustacea, diapause, subfossils, eutrophication, pollution

## 1. INTRODUCTION

"Environmental stress" is an expression widely used by ecologists to indicate externally applied forces detrimental to ecosystems (Odum *et al.* 1979; Odum 1985). Environmental stress disrupts ecosystem homeostasis and sometimes changes biological structure (e.g., species composition) and function (e.g., metabolism). According to Odum (1969, 1985), external disturbances of low predictability to which organisms have not pre-adapted are most likely to have negative consequences for energetics, nutrient cycling, community structure or other system-level properties. Elliot & Quintino (2007) describe environmental stress as a perturbation that reduces the ability of biological systems to organize, function, and survive, with possible disruption at the level of cell, individual, population, community, or ecosystem.

The influence of environmental stress on the biological functioning of cladocerans (Crustacea) is relatively well established for the widely used genus *Daphnia*, family Daphniidae. For example, Hanazato & Dodson (1995) and Boersma *et al.* (1999) have shown that the life-history characteristics (e.g., growth rate and body size) of *Daphnia* are affected by exposure to predator kairomones, low oxygen and pesticides. Also, sexual reproduction and subsequent diapause in *Daphnia* species have been related to environmental stressors, such as shortening day-length, decline in food availability and increased fish predation (Stross & Hill 1965; Hobæk & Larsson 1990; Pijanowska & Stolpe 1996).

For other species less common of family Chydoridae, similar relationships have been implied to be present (Nevalainen & Sarmaja-Korjonen 2008) but are yet to be adequately established.

In the current study, we use paleolimnological data to evaluate long-term changes in the relative importance of sexual *versus* asexual reproduction in populations of *Alonella nana* (Baird) and *Alona affinis* (Leydig). Sexual reproduction was reconstructed by means of subfossil ephippia of the two species. From previous studies we knew that one of the study lakes had undergone eutrophication (Lake Hampträsk), while atmospheric pollution, involving aluminum and resulting in severe acidification, had occurred in another (Lake Pieni Majaslampi). A third lake (Iso Lehmälampi), for which previous work (Nevalainen *et al.* 2008) indicated a lack of exposure to serious environmental stressors over temporal resolution of the examined sediment core, was included as a reference for the reproductive changes. Our goal was to investigate whether changes in sexual reproduction occur in populations under stress. We hypothesized that sexual reproduction, resulting in diapausal resting eggs and renewed genotypes, could be used as a mechanism for maintaining population homeostasis; i.e. the ability to perform fundamental functions and to sustain vital properties under environmental stress. In addition to the high viability of the resting eggs after dormancy under environmentally unfavorable periods, sexual reproduction increases genetic diversity *via* meiosis and fertilization (Frey

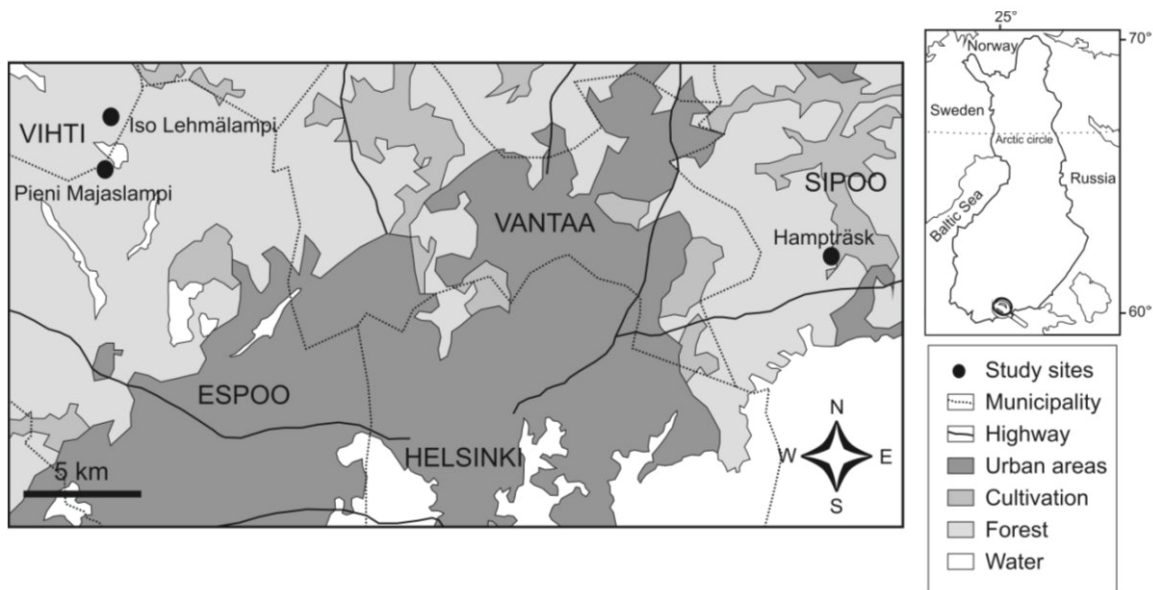


Fig. 1. Location of the study lakes in southern Finland.

1982), increasing also the likelihood that the next generations include stress-tolerant members.

## 2. METHODS

Study lakes Hamträsk (60°17'N, 25°15'E), Iso Lehmälampi (60°20'N, 24°36'E), and Pieni Majaslampi (60°19'N, 24°35'E) are located in southern Finland very close to one another (Fig. 1). The basic limnological variables from the study lakes are presented in table 1. Hamträsk is a eutrophic lowland lake, lying in a clay basin. The lake is shallow (max. depth ~2.5 m) and small (surface area ~3.8 ha). The lake has suffered from nutrient enrichment during past centuries due to increased agricultural land-use in the catchment (Sarmaja-Korjonen 1992; Luoto *et al.* 2008). Lakes Iso Lehmälampi (max. depth 8.1 m, surface area ~5.1 ha) and Pieni Majaslampi (~6.6 m, ~1.1 ha) are oligotrophic and acidic upland lakes lying on granitic bedrock. Due to the limnological and catchment characters, the two lakes can be considered to be sensitive to atmospheric loading (Virkanen *et al.* 1997) and they acidified badly during 1980s (Verta *et al.* 1990; Nyberg *et al.* 2010). Lake selection was based on previous studies (Verta *et al.* 1990; Virkanen *et al.* 1997; Luoto *et al.* 2008; Luoto & Salonen 2010), which indicated a history of severe anthropogenic disturbances (eutrophication and atmospheric pollutants) in them.

Short sediment cores were collected through the ice of lakes Hamträsk (46 cm) and Iso Lehmälampi (24 cm) with a Limnos gravity corer (Kansanen *et al.* 1991) during late winter of 2005. The cores were subsampled in the field at 1 cm intervals and stored in a cold room (at 4 °C and in the dark) for later microfossil analyses. The topmost 14 cm of the sediment core from Lake Pieni Majaslampi was collected with a freeze corer (cf.

Rymer & Neale 1981) through the ice during late winter 2006 and subsampled in the laboratory at 0.5 cm intervals. Several samples from Lakes Hamträsk (at depths 16, 22, 29, 32, 35, 44 cm) and Iso Lehmälampi (at depths 13, 18, 24 cm) were radiocarbon dated (AMS technique) using terrestrial macrofossils and bulk sediment samples at the Poznań Radiocarbon Laboratory in Poland (Tab. 2). The age-depth models used in age estimations of the present study were originally presented by Luoto *et al.* (2008) for Lake Hamträsk and by Nevalainen *et al.* (2008) for Lake Iso Lehmälampi. Instead, the sediment core (12 samples) from Lake Pieni Majaslampi was dated at the Laboratory of Quaternary Geochronology, Institute of Geological Sciences, Poland, using <sup>210</sup>Pb methodology and the CRS age-depth model (Tab. 2, Nevalainen 2008a).

