

Does oxygen availability regulate sexual reproduction in local populations of the littoral cladoceran *Alonella nana*?

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Abstract We investigated sexual reproduction patterns of a ubiquitous littoral cladoceran *Alonella nana* (Anomopoda, Chydoridae), using ecological and paleoecological approaches to clarify the forcing mechanisms behind its sexual reproduction. Contemporary sampling of *A. nana* populations in two environmentally different lakes showed abundant sexual reproduction in the lake with eutrophic, oxygen-deficient conditions and scarce gamogenesis in the oligotrophic, well-oxygenated lake. Sediment core studies from the same lakes indicated that comparable sexual reproduction patterns had prevailed for many centuries. However, in eutrophic Lake Hamträsk, *A. nana* increased its sexual reproduction from 1700 AD onward, consistently with decreasing trend in chironomid-inferred oxygen. The core samples, together with a surface sediment dataset of 25 lakes from southern and central Finland, showed a negative correlation between ephippia of *A. nana* and winter oxygen, although the surface sediment data per se did not show any significant

correlation. When the dataset was scaled locally to include lakes in close proximity in southern Finland, the correlation became clearer. The results imply that the spatial and temporal variations in sexual reproduction of *A. nana* populations may partly be explained by differences in oxygen levels.

Keywords *Alonella nana* · Chydorids · Diapause · Finland · Paleoecology

The basic patterns of chydorid (Cladocera, Chydoridae) reproduction are generally known; the life cycles of chydorids alternate between active and dormant stages, due to seasonality, and are controlled by photoperiod and water temperature (Shan, 1974; Frey, 1982; Koksvik, 1995; Nevalainen, 2008a). At the end of the active period, induced by deteriorating environmental conditions associated with the oncoming winter, chydorids begin to reproduce sexually. Sexual reproduction results in a diapause through resting egg production and aims to provide a mechanism for the reestablishment of populations after seasonal or aperiodic stresses, a means for passive dispersal, and to generate new genotypes (Frey, 1982). Resting eggs are enclosed by ephippia, which are modified and thickened carapaces, and are very tolerant to environmental deterioration, and chydorids emerge from resting eggs when environmental conditions are favorable. The reproduction

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patterns of chydorids can also be examined by paleoecological approaches using subfossil chydorid assemblages (Sarmaja-Korjonen, 2003, 2004), because chitinous chydorid carapaces (indicating asexual reproduction) and ephippia (indicating sexual reproduction) are preserved well and are identifiable to species level in lake sediments.

Of the chydorids, *Alonella nana* (Baird) is a common and widely distributed species in the Holarctic and thrives in a wide range of aquatic environments and habitats (Fryer, 1968; Whiteside, 1970; Duigan, 1992; de Eyto et al., 2003; Nevalainen & Sarmaja-Korjonen, 2008a, b). Although chydorids are a moderately well-studied group, many aspects of their population ecology such as reproduction behavior remain narrowly examined. In the case of *A. nana*, only a few records of its reproduction cycle exist, showing that the species is perennial in Estonia, Germany, and Norway (Mäemets, 1961; Flössner, 1964; Koksvik, 1995) and has a low incidence of sexual reproduction (Järnefelt, 1956; Frey, 1982; Koksvik, 1995). In accordance, two distinct studies from southern Finland (Luoto et al., 2008; Nevalainen & Sarmaja-Korjonen, 2008a) reported dualistic reproduction behavior in *A. nana*, since it showed very high or extremely low sexual intensity in different types of lakes. Consequently, in this study we hypothesize that dissolved hypolimnetic oxygen directly (metabolism) or indirectly (resource availability) regulates sexual reproduction in *A. nana* populations. We combined contemporary and sedimentary data to examine its reproduction, and explored the relationship between its sexual reproduction and dissolved hypolimnetic winter oxygen.

