J. FAC. SCI., SHINSHU UNIVERSITY, Vol. 35, No. 1, pp.31-42 2000

Contribution of Resting Eggs and Overwintering Free-living Individuals to Establishment of Spring Daphnia Populations : A Suggestion Obtained from Studies using Experimental Ponds

Takayuki HANAZATO

Suwa Hydrobiological Station, Shinshu University, 5-2-4 Kogandori, Suwa 392-0027, Japan

(Received July 15, 2000)

Abstract

In outdoor experimental ponds, where overwintering individuals of zooplankton had been controlled with an insecticide, the following events were observed during winter and the following spring : (1) *Daphnia* survived through the winter as free-living individuals ; (2) *Daphnia* ceased egg production in mid-winter, but restarted it in late winter or early spring ; (3) the spring peak of the *Daphnia* population established by overwintering free-living individuals appeared nearly a month earlier than that by individuals hatched from resting eggs. Although resting eggs are often thought to play an important role in establishing spring populations of *Daphnia,* the experimental results suggest that overwintering free-living individuals can also contribute to it. The significance of the overwintering stages in *Daphnia* population dynamics is discussed.

1 Introduction

Cladocerans usually reproduce asexually, but perform sexual reproduction and produce resting eggs when exposed to unfavorable conditions. The cladoceran populations thus survive harsh environments as resting eggs. When the unfavorable conditions have gone, new populations of free-living individuals are established from the individuals that hatch from the resting eggs. Thus, resting eggs play an important role in cladoceran population dynamics and studies have been reported on the factors controlling the production and hatching of these eggs[1-8].

The cladoceran *Daphnia* is one of the most important components of lake ecosystems, because it often forms a high biomass and is an effective grazer on phytoplankton (a major primary producer in the ecosystems) and a favorable food item of various invertebrate and vertebrate predators[9]. The population dynamics of *Daphnia* in lakes during the growing season have therefore been studied well, although those of the cold season much less so. Laboratory studies have been used to assess the factors inducing sexual reproduction and hatching of the resting eggs.

Sexual reproduction (production of males and resting eggs) is induced by lowered temperature, a short daylength, food shortage, and crowding[2,4,5,7]. Some of these factors are those observed in fall, suggesting that the cladocerans perform sexual reproduction in that season.

On the other hand, resting eggs in laboratory studies are activated by treatment with increased photoperiod and increased temperature[1-3,6,8]. These factors appear in spring, suggesting that breaking of the diapause occurs at that time.

It is therefore widely considered that *Daphnia* populations survive the winter as resting eggs, and that new populations of free-living individuals are established by the individuals that hatch from resting eggs in spring[10]. *Daphnia* populations often decrease markedly in the fall and become nearly extinct in winter, but show a great recovery in spring[11-13].

However, free-living *Daphnia* individuals have often been observed to overwinter although the population density during winter was the lowest of the year[14-16]. This shows that lake conditions in winter do not always interfere with the survival of free-living individuals. If the free-living *Daphnia* overwinter, it seems likely that they can contribute to the spring population.

The question thus arises as to which form contributes more to the establishment of the spring populations, the resting eggs or the overwintering free-living individuals?

During a study on the effects of an insecticide on winter plankton communities in outdoor experimental ponds[17], I obtained interesting results relevant to this question. The purpose of the present paper is to report these results and to discuss the significance of the two forms of *Daphnia* for the development of spring populations.

2 Experimental Design

Outdoor experimental ponds of two types were used for the two experiments: large (4.1x 5.1m horizontally, 1.5m deep) and small (1.5x 2m horizontally, 0.7m deep). Two large ponds were used in the first experiment, and six small ponds in the second experiment. They were lined with polyethylene film to avoid any influence from previous experiments. In order to establish plankton communities, bottom mud was taken from Takahamairi Bay of the eutrophic Lake Kasumigaura (Japan: 36°24′N, 140°24′E). These sediments contained resting stages of phytoplankton and zooplankton. An 80-kg quantity was placed in each of the large ponds or 5kg in each of the small ponds. Ground water was then poured to a depth of 1.3m for the large ponds (27.2m^3 of water/pond) or 0.5m for the small ponds (1.5m^3 of water/pond) and the experiment was started.

The first experiment was initiated on 18 October 1985 (day 1) and terminated on 28 April 1986 (day 192). One of the two large ponds (Pond A) received no treatment and served as a control. Another pond (Pond B) was treated with the insecticide carbaryl at a target concentration of 1mg l^{-1} on 18 November 1985 (day 31 after the start of the experiment). Carbaryl is easily decomposed in water at higher temperatures. 90% of the chemical applied to the same experimental ponds was dissipated within 1.18 day at 20°C, but the rate of dissipation declined with decreasing water temperature[18]. In this experiment, the chemical was applied to Pond B when the water temperature was as low as 10°C and therefore the dissipation rate of the chemical would have been lower. However, the chemical disappeared in the pond water within a month, which was confirmed by a laboratory bioassay using the cladoceran *Moina micrura*.

The second experiment was carried out with six small ponds (Ponds 1, 2, 3, 4, 5, 6) from 29 September 1987 to 30 May 1988 (day 244). Pond 1 was kept as a control receiving no treatment. Pond 2 was treated with carbaryl at 1 mg l^{-1} (high dose) on 19 October 1987 (day 20 after the start of the experiment). Pond 3 received the high dose of carbaryl on 18 January 1988 (day 111). Pond 4 was treated twice (19 October and 18 January) with the high dose of carbaryl. In these two ponds (Ponds 3 and 4), the pond water was replaced with ground water on 7 March 1988, because the bioassay with *Daphnia ambigua* showed that the residual carbaryl retained its toxicity to kill *Daphnia* until 4 March 1988. The toxicity disappeared after this treatment. The remaining two ponds (Ponds 5 and 6) received carbaryl at 0.5mg l^{-1} (low dose) on 19 October 1987 and 18 January 1988.

Water temperature, pH, dissolved oxygen concentration, chlorophyll *a* concentrations ware analyzed routinely and changes in zooplankton abundance and community structure were monitored one to three times a week. Detailed information on the methods employed has been given in Hanazato and Yasuno[17].

3 Population Dynamics of *Daphnia* in the Ponds

First experiment

Water temperature was 15°C at the start of the experiment and decreased steadily to 4°C in mid-December (Fig. 1). Subsequently, the temperature stayed at 4°C until late February, and then rose gradually with time. During the mid-winter period (January -February), the pond was often covered with thin ice, but only from midnight to early morning.

Chlorophyll *a* concentration showed a large and sharp peak on day 10, but remained at around 5μg l^{-1} subsequently (Fig. 1). The concentration in Pond B showed a similar change to that in Pond A, but it built up a small peak after the chemical application.

D. ambigua dominated the zooplankton community in Pond A (Fig. 2). The population reached a peak of 68 individuals l^{-1} in late November and then maintained a density of 20-50 individuals l^{-1} until late January, when there was a decrease in the

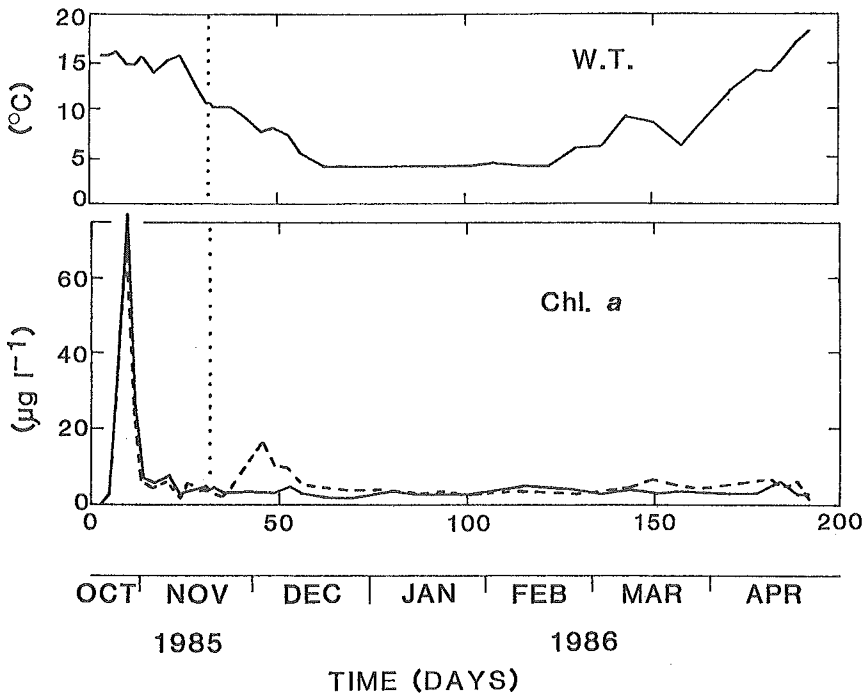

Fig. 1 : Changes in water temperature (W.T. ; °C) and chlorophyll *a* concentration ($\mu g\ l^{-1}$) in Pond A (solid line) and in Pond B (broken line) in the first experiment. Vertical dotted line indicates the treatment day (day 31).

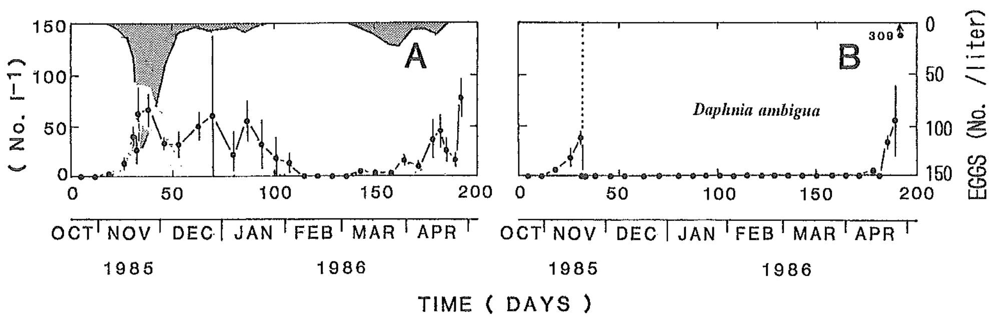

Fig. 2 : Changes in density of *Daphnia ambigua* in Ponds A and B in the first experiment. Vertical bars show 95% CI. The total number of eggs per liter of *Daphnia* in Pond A is shown by shaded areas. Vertical dotted line in Pond B shows the treatment day.

population. Eggs were produced intensively before the time of the maximum density. Subsequently, the egg production was greatly reduced, but did not cease. Reproduction did, however, finally stop in late January. Although no eggs were produced until early March, free-living individuals never disappeared during the winter, though the density was <3 individuals l^{-1}. Egg production began in early March and this was followed by an increased population density of free-living individuals.

In the treated Pond B, all the individuals were eliminated from the water by application of the chemical, and recovery was not observed until mid-April (Fig. 2). Subsequently, a large population developed in late April presumably from the individuals which had hatched from resting eggs.

Second experiment

Water temperature and chlorophyll *a* concentration in the ponds changed similarly to the ponds of the first experiment (Fig. 3).

Daphnia galeata as well as *D. ambigua* appeared in the ponds in this experiment. *D. galeata* invaded Lake Kasumigaura and dominated the zooplankton community for the first time in the fall of 1986[19] and probably laid resting eggs on sediment, which explains why this species appeared in the second experiment but not the first experiment.

In the control Pond 1, *D. ambigua* appeared in October, but was replaced by *D. galeata* (Fig. 4). This replacement might have been a result of competition between the two species. Probably because larger cladocerans tend to be superior in competition than smaller ones[20,21], the larger *D. galeata* should be a superior competitor to the smaller *D. ambigua*. *D. galeata,* which produced eggs in the fall, stopped egg production

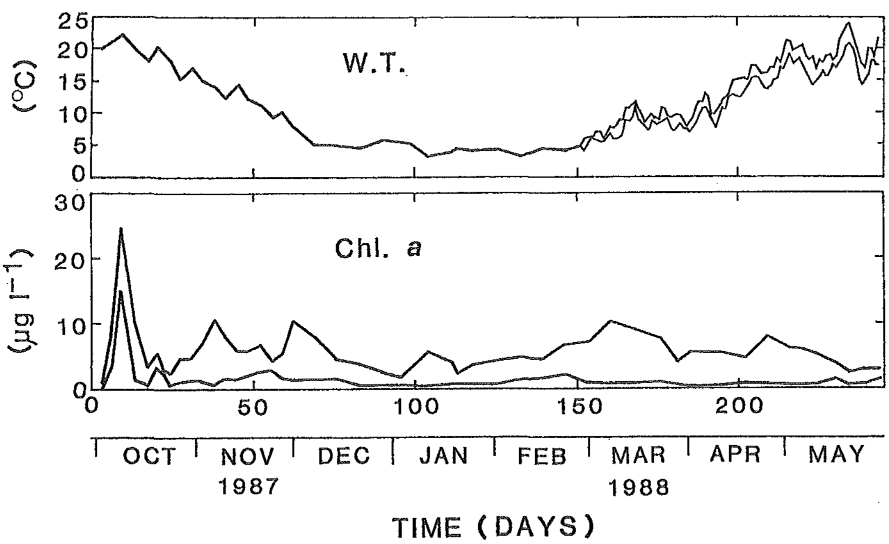

Fig. 3 : Changes in water temperature (W.T. ; °C) in Pond 1 and daily maximum and minimum chlorophyll *a* concentration ($\mu g\ l^{-1}$) among all the ponds in the second experiment. Water temperature was measured at the sampling time until day 151. Thereafter diurnal changes in water temperature were monitored at one-hour intervals. Daily maximum and minimum temperatures are shown.