In the laboratory, the sediment samples were prepared for subfossil Cladocera analysis using the procedures described in Szeroczyńska and Sarmaja-Korjonen (2007). For ephippium analysis (Sarmaja-Korjonen 2003, 2004), subfossil chydorid remains were counted until a minimum of 200 chydorid carapaces + ephippia were enumerated in each sample. In the present study, only data from carapaces and ephippia of *Alonella nana* and *Alona affinis* were used. From the relative proportions of carapaces and ephippia, we obtained rough estimates of the relative proportion of asexual *versus* sexual reproduction over time. We used smoothing spline technique (smooth 2.0) to illustrate general temporal trends in sexual reproduction using the program PAST (Hammer *et al.* 2001). Cladocera stratigraphies of the three lakes are provided by Luoto *et al.* (2008), Nevalainen *et al.* (2008) and Nevalainen (2008a). Chydorid ephippia stratigraphies from lakes Hamträsk and Pieni Majaslampi are presented in Luoto *et al.* (2008) and Nevalainen (2008a), respectively. Data on the

**Tab. 1.** Basic limnological properties of the study lakes. The measurements were performed *in situ* from the littoral zones in three seasons during ecological monitoring of modern chydorid communities in 2005.

	Season	Lakes		
		Hamträsk	Pieni Majaslampi	Iso Lehmälampi
pH	Spring	7.0	5.1	5.3
	Summer	6.6	4.9	5.1
	Winter	5.4	4.8	5.8
Conductivity ( $\mu\text{S cm}^{-1}$ )	Spring	41	11	14
	Summer	49	16	17
	Winter	44	20	20
Oxygen ( $\text{mg L}^{-1}$ )	Spring	9.0	9.9	8.4
	Summer	9.2	9.7	8.9
	Winter	5.4	11.0	18.1
Color Pt ( $\text{mg L}^{-1}$ )	Spring	75	5	20
	Summer	75	25	30
	Winter	75	15	40
TP ( $\text{mg L}^{-1}$ )	Spring	30	<10	<10
	Summer	28	<10	25
	Winter	13	<10	10
TN ( $\text{mg L}^{-1}$ )	Spring	981	227	343
	Summer	1123	191	382
	Winter	808	217	457

**Tab. 2.** Results of  $^{14}\text{C}$ - and  $^{210}\text{Pb}$ -dating (CRS model) for cores of the study lakes. For Lake Hamträsk, the chronology was estimated based on the terrestrial macrofossils, as the bulk sediment dates were considered unreliable (see Luoto *et al.* 2008).

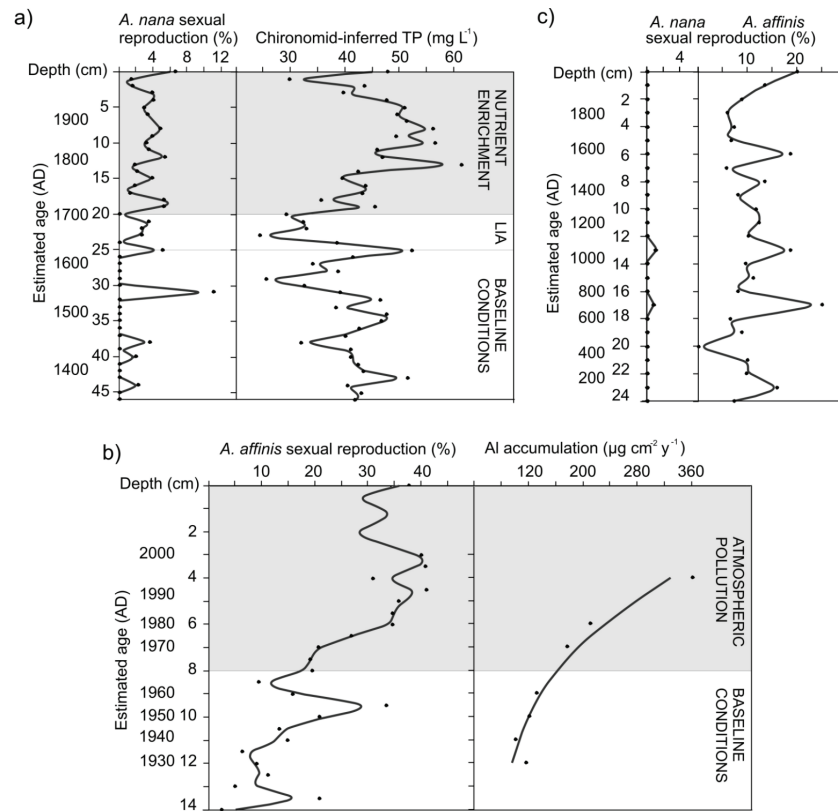
	Depth (cm)	Dated material	Age $\pm$ (AD)	Remarks
Iso Lehmälampi ( $^{14}\text{C}$ )	13	Piece of tree	1095 $\pm$ 46	
	18	Bulk	512 $\pm$ 53	
	24	Bulk	89 $\pm$ 28	
Hamträsk ( $^{14}\text{C}$ )	16	Bulk		Too young
	22	Seed		Too small
	22	Bulk	1346 $\pm$ 37	
	29	Catkin scale	1489 $\pm$ 115	Small (0.12 mg C)
	32	Bulk	1369 $\pm$ 42	
	35	Seed	1550 $\pm$ 61	Small (0.15 mg C)
Pieni Majaslampi ( $^{210}\text{Pb}$ )	44	Seed	1362 $\pm$ 40	
	1		2005 $\pm$ 0.5	
	2		2004 $\pm$ 0.3	
	3		2002 $\pm$ 1	
	4		1994 $\pm$ 2	
	5		1987 $\pm$ 3	
	6		1978 $\pm$ 2	
	7		1969 $\pm$ 5	
	8		1966 $\pm$ 2	
	9		1961 $\pm$ 2	
10		1951 $\pm$ 3		
11		1941 $\pm$ 5		
12		1931 $\pm$ 25		