The 25 small and shallow study lakes are located in southern and central Finland. Dissolved winter oxygen varies in the lakes between 0.5 and 18.1 mg l⁻¹, pH between 4.2 and 8.0, and conductivity between 8 and 312 μS cm⁻¹. Two lakes were used as case study sites; Lake Hampträsk (60°17' N, 25°15' E) is a meso-eutrophic lake with human settlement in its catchment (winter oxygen in 2005 5.4 mg l⁻¹) and Lake Iso Lehmälampi (60°20' N, 24°36' E) is an acidic and oligotrophic forest lake, having high year-round oxygen concentrations (winter oxygen in 2005 18.1 mg l⁻¹, being the highest among the study lakes).

Contemporary sampling of chydorids was performed weekly during the autumnal sexual reproduction period

in lakes Hampträsk and Iso Lehmälampi. The samples were taken with 100-μm plankton net, which was swept back and forth over the lakeshore and mounted in glycerine jelly on preparation slides in the laboratory. Chydorid individuals (parthenogenetic females, gamogenetic females, and males) were counted under a light microscope, until a minimum of 100 were encountered or four preparation slides (24 × 50 mm) were scanned through. The relative proportions of gamogenetic *A. nana* individuals were calculated from the total number of all *A. nana* individuals in each sample. The proportions of autumnal gamogenesis in the chydorid communities are originally presented by Nevalainen & Sarmaja-Korjonen (2008a).

Short sediment sequences were cored with a Limnos gravity corer from Lake Hampträsk (46 cm) and Lake Iso Lehmälampi (24 cm) through ice in 2005, and the sediments were subsampled at 1-cm intervals in the field. Surface sediments (topmost 1–2 cm) from the remaining 23 lakes were sampled with a Limnos corer through ice in 2005. In the laboratory, the samples were prepared for subfossil Cladocera analyses for concentration calculations, according to Szeroczyńska & Sarmaja-Korjonen (2007). The slides were examined under a light microscope until a minimum of 200 chydorid carapaces and ephippia were enumerated. In the surface sediment and sediment core samples, the relative proportions of *A. nana* carapaces and ephippia were calculated from the sum of *A. nana* carapaces added to *A. nana* ephippia. The proportions of ephippia of all chydorid species in the chydorid community of Lake Hampträsk are presented in Luoto et al. (2008). A previously available chironomid-based inference model for late-winter hypolimnetic oxygen (Luoto & Salonen, 2010) from southern and central Finland was used to provide the oxygen inferences for the sediment sequences. Pearson's correlation (*r*) was used to test the relationship between dissolved hypolimnetic oxygen and proportion of ephippia of *A. nana* in the surface sediment and sediment core samples.

The results showed that *A. nana* was very abundant in the autumnal communities in lakes Hampträsk and Iso Lehmälampi (Fig. 1a), illustrating that it thrives in both of these limnologically different lakes. However, *A. nana* reproduced with very high sexual intensity in Hampträsk and with extremely low intensity in Iso Lehmälampi (Fig. 1a). Previous