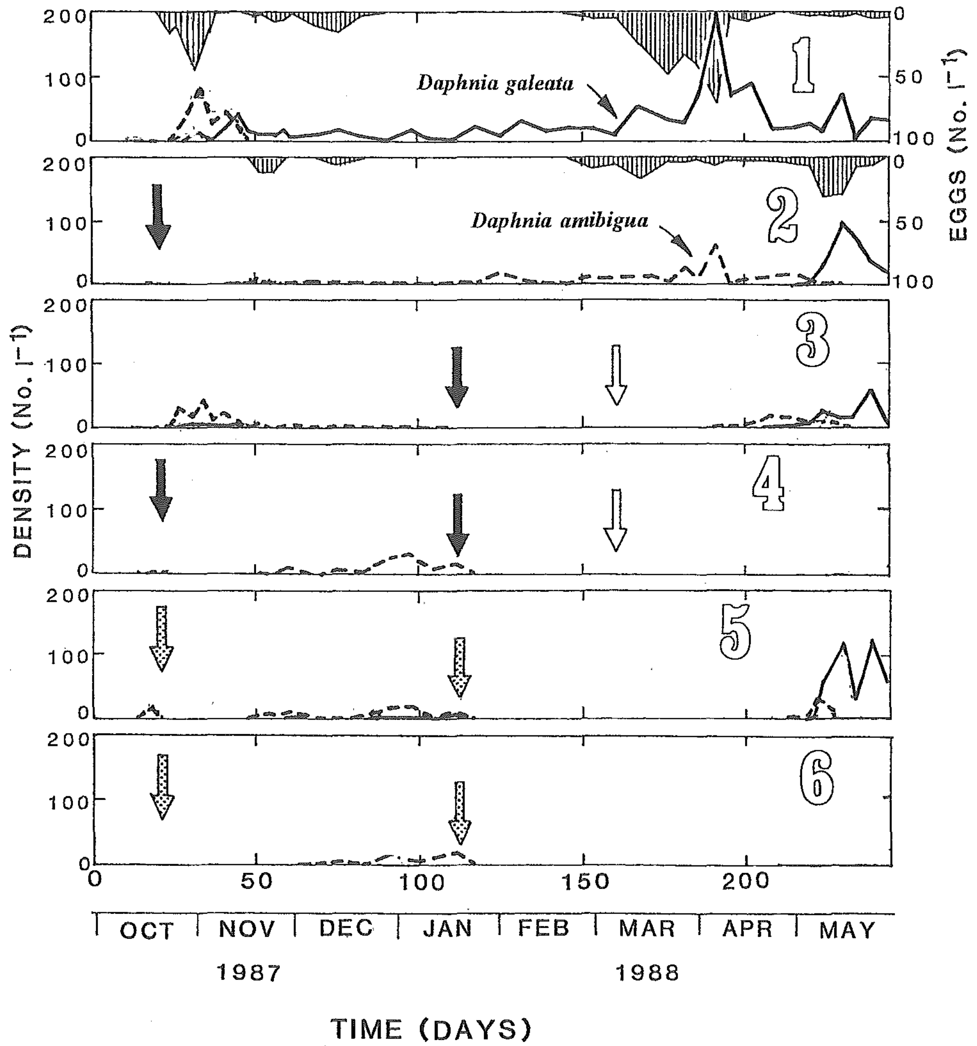

Fig. 4 : Changes in density of *Daphnia galeata* (solid line) and *Daphnia ambigua* (broken line) in Ponds 1, 2, 3, 4, 5 and 6 in the second experiment. In Ponds 1 and 2, the total numbers of eggs per liter of all *Daphnia* species combined are shown by shaded areas. Solid arrows and shaded arrows indicate the treatment days with 1mg l^{-1} carbaryl and 0.5mg l^{-1} carbaryl, respectively. Open arrows show the day when the pond water was replaced with ground water.

in late December. However, *D. galeata* individuals survived the winter at a constant density of 5-20 individuals l^{-1}. Egg production commenced in late February, this being followed by an increased population density, with a peak of 200 individuals l^{-1} in early April.

In Pond 2, where carbaryl was applied in October, the chemical killed all the

Daphnia individuals (Fig. 4). However, *D. ambigua* reappeared in November and overwintered at densities of <20 individuals l^{-1}. These finally led to a peak in early April. Egg production stopped in late December, but restarted in late February. Thus, the population dynamics of *D. ambigua* in this pond was quite similar to those of *D. galeata* in Pond 1. *D. ambigua* was, however, finally replaced by *D. galeata,* which appeared in late April and had a marked peak in mid-May. The spring *D. galeata* population must therefore have been established from resting eggs.

In the other ponds, where the chemical was applied at either a low or a high dose in January, no *Daphnia* was found during the winter (Fig. 4). Its reappearance of *Daphnia* was observed in Ponds 3 and 5 in April or May and large populations occurred in May. The spring populations must therefore have been established from resting eggs.

4 Discussion

In the present experiments, the following events were clearly observed (Fig. 5). (1) *Daphnia* survived through the winter as free-living individuals. (2) *Daphnia* ceased egg production in mid-winter, but restarted it in late winter or early spring. (3) The spring peak established by overwintering free-living individuals appeared nearly a month earlier than that by individuals which had hatched from resting eggs.

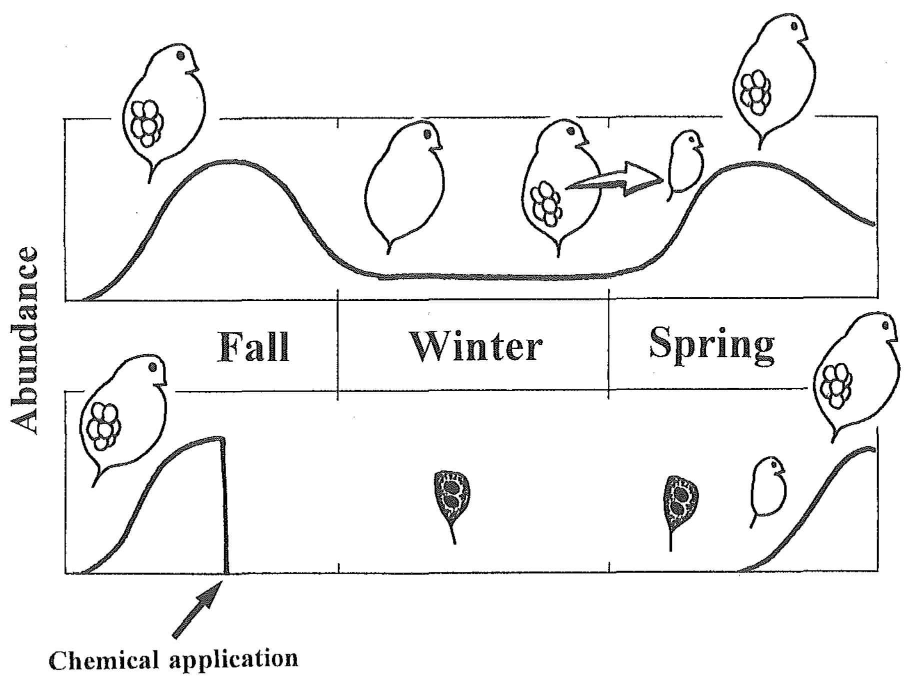

Fig. 5 : Scheme demonstrating winter dynamics of *Daphnia* populations in the control and treated ponds.

Because *Daphnia* populations did not perform reproduction in mid-winter, the overwintered free-living individuals must have been born in late fall or early winter. Low temperatures reduce the growth rate and prolong the life span of *Daphnia*. Yamada *et al.*[22] have investigated the life span of *D. pulex* cultured in the laboratory at temperatures ranging from 10°C to 25°C. The maximum life span increased with decreasing temperature from 35 days at 25°C to 182 days at 10°C. The life span must therefore be much longer at even lower temperatures such as 4°C. This indicates that *Daphnia* can overwinter as free-living individuals without reproduction.

In the present experiments, both *D. galeata* and *D. ambigua* stopped egg production in late December-January, when water temperature was at its lowest (4°C). It may therefore be assumed that the low temperature of 4 caused *Daphnia* to stop reproduction. Egg production began again in late February, when the water temperature started to increase from its lowest value, suggesting that the increase in temperature may have been the stimulus which led to egg production being restarted. However, evidence to support these assumptions will require further experiments.

The time of occurrence of the spring *Daphnia* population differed between the populations established from overwintering free-living individuals and from resting eggs: the former was a month earlier than the latter. This may be due partly to the difference in time required by the two overwintering life stages to release offspring[3]. The overwintering free-living individuals (adult females), which have not been reproducing, start to develop the spring population by producing eggs, while in the case for resting eggs, the development of the spring population should be initiated by hatching of the resting eggs. For the free-living individuals, the time required to release the first offspring after the start of reproduction is nearly the same as the development time for eggs. On the other hand, the time is equal to the age at first parturition (maturation time+egg development time) for the individuals originating from resting eggs. Hanazato and Yasuno[23] determined egg development time (ED) and age at first parturition (AFP) of *D. longispina* cultured at different temperatures. At 10°C, for example, ED was 8.5 days and AFP was 48 days. Thus, the difference between the two parameters is 39.5 days. This difference is 15.3 days at 12°C (ED=6.7d, AFP=22d) and 9.7 days at 15°C (ED=4.3d, FP=14d). Thus, it decreases with increasing temperature. In both the first and second experiments, egg production by the overwintering free-living individuals was initiated in February at 9 and 6°C, respectively. Assuming that the egg production by free-living individuals and hatching of the resting eggs occurs at the same time, that the temperature is constant at 6-9°C, and that ED and AFP of *D. galeata* and *D. ambigua* are the same as those of *D. longispina*, the difference between ED and AFP should be longer than 39.5 days (the difference at 10°C obtained in the laboratory experiment for *D. longispina*). This may explain much of the time difference (nearly a month) between when the spring populations were established from overwintering free-living individuals and when established from resting eggs. Unfortunately there are no data on the time when the resting eggs hatched in the experimental ponds. It is known that a combination of temperature and light conditions is required to activate resting eggs[1,6,8], but that there are inter and intraspecies differences in the response of resting eggs to these conditions[6,24]. It is highly desirable to obtain for many lakes the time when resting eggs start to hatch, as well as the time when overwintering free-living individuals start egg production.

Overwintering free-living individuals established the spring peak earlier than did the individuals from resting eggs. The former individuals may confer a great advantage to the *Daphnia* population over other zooplankton species, because the early established population can obtain food resources earlier than other species. Small cladocerans and rotifers were abundant in spring in the present experimental ponds where carbaryl had eliminated overwintering *Daphnia,* while they scarcely appeared in spring in the untreated control ponds where *Daphnia* overwintered and established the early spring population[17]. Thus, the presence or absence of overwintering free-living individuals of *Daphnia* affects the spring zooplankton community. Furthermore, it may control the time of occurrence of spring clear-water phase in lakes, which is a phenomenon induced by large spring populations of *Daphnia* grazing heavily on phytoplankton[10,25,26].