abundance of *A. nana* (ephippia and carapaces) in lakes Hamträsk and Iso Lehmälampi are discussed in Nevalainen *et al.* (2011).

Spearman's rank correlation coefficient ( $r_s$ ) was used to test statistical significance of relationships between sexual reproduction in *Alonella nana* and chironomid-inferred total phosphorus in Lake Hamträsk and sexual reproduction of *Alona affinis* and aluminum accumulation in Lake Pieni Majaslampi. A moving average (MA) technique with 2-intervals, conventionally used for

smoothing data in time series analysis (Shumway & Stoffer 2006), was applied to the data on sexual reproduction and environmental variables; MA values were then additionally used for correlation analysis.

The chironomid-based TP inference model applied to the Hamträsk core was based on data from 41 shallow lakes in Finland (reduced model) having a coefficient of determination ( $r^2_{\text{jack}}$ ) of 0.92 and a root mean square error of prediction (RMSEP) of  $7.3 \mu\text{g L}^{-1}$  (Luoto 2011). Data on sedimentary Al accumulation in Lake



**Fig. 2.** Long-term trends (smoothing done with smoothing spline method) in incidence of sexual reproduction in *Alonella nana* and *Alonella affinis* as reconstructed from ephippia in sediment cores from a) Lake Hamträsk (past ~700 y), b) Lake Pieni Majaslampi (past ~100 y), and c) Lake Iso Lehmälampi (past ~2000 y). Periods of environmental stress (eutrophication in Lake Hamträsk, atmospheric pollution in Lake Pieni Majaslampi) are indicated as gray bands, while trends in the pollutant (chironomid-inferred total phosphorus and aluminum accumulation rate) are plotted alongside the ephippia data. The culmination period (Luoto *et al.* 2008) of the Little Ice Age (LIA) is marked for the Hamträsk core.

Pieni Majaslampi in ~1990, 1980, 1970, 1960, 1950, 1940, and 1930 AD were taken from Virkanen *et al.* (1997), determined with atomic absorption spectrophotometry.

