Role of Nutritional Status in Vertical Distribution of *Daphnia galeata*

May 1998

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Contents

Chapter 1. Introduction

Zooplankton are found in various aquatic habitats from ponds, lakes to ocean. In these habitats, zooplankton affect bacteria and algae through grazing (Haney 1973; Lampert and Taylor 1984, 1985; Pinto-Coelho 1991) and nutrients regeneration (Lehman and Sandgren 1982; Olsen and Østghust 1985; Elser and Goldman 1991), and are affected by fish and other invertebrates through predation and competition (Kerfoot and Sih 1987). Several studies have shown that strength of these species interactions relates with distribution and migration of zooplankton within a habitat (Urabe 1990; Lampert and Taylor 1984, 1985). In addition to these interactions, zooplankton contribute material transport through vertical migration (Longhurst and Harrison 1989; Longhurst *et al.* 1990; Longhurst 1991). Thus, investigation of vertical migration and distribution of zooplankton are essential to clarify processes determining food web structure and rate of material cycling in aquatic ecosystems.

In the previous studies, vertical distribution of zooplankton has been explained by environmental factors, such that they choose specific depth to avoid harmful factors and to gain more resources relative to loss. Approach of these studies can be divided into two categories, short-term individual level analyses and long-term population level analyses. The former have focused on diel vertical migration and the latter have focused on seasonal changes in vertical distribution.

Diel vertical migration is general phenomenon in marine and freshwater zooplankton. Usually, zooplankton ascend at dusk after staying at the deeper layer during daytime, and then descend at dawn after staying at the upper layer during nighttime (Cushing 1951; Hutchinson 1967). Various hypotheses have been proposed to explain why zooplankton descend to cool and food-poor deep layer during daytime, including avoidance from intense light (Siebeck

1978), necessity of low temperature in efficient reproduction (McLaren 1963 and 1974), conservation of energy loss (Geller 1986) and avoidance from predators (Zaret and Suffern 1976). Among these, avoidance from predators has been most supported as an explanation for diel vertical migration (Stich and Lampert 1981; Wright *et al.* 1980). Since most planktivorous fish search zooplankton visually (O'Brien 1979), they distribute and feed on zooplankton in light surface layer where phytoplankton, a major food source for zooplankton, are abundant. Thus, predator avoidance hypothesis explains the diel vertical migration to avoid visually searching predators during daytime in expense of food gain. However, Huntley and Brooks (1982) reported that zooplankton stay at the upper layer throughout a day in spite of presence of visually searching predators when food supply was limited. This fact suggests that whether zooplankton perform diel vertical migration or stay at the upper layer throughout a day is dependent not only on predation pressure but also food gain (Johnsen and Jakobsen 1987; Lampert 1989).

A number of studies have examined seasonal changes in vertical distribution of zooplankton. These studies suggest that zooplankton change their vertical distribution according to food abundance (Cummigs 1983; George 1983; Paffenhöfer 1983; Sameoto 1984; Leibold 1990), predation pressