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Effect of high population density on growth and reproduction of *Daphnia pulex* DeGeer

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Abstract: *Daphnia pulex* was reared at two different densities (1 and 30 indiv. [50 ml]$^{-1}$) in order to investigate the effect of high population density on the growth and reproduction of individuals with food supplied in excess. The animals in high population density cultures showed similar patterns of growth and similar age at first reproduction compared with those in low density cultures. In the high density cultures, however, the carapace length was significantly smaller after maturation, and the cumulative number of neonates produced for 15 d from first reproduction was also significantly lower. Ephippial eggs were observed only in high density cultures. These results suggest that high population density depresses both growth in adult instars and parthenogenetic egg production in *D. pulex* and induced ephippial egg production even under conditions of sufficient food supply.

Key words: crowding effect, *Daphnia*, growth, reproduction

Introduction

Daphiniid cladocerans are known to thrive in freshwater environments. In field studies of the population dynamics of *Daphnia*, a striking transition from a low-density spring population with a large mean brood size to a high-density summer population with much lower egg production has frequently been observed (e.g. Brooks 1946; Kerfoot et al. 1985). Some of these studies concluded that small brood size in a high density population was a consequence of a reduction in food supply (Clark & Carter 1974; Hebert 1978). In laboratory studies, a similar relationship between population density and egg production has been shown (e.g. Frank et al. 1957; Kerfoot et al. 1985). Recently, however, growth and reproduction in daphniids have been shown to be affected by high population densities or by addition of water from crowded cultures, even under sufficient food concentrations (Seitz 1984; Guisande 1993; Matveev 1993; Burns 1995). Information on such effects is still scarce. Furthermore, there have been no studies on the effect of crowding through the whole life of the animal.

In this study, *Daphnia* pulex individuals were reared at two different densities in the laboratory to examine the effect of high population density on their growth and reproduction under conditions of sufficient food supply. Somatic growth from hatching to adulthood, days at first reproduction, and number of neonates produced by the animals during the experiments were monitored.

Materials and Methods

Daphnia pulex, which had been maintained in the Plankton Laboratory, Hokkaido University for about three years, were used. A clone was established from a single female isolated from the culture. Water used in stock and experimental cultures was collected from Lake Ohnuma, near our laboratory and then filtered through a glass-fiber filter (Whatman GF/C). The stock cultures were maintained on *Chlamydomonas reinhardi* (IAM, strain no. C–9) at a concentration of $>5\times10^5$ cells ml^{-1} at 20°C, 12L : 12D. *C. reinhardi* was grown on the medium described by Ichimura (1971) at 20°C, 12L : 12D, 4,000 lux. After the cells from 7 d-old cultures were centrifuged and washed with filtered lake water, the cell concentration was calculated from hemacytometer counts before addition into the experimental cultures.

Two series of experiments for low and high population densities, i.e. 1 and 30 indiv. (50 ml)$^{-1}$, respectively, were made at 20°C, 12L : 12D, 60 lux. These densities of cladocerans are within the range that can be observed in natural environments (e.g. up to 1000 indiv. l^{-1}, Barker & Hebert 1990), and have been used for laboratory studies (e.g. Stross & Hill 1968). We used different food concentrations corresponding to the low- and high-density conditions in order to insure sufficient food supply. In the low density experiment, a neonate born within a 24-h period in the stock culture was placed in a 50-ml jar containing 50 ml of filtered lake water and the food alga at 10^5 cells ml^{-1}. In the high density experiment, the 30 neonates were placed in a 50-ml jar containing 50-ml filtered lake water and the food alga at 5×10^5 cells ml^{-1}. The animals were transferred to new jars containing fresh food suspensions at 24-h intervals. According to Lampart & Schober (1980) and Urabe (1988), the lowest food concentration at which the maximum egg production rate can be maintained by *Daphnia* of 2-mm length is at about 2.4×10^4 cells ml^{-1} of *C. reinhardi*. A preliminary experiment showed that the concentration of food that remained after a 24-h period did not fall below this concentration in either of the density experiments.

Growth and reproduction of the animals in both density cultures were checked at 24-h intervals. The animal was placed on a slide glass and its carapace length was measured with an optical micrometer attached to a dissecting microscope. When newly born neonates or ephippial eggs were produced, the number of them in the jar was also recorded (at a 48-h interval in the case of the 1 indiv. [50ml]$^{-1}$ cultures). Following this, the individuals were removed in order to insure a constant population density. Carapace length was measured over a period of 25 d. The number of neonates and ephippia (i.e. ephippial eggs) were counted until 15 d after first reproduction. Of the low and high density experiments, there are 23 and 13 replicates, respectively.