### 3. RESULTS

The <sup>14</sup>C-dating of the Iso Lehmälampi core was based on one terrestrial macrofossil and two bulk sediment samples, which were in chronological order and had small estimation errors (Tab. 2), providing a valid chronology. According to the dating results, the 24 cm sediment section represents past ~2000 years. On the contrary, the bulk dates from Lake Hamträsk showed inconsistent dates when compared to the dates from terrestrial macrofossils (Tab. 2). Because Lake Hamträsk has been affected for centuries by agricultural land-use, the bulk sediments may have contained older carbon from allochthonous source and therefore the chronology presented here follows the age estimations based on macrofossil dating results (see Luoto *et al.* 2008 for further discussion). Based on the terrestrial macrofossil dates, the 46 cm sediment profile represents the time period from ~1300 AD until present. The chronology of the Pieni Majaslampi core, based on <sup>210</sup>Pb-dating, may

also contain some problems, since <sup>210</sup>Pb specific activity was uniform in the uppermost 9 cm of the core (not shown here). This may be due to sediment mixing or alternatively be a result of post-depositional remobilization and transport of lead along the sediment column without any mixing. However, since there were clear faunal changes within the 9 cm section (Nevalainen 2008a, Fig. 2) it is likely that uninterrupted sedimentation has prevailed in the lake allowing reliable age estimation of the core (Tab. 2). According to the CRS model, the 14 cm section represents roughly the past ~100 years.

Long-term (~700 years) changes in the proportion of asexual/sexual reproduction of *Alonella nana* and *Alonella affinis* populations from the three study lakes are illustrated in figure 2. Sexual reproduction by *A. nana* in Lake Hamträsk was very low and sporadic prior to lake eutrophication (Fig. 2a), although there was a temporary increase (up to ~10%) at ca 1550 AD (30 cm depth in the core). From 20 cm depth (~1700 AD) to the topmost sediment section, the incidence of sexual reproduction by *A. nana* increased, so that surface ephippia made ~6%. The proportion of sexual reproduction was generally ~5% and the average for the core was 2.2%. The chironomid-inferred TP concen-

trations in the core varied between 24 (at 23 cm) and 61  $\mu\text{g L}^{-1}$  (at 13 cm) (Fig. 2a). A general decline in TP was observed prior to 1700 AD (during the LIA), after which an increasing trend was recorded between ~1800 and 1900 AD.

In Lake Pieni Majaslampi *Alona affinis* reproduced sexually with much higher proportions (average 23.9%) during the past ~100 years as *A. nana* in Hamträsk. The proportion of sexual reproduction in *A. affinis* increased abruptly up to about 30% at a sediment depth of 10 cm, which corresponded to a date of about 1950 AD. There was a sharp decrease from ca 1950 to 1960 AD, before rising more steadfastly after ~1960 AD. The highest values (~40%) were recorded in the late 1980s and early 1990s (Fig. 2b). Corresponding values for Al accumulation rate were around 100  $\mu\text{g cm}^{-2} \text{y}^{-1}$  in the 1930 and 1940s, rising rapidly after 1950s to exceed 350  $\mu\text{g cm}^{-2} \text{y}^{-1}$  in the 1990s (Virkanen *et al.* 1997, Fig. 2b).

Both *A. nana* and *A. affinis* were present in sediments from Lake Iso Lehmälampi but *A. nana* ephippia were found in low numbers and only at two sediment depths, 13 and 17 cm, suggesting that this population reproduced almost exclusively asexually. For *A. affinis*, we calculated an average incidence of sexual reproduction of 10.8%. No temporal pattern of ephippia production was discernable over the roughly 2000 years assessed (Fig. 2c).

The Spearman's rank correlation coefficient for *A. nana* and chironomid-inferred TP was moderate ( $r_s = 0.27$ ,  $p = 0.07$ ,  $n = 47$ ), and non-significant at  $p \leq 0.05$ . For *A. affinis* and Al accumulation rate however, it was high and statistically significant ( $r_s = 0.82$ ,  $p = 0.034$ ,  $n = 7$ ). When 2-interval moving averages for species and environmental data were used, all correlations increased in strength and were significant at  $p \leq 0.05$  (Tab. 3).

**Tab. 3.** Spearman's correlation coefficients ( $r_s$ ) and significance levels ( $p$ ) for relationships between sexual reproduction in *Alonella nana* and chironomid-inferred total phosphorus in Lake Hamträsk and sexual reproduction in *Alona affinis* and aluminum accumulation rate in Lake Pieni Majaslampi. Correlations based on 2-interval moving average values for sexual reproduction and environmental variables are marked in boldface.