It is thus important to understand the population dynamics of *Daphnia* in winter.

If *Daphnia* cease egg production in winter in many lakes as seen in the present experimental ponds, the free-living individuals born in late fall or early winter must survive during winter to maintain the overwintering population. In the present experiment, the period when *Daphnia* did not have eggs was about two months, which may be short enough for individuals to survive at 4°C. However, this experiment was performed in Tsukuba, Japan (36°03′N, 140°08′E), where the winter is not so severe. *Daphnia* inhabits lakes at higher latitudes and higher altitudes as well, where environments are colder and the winter is much longer than in the present experimental ponds. It is questionable whether *Daphnia* individuals can survive the longer winter. This should be tested.

Another significant factor controlling the survival of overwintering free-living *Daphnia* may be predation. The predation pressure on *Daphnia* populations is high in summer because the rate of predation by vertebrates and invertebrates depends on temperature, higher rates occurring at higher temperatures. However, it seems likely that the predation pressure is also high in winter, because the feeding activity of predators and population growth rate of *Daphnia* are both low due to the low temperature. If the *Daphnia* population, which has a low growth rate, cannot compensate for the population loss due to predation, predation then becomes an important factor causing a high mortality of the *Daphnia* population. The impact of predation on winter *Daphnia* populations therefore requires analysis. Predator-prey relationships in zooplankton communities under different temperatures (from winter temperatures to summer temperatures) have scarcely been studied[27].

It is considered that resting eggs contribute to the establishment of spring population if no free-living individuals are obtained by normal sampling in lakes in winter. However, it is very difficult to show that there are no overwintering free-living individuals, because it is still possible that they are distributed in winter in regions such as the lake bottom and among vegetation where zooplankton are not sampled by the usual sampling procedure. The present experiment suggests that the overwintering free-living individuals can contribute significantly to the establishment of a spring population, even though the overwintering population density is low. The winter distribution of *Daphnia* in lakes should therefore be analyzed to evaluate the significance of overwintering free-living *Daphnia*.

I have emphasized the significance of overwintering free-living individuals of *Daphnia* for the spring populations. However, this does not rule out the importance of resting eggs for the *Daphnia* populations. Resting eggs have a diverse genetic composition because they are produced sexually, and they contribute to the maintenance of a high genetic diversity in the population. The replacement of the *Daphnia* population originating from overwintering free-living individuals by that originating from resting eggs should often occur in lakes where environmental changes are pronounced.

Acknowledgments

This study was partly supported by a Grant-in-Aid (No. 10480144) to T. Hanazato from the Ministry of Education, Science and Culture, Japan.

References

[1] STROSS, R.G. (1966) Light and temperature requirements for diapause development and release in *Daphnia*. *Ecology* **47**, 368-374.

[2] STROSS, R.G. (1987) Photoperiodism and phased growth in *Daphnia* populations: Coactions in perspective. *Mem. Ist. Ital. Idrobiol.* **45**, 413-437.

[3] STROSS, R.G. and J.C. HILL (1968) Photoperiod control of winter diapause in the freshwater crustacean, *Daphnia*. *Biol. Bull.* **134**, 176-178.

[4] BUNNER, H.C. and K. HALCROW (1977) Experimental induction of the production of ephippia by *Daphnia magna* Straus (Cladocera). *Crustacea* **32**, 77-86.

[5] FERARI, D.C. and P.D.N. HEBERT (1982) The induction of sexual reproduction in *Daphnia magna* : genetic differences between arctic and temperate populations. *Can. J.*

Zool. **60**, 2143-2148.

[6] SCHWARTS, S.S. and P.D.N. HEBERT (1987) Methods for the activation of the resting eggs of *Daphnia*. *Freshwat. Biol.* **17**, 373-379.

[7] HOBAEK, A. and P. LARSSON (1990) Sex determination in *Daphnia magna*. *Ecology* **71**, 2255-2268.