Results

The relationship between age and mean carapace length of the animals at the two densities is shown in Fig. 1. During the experiments, the gross pattern of the increase in carapace length was very similar under both density conditions. However, the mean carapace length in the high density experiment was significantly smaller, ca. 8 %, than that in the low density experiment after 10 d from the beginning of the experiment (*t*-test, $p<0.05$), though there were significant differences in carapace length between the two densities also from day 1 to 3.

The animals required about 8 d to mature in both of the density experiments, and there was no significant difference in age at first reproduction (AFR) under the different density cultures (*t*-test, $p>0.1$, Table 1). However, the cumulative number of neonates produced by a female

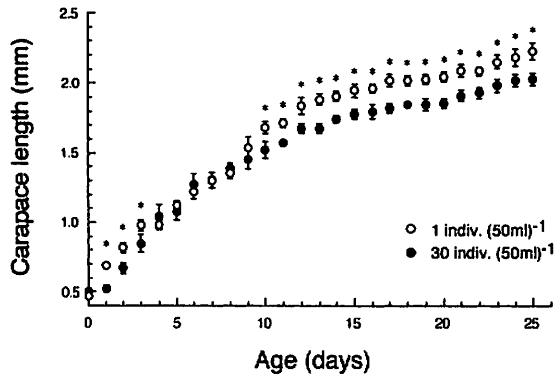

Fig. 1. Time sequence of the increase in carapace length of *Daphnia pulex* at culture densities of 1 and 30 indiv. $(50\,ml)^{-1}$. Vertical bars represent 95 % confidence interval. Asterisks indicate significant difference in carapace length between the two density cultures (t-test, $p<0.05$).

thereafter was significantly different between the two density cultures (t-test, $p<0.01$, Table 1). At the low density, the mean cumulative number of neonates increased with age and reached about 69 indiv. $female^{-1}$ after 15 d, while the number under the high density conditions was significantly lower, reaching only 30 indiv. $female^{-1}$ during the same period (Fig. 2, Table 1). Although smaller adults produced a small number of neonates and larger ones were more productive at both densities, two to three fold more neonates were produced by a female in the low density experiment compared to those produced by the same sized female in the high density one (Fig. 3).

Ephippia were found only in the high density experiment (Table 1). Cumulative numbers of ephippia produced during the 15 d varied among the replicates and ranged from 0 to 9. This high variability might affect the number of neonates produced, because the brood chamber is covered with several protective membranes when daphniids produce ephippial eggs, and parthenogenetic egg production may be hindered. However, there was no significant correlation between the cumulative number of neonates and ephippia among replicates (Spearman's rank correlation test, $\rho=-0.45$, $p=0.08$). Thus, the number of neonates would not have been affected significantly by ephippial egg production in the present experiments. This was probably due to the preponderance of neonates over ephippia.

Table 1. Age at first reproduction (AFR), cumulative number of neonates and ephippial eggs for the 15 d from first reproduction at each population density. Data are expressed as mean±s.d.

Reproductive parameters	Density (indiv. $[50\,ml]^{-1}$)		t value
	1	30	
AFR (days)	8.15±1.01 n=26	8.69±1.25 n=13	−1.45*
Neonates ($female^{-1}$)	68.61±13.42 n=23	30.12±6.36 n=13	11.63**
Ephippial eggs ($female^{-1}$)	NF n=23	0.09±0.08 n=13	—

* Do not differ significantly between the two densities (df=34, $p>0.1$). ** Differ significantly between the two densities (df=33, $p<0.01$). NF: not found.

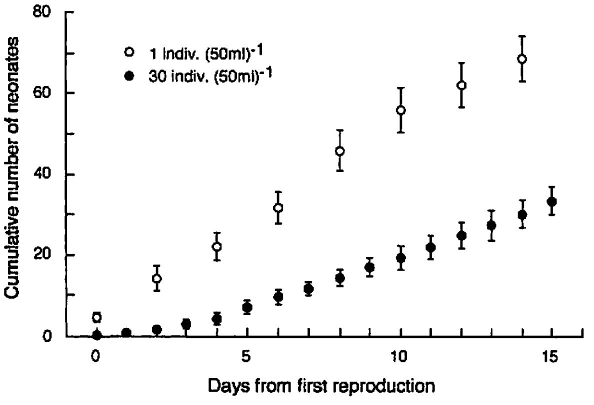

Fig. 2. Relationship between days from first reproduction and mean cumulative number of neonates per female at culture densities of 1 (open circles) and 30 (closed circles) indiv. $(50\,ml)^{-1}$. Vertical bars represent 95% confidence interval based on the number of replicates using averages of the results for the 30-animals experiments in each replicate.