	$r_s$	$p$
<i>Alonella nana</i> (Hamträsk)	0.27	0.07
	<b>0.30</b>	<b>0.041</b>
<i>Alona affinis</i> (Pieni Majaslampi)	0.82	0.034
	<b>0.93</b>	<b>0.007</b>

#### 4. DISCUSSION AND CONCLUSIONS

The role of sexual reproduction in cyclical parthenogens is well established (Maynard Smith 1968; Frey 1982; Rispe & Pierre 1998; Pękalski 2000). Its occurrence still attracts the attention of scientists, probably because it can be regarded as a paradox (Hurst & Peck 1996; Doncaster *et al.* 2000); males contribute to popu-

lation growth only through females. Sexual reproduction is, in fact, a mechanism through which populations buffer the risk and damage of unexpected external environmental perturbations. It allows a temporary escape from unfavorable conditions through diapause and an increase of the population fitness as the greater genetic variability of the following generation permits selection of better adapted organisms (Lynch & Gabriel 1983; Hurst & Peck 1996). Thus, it is a reaction to stress, as defined by Odum (1985). Spatial variations in the proportion of cladoceran sexual reproduction are obvious in the literature (e.g., Cáceres & Tessier 2004; Nevalainen 2008b), indicating local adaptations among populations. The present study shows that even adjacent cladoceran populations may exhibit strong variations in their reproductive strategies when local environmental conditions are different (cf. Cáceres & Tessier 2004; Nevalainen 2008b).

Our paleolimnological data provide evidence for temporal changes in the reproductive strategies of chydorid species, and suggest that they are coincident with long-term environmental change. In Lake Iso Lehmälampi, our reference system, co-occurring *A. nana* and *A. affinis* populations maintained relatively consistent distributions of sexually and asexually reproducing individuals over two millennia (Fig. 2c). This is in contrast with increasing incidence of sexual reproduction in the other two lakes that have been exposed to serious environmental stress (Fig. 2a, 2b). Sarmaja-Korjonen (2003) anticipated this response of chydorids to environmental stressors after noting a sudden increase in the proportion of ephippia of *A. affinis* and *A. nana* in two sediment cores from lakes in southern Finland. She hypothesized that increased nutrient input and/or altered food-web structure were forcing factors in the change. The increase in sexual reproduction that we observed in the two polluted study lakes was also coincident with the onset of anthropogenic stresses. At ~1700 AD an increasing land-use brought more nutrients into Lake Hamträsk (Luoto *et al.* 2008; Luoto & Salonen 2010) and, as a consequence, *A. nana* increased ephippia production, instead in Lake Pieni Majaslampi *A. affinis* increased sexual reproduction at ~1960 AD as atmospheric pollution became more severe (Virkanen *et al.* 1997) and resulted in acidification and dramatic food-web changes. In both cases, we found statistically significant correlations between proportions of sexual reproduction and the magnitude of the stressor (Tab. 3). Furthermore, both perturbations (eutrophication and atmospheric pollution) are well-documented stressors for aquatic ecosystems (Schindler *et al.* 1985; Cattaneo *et al.* 1998; Manca *et al.* 2007; Jeppesen *et al.* 2009), and are expected to influence directly or *via* indirect forcing mechanisms on population homeostasis of chydorids.

In our reference lake (Iso Lehmälampi), no distinct trends were found in the reproduction patterns of *A.*

*nana* and *A. affinis* (Fig. 2c). Lake Iso Lehmälampi, although it is known to have suffered from heavy acidification due to atmospheric loading during 1980s, showed no clear indication of anthropogenic stress-induced environmental forcing on chydorids (Nevalainen *et al.* 2008). This is most likely due to the rough temporal resolution of the sediment core; the 24 cm sediment core, subsampled at 1 cm resolution, appeared to represent past 2000 years (Tab. 2) and therefore the most recent anthropogenic impacts are not clearly reflected in the stratigraphy. However, it is worth mentioning that sexual reproduction in *A. affinis* increased markedly in the most recent sediment samples (0–2 cm, ~1900 AD onward) and this can be indicative of environmental stress related to atmospheric loading (Fig. 2c).