[8] DE MEESTER, L. and H. DE JAGER (1993) Hatching of *Daphnia* sexual eggs. II. The effect of age and a second stimulus. *Freshwat. Biol.* **30**, 227-233.

[9] LARSSON, P. and S.I. DODSON (1993) Chemical communication in planktonic animals. *Arch. Hydriobiol.* **129**, 129-155.

[10] SOMMER, U., Z.M. GLIWICZ, W. LAMPERT and A. DUNCAN (1986) The PEG-model of seasonal succession of planktonic events in fresh waters. *Arch. Hydrobiol.* **106**, 433-471.

[11] KWIK, J.K. and J.C.H. CARTER 1975. Population dynamics of limnetic Cladocera in a beaver pond. *J. Fish. Res. Board Can.* **32**, 341-346.

[12] VIJVERBERG, J. and A.F. RICHTER (1982) Population dynamics and production of *Daphnia hyalina* Leydig and *Daphnia cucullata* Sars in Tjeukemeer. *Hydrobiologia* **95**, 235-259.

[13] HART, R.C. (1987) Population dynamics and production of five crustacean zooplankters in a subtropical reservoir during years of contrasting turbidity. *Freshwat. Biol.* **18**, 287-318.

[14] GEORGE, D.G. and R.W. EDWARDS (1974) Population dynamics and production of *Daphnia hyalina* in a eutrophic reservoir. *Freshwat. Biol.* **4**, 445-465.

[15] JONES, H.R., T.J. LACK and C.S. JONES (1979) Population dynamics and production of *Daphnia hyalina* var. *lacustris* in Farmoor I, a shallow eutrophic reservoir. *J. Plankton Res.* **1**, 45-65.

[16] MASON, C.F. and M.M. ABDUL-HUSSEIN (1991) Population dynamics and production of *Daphnia hyalina* and *Bosmina longirostris* in a shallow, eutrophic reservoir. *Freshwat. Biol.* **25**, 243-260.

[17] HANAZATO, T. and M. YASUNO (1989) Influence of Overwintering *Daphnia* on spring zooplankton communities : An experimental study. *Ecol. Res.* **4**, 323-338.

[18] HANAZATO, T. and M. YASUNO (1990) Influence of time of application of an insecticide on recovery patterns of a zooplankton community in experimental ponds. *Arch. Environ. Contam. Toxicol.* **19**, 77-83.

[19] HANAZATO, T. and M. AIZAKI (1991) Changes in species composition of cladoceran community in Lake Kasumigaura during 1986-1989 : Occurrence of *Daphnia galeata* and its effect on algal biomass. *Jpn. J. Limnol.* **52**, 45-55.

[20] GLIWICZ, Z.M. (1990) Food thresholds and body size in cladocerans. *Nature* **343**, 638-640.

[21] HANAZATO, T. and M. YASUNO (1991) Insights into competition in zooplankton communities derived from studies using experimental ponds. *Verh. Internat. Ver. Limnol.* **24**, 1572-1580.

[22] YAMADA, H., Y. TAKIMOTO and J. MIYAMOTO (1983) OECD test guidelines for

ecotoxicology. Seitaikagaku (Ecological chemistry) **6**, 21-28 (in Japanese).

[23] HANAZATO, T. and M. YASUNO (1985) Effect of temperature in the laboratory studies on growth, egg development and first parturition of five species of Cladocera. *Jpn. J. Limnol.* **46**, 185-191.

[24] DE MEESTER, L. and H. De JAGER (1993) Hatching of *Daphnia* sexual eggs. I. Intraspecific differences in the hatching responses of *D. magna* eggs. Freshwat. Biol. **30**, 219-226.

[25] LAMPERT, W., W. FLECKNER, H. RAI and B.E. TAYLOR (1986) Phytoplankton control by grazing zooplankton : A study on the spring clear-water phase. *Limnol. Oceanogr.* **31**, 478-490.

[26] LAMPERT, W. (1988). The relationship between zooplankton biomass and grazing : A review. *Limnologica* **19**, 11-20.

[27] DODSON, S.I. and A.E. WAGNER 1996. Temperature affects selectivity of *Chaoborus* larvae-eating *Daphnia. Hydrobiologia* **325**, 157-161.