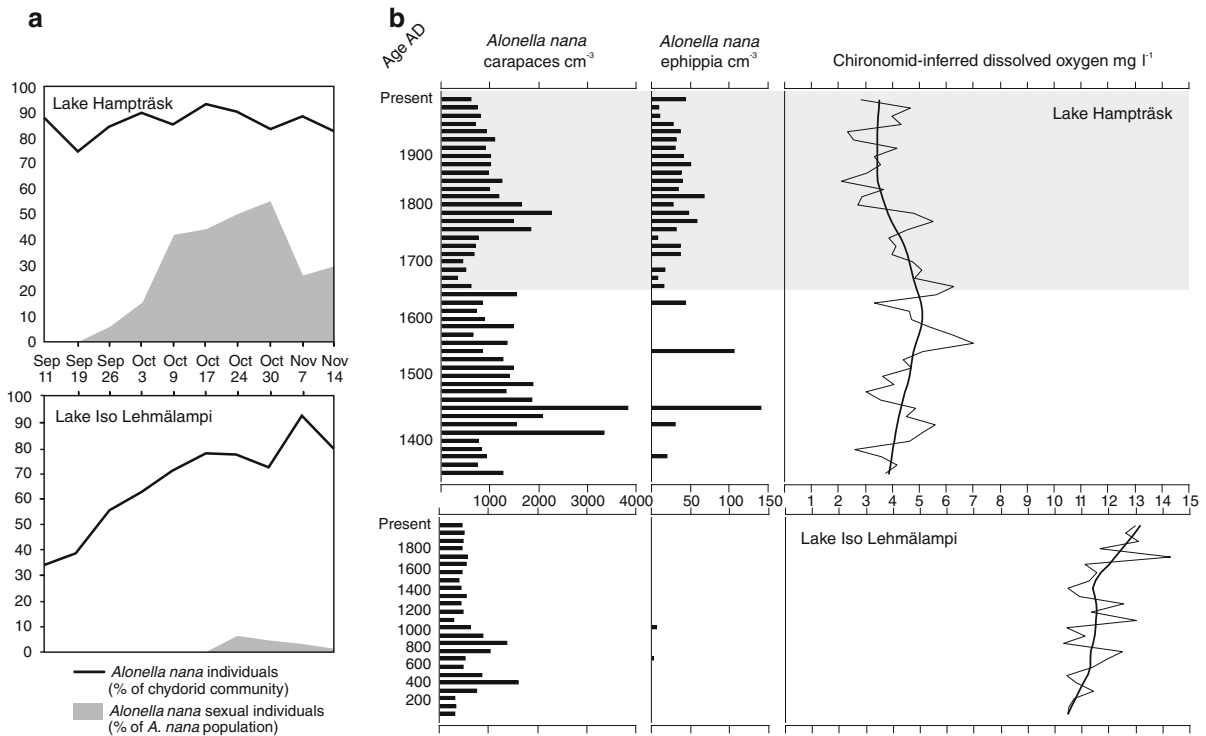


Fig. 1 Modern and past reproduction in populations of *A. nana* in lakes Hamträsk and Iso Lehmälampi. **a** Percentage proportions of *A. nana* individuals of the chydorid communities and sexual individuals (males and sexual females) of the *A. nana* populations during the autumnal sexual reproduction period. **b** Numbers of *A. nana* carapaces (asexual reproduction) and ephippia (sexual reproduction) together with chironomid-

inferred dissolved oxygen reconstructions, applied through a lowness degree of smooth (span 0.45), in the sediment cores. The gray color indicates the period of intensified sexual reproduction in *A. nana* in Lake Hamträsk, and the age estimates are based on radiocarbon dates by Luoto et al. (2008) for Lake Hamträsk and by Nevalainen et al. (2008) for Lake Iso Lehmälampi

studies have shown that the intensity of sexual reproduction in chydorid species may vary among populations, probably indicating local adaptations (Frey, 1982; Nevalainen, 2008b; Nevalainen & Sarmaja-Korjonen, 2008a, b). Diapause is for survival of populations under environmental deterioration, such as winter or drought, when active individuals would not survive (e.g., Frey, 1982). The hypothesis of Lynch (1983) and Cáceres & Tessier (2004) on *Daphnia* Müller (Daphniidae) suggests that populations with high probability of persistence year-round have a low incidence of diapause (e.g., permanent waters). A high incidence is typical for populations exhibiting seasonal changes in abundance (e.g., temporary pools), thus being linked with the physical environment of the populations. High incidence of diapause may occur in *Daphnia* also under high predation pressure

(Ślusarczyk, 1995, 2001), lowering the probability of populations to persist year-round. Active chydorids have been encountered under ice (Heywood, 1967; Keen, 1973), and *A. nana* is reported perennial in Estonia and Norway (Mäemets, 1961; Koksvik, 1995). Therefore, some *A. nana* populations may be perennial in the southern parts of Finland, despite the icecover. In this respect, the monitoring (Fig. 1a) may suggest that the variation in sexual reproduction among the two populations may be explained by the physical environment in the lakes; high incidence of sexual reproduction in the lake with low oxygen during winter and low probability of survival (Lynch, 1983; Cáceres & Tessier, 2004), and low incidence in the lake with elevated winter oxygen and high probability of survival.