Evidence for a serious increase in the trophic state of Lake Hamträsk was provided by Luoto *et al.* (2008), who reconstructed the lake's trophic history in a multi-proxy paleolimnological investigation. They attributed the change to an increase in agricultural land use in the lake catchment beginning around 1800 AD. Furthermore, in-lake oxygen concentration, inferred from sedimentary chironomid-stratigraphy, declined concurrent with eutrophication (Luoto & Salonen 2010). Rising trophic state also was evident in the current study from the chironomid-inferred TP reconstruction from the sediment core (Fig. 2a). We interpret the increase in sexual reproduction of *A. nana* population as a response to eutrophication stress, which, most likely, acted both directly (by disturbing population homeostasis e.g. *via* decreased oxygen concentration in bottom waters) and indirectly (changes in food-web). Our interpretation is supported by the fact that the proportion of sexual reproduction of *A. nana* is correlated with the inferred TP in-lake concentration (Tab. 3).

Virkanen *et al.* (1997) found that Lake Pieni Majaslampi received heavy atmospheric loading of several biologically harmful metals (e.g., Al, Cd, Pb), and that the influx increased markedly between the 1960s and 1980s. Trends in aluminum accumulation in the sediment core of the lake clearly confirm this atmospheric pollution (Fig. 2b, Virkanen *et al.* 1997). Virkanen *et al.* (1997) mainly attributed the increase in sedimentary aluminum accumulation to dry precipitation of particles from the atmosphere. During the 1980s, however, Lake Pieni Majaslampi was affected severely by acid precipitation, which decreased pH (~4.5), increased labile-aluminum concentrations in the water, and severely damaged aquatic communities (Nyberg *et al.* 2010). For example, the natural perch (*Perca fluviatilis*) population was extirpated and the food-web changed as invertebrates were established as top-predators (Nyberg *et al.* 2010). Aluminum is a toxic metal having direct effects on aquatic biota and also cascading indirect effects (Havens 1990, Nilssen & Sandøy 1990, Sparling & Lowe 1996, Soucek *et al.* 2001). The increase in sexual reproduction of *A. affinis*,

coincident with the elevated Al accumulation and onset of acidification during 1970–1980s (Fig. 2), may thus have been a homeostatic response to either direct (physiological) stress under changed chemical conditions or to stresses induced through indirect effects, such as altered food-resources and increased benthic invertebrate predation. Most likely, it involved both pathways, and could have been aggravated by the toxicity of metals co-occurring with aluminum.

Cladocera, primarily Daphniidae, have been used in aquatic toxicology as test organisms because of their short life cycle, easy culturing, and sensitivity to chemicals (Münzinger & Mocelli 1991; Muysen *et al.* 2002; Lam & Wang 2008; Ponti *et al.* 2010). In the case of chydorids, however, toxicological studies have been rare (Koivisto *et al.* 1992; Bossuyt & Janssen 2005). To our knowledge, the only publication reporting effects of toxins on chydorid sexual reproduction was published a long time ago by Kiser *et al.* (1963) who found that the annual induction of sexual reproduction in chydorids occurred earlier than previously observed at similar latitudes after toxic pesticide (rotenone) was discharged into the lake. Kiser *et al.* (1963) implied for the first time that sexual reproduction might be regarded as a response of chydorids to environmental stress caused by pollution. We further suggest that this response may be a general reaction to the disturbance of population homeostasis, because similar effects were observed in the present study following two very different types of anthropogenic impacts, eutrophication and metal pollution (Fig. 2). It appears that changes in the water chemistry of the study lakes along with cascading ecological consequences (deteriorated oxygen, altered predation regime) forced chydorid populations to undergo intensified sexual reproduction to escape unfavorable conditions and ensure the survival of future populations through genetic adaptation to the new environmental ranges. Sexual reproduction seems to have a vigorous role in maintaining population homeostasis in cladocerans, whether providing diapause and preserving the vitality of future populations or improving genetic heterogeneity under unexpected environmental stresses. In addition to using subfossil cladocera assemblages as indicators of multiple anthropogenic environmental stressors (DeSellas *et al.* 2011), we emphasize the importance and usability of subfossil ephippia in paleolimnological investigations for providing information about periods of environmental disturbance.

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