Discussion

Similar body size in juveniles and AFR between the two different density treatments in this study suggest that juvenile growth and maturation of *Daphnia pulex* is not affected by population density in the presence of an excess of food. Since growth and age at maturity in *Daphnia* have been shown to be affected by food concentration (Taylor 1985; Urabe 1988), the two different cultures used in our study would be under similar nutritional conditions, at least during the juvenile instars. Growth in the adult instars was slightly depressed in the high density experiment even under an excess of food. This implies that the crowding effect on growth in *D. pulex* could change ontogenetically, probably relating to egg production as in the other cladoceran species, *Simocepharus vetulus* (Lee & Ban, unpublished data).

The number of neonates produced by females of similar carapace length was lower in the higher density culture. The reproductive rates of cladocerans are affected by temperature (Hall 1964; Bottrell 1975; Hanazato & Yasuno 1985; Korpelainen 1986), food concentration (Hall 1964; Vijverberg 1976; Urabe 1988), photoperiod (Korpelainen 1986) and population density (Guisande 1993; Burns 1995). In the present experiments, both temperature and photoperiod were held constant, and the food concentration was sufficient. Therefore, high daphniid population density is considered to have depressed the production of neonates.

Helgen (1987) showed that crowded *D. pulex* at 270 indiv. l^{-1} fed more slowly than at 30

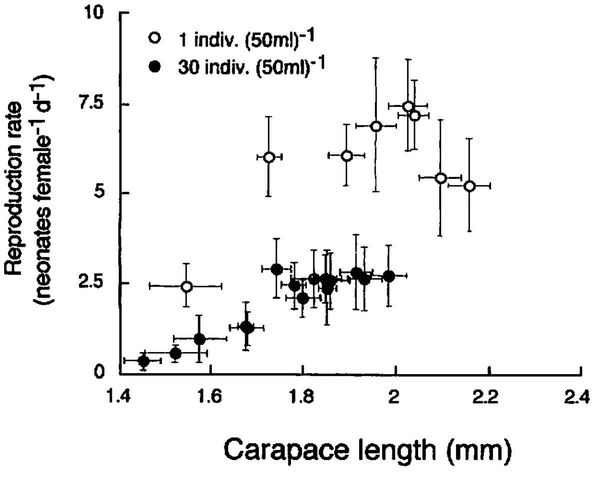

Fig. 3. Relationship between carapace length and reproduction rate (neonates female^{-1} d^{-1}) of *Daphnia pulex* at culture densities of 1 (open circles) and 30 (closed circles) indiv. $(50\,ml)^{-1}$. Vertical and horizontal bars represent 95% confidence interval.

indiv. l^{-1}. In *D. carinata*, water preconditioned with 3–67 daphniids l^{-1} for 30 h had the effect of reducing feeding rates of conspecifics (Matveev 1993). These observations support the idea that reduction in the feeding rate of *Daphnia* induced by crowding may consequentially change their growth and reproductive patterns (Burns 1995). In the present study, *D. pulex* in the high density cultures produced fewer offspring than in the low density ones. The animals in the high density cultures may have had a less energy due to a lower feeding rate than in the low density cultures. On the other hand, crowding may also reduce metabolic rate and/or assimilation efficiency in *Daphnia*. However, there are few studies as yet addressing this concern. More detailed studies are needed to fully understand this crowding effect.

In this study, ephippial eggs were produced only in high density conditions. This implies that high population density induces ephippial egg production. Previous studies have implied or suggested that ephippial egg formation is caused by extreme starvation (Berg 1934; Mortimer 1936; Banta et al. 1939), a rapid decline in food supply (Slobodkin 1954; von Dehn 1956; D'Abramo 1980) or short-day photoperiods in conjunction with higher density conditions (Stross & Hill 1965, 1968). These stimuli are all density-dependent factors, but the experimental techniques used in most previous studies were unable to separate the effects of several density-dependent factors (Hobæk & Lasson 1990). By using a flow-through technique, Kleiven et al. (1992) was successful in separating two density-dependent factors, i.e. food limitation and the accumulation of their own metabolic product. They showed that both factors were necessary to trigger ephippial egg production in *Daphnia magna* in addition to a photoperiod cue, but that no ephippial eggs were produced when only the two stimuli were present. The present experiments suggest that *D. pulex* individuals may produce ephippial eggs in high density conditions even under an excess of food and a normal photoperiod, though the flow-through technique was not used. Probably, the response of ephippial egg production to a given density-dependent factor differs among daphniid species. Recently, Burns (1995) suggested that species-specific differences in daphniid response to population density may depend on their body size; small sized daphniids may be more sensitive in their response to high density conditions. Our *D. pulex* is smaller in body size than *D. magna*. To understand differences in response among species, body size may be an important factor.

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