Furthermore, the reproduction patterns shown by the contemporary data have prevailed in the lakes in

the past as well (Fig. 1b). *A. nana* has been an abundant part of the chydorid fauna in lakes Hampträsk and Iso Lehmälampi throughout the sedimentary records. Sexual reproduction in *A. nana* in Iso Lehmälampi rarely occurred, indicating that similar sexual reproduction patterns as observed in the modern samples (Fig. 1a) have prevailed for 2,000 years. The oxygen conditions in the lake have also been constantly favorable, since the chironomid-inferred dissolved oxygen concentrations were continuously high (Fig. 1b). However, the past sexual reproduction patterns in *A. nana* in Hampträsk clearly changed after 1700 AD, when ephippia began to appear constantly and increased in abundance. Simultaneously, the trend in chironomid-inferred dissolved oxygen decreased from the improved oxygen conditions associated with decreased production during the climate cooling of the Little Ice Age (Luoto et al., 2008; Luoto & Salonen, 2010). These changes were probably caused by the climatic warming and eutrophication of the lake due to increased land use, reducing the dissolved late-winter oxygen availability (Luoto et al., 2008) and acting as an environmental stressor to the species (Sarmaja-Korjonen, 2003).

In general, cladoceran sexual reproduction is believed to be under environmental control, although studied mostly with planktonic species, including water temperature, day length, predation pressure, crowding, and food depletion (e.g., Stross & Hill, 1965, 1968; Hobæk & Larsson, 1990; Larsson, 1991; Kleiven et al., 1992; Ślusarczyk, 1995, 2001). In chydorids, climatic signals are believed to be the main inducers and regulators of sexual reproduction (Shan & Frey, 1968; Shan, 1974; Kubersky, 1977). In the present results, the proportions of ephippia of *A. nana* and dissolved hypolimnetic oxygen in the surface sediment dataset showed no correlation ($r = 0.21$, $P = 0.33$, and $N = 25$), but when calculated for all sedimentary, both surface and core samples ($N = 97$), there was a clear correlation ($r = 0.49$, $P = <0.001$). However, the combination of the core samples to calculate the correlation includes the potential problem of pseudoreplication (Hurlbert, 1984), as the core samples are not independent from each other. Therefore, the temporal pseudoreplication may act as a possible source of error in this statistical inference hampering the

relationship, although two independent “treatments” (i.e., *A. nana* populations in the sediment cores) may somewhat smooth down the potentiality of pseudoreplication. Pseudoreplication is an issue not often addressed in paleoecology and could be tackled, for example, by using multiple samples from same depths from a sediment core, as done by Kerfoot & Weider (2004). Furthermore, the relationship between ephippia and dissolved oxygen became stronger when only local populations in southern Finland (within a radius of ca. 25 km) were included in the analysis ($r = 0.82$, $P < 0.01$, and $N = 9$). These results may imply that non-climatic environmental factors also partly regulate sexual reproduction in local populations of chydorids. However, it must be kept in mind that correlation does not necessarily indicate causality and “spurious correlations” may be obtained without a true connection between two functions (Aldrich, 1995).

If the above-mentioned correlation between oxygen and sexual reproduction would mean causality, this type of local response would provide evolutionary advantage, because it improves the ability of the populations to adapt to changing environmental conditions due to the genetic variability created in sex (Maynard Smith, 1968; Pękaliski, 2000). Nevertheless, further exploration is required to determine accurate relationship between oxygen conditions and sexual reproduction in chydorids, and hence, data from controlled common garden experiments would provide mechanistic evidence for the role of oxygen in the control of sexual reproduction. In addition, to elucidate whether the observed variations (Fig. 1a) in reproduction among *A. nana* populations are caused by specific genetic structures or by phenotypic plasticity (cf. Lynch, 1983), extensive studies are required. In conclusion, the combination of ecological and paleoecological data in this study provided the means for investigating the reproduction patterns in *A. nana* populations at different spatial and temporal scales, providing some new insights on its dualistic sexual reproduction. This study also demonstrated that paleoecological studies may provide valuable data on the population ecology of individual chydorid species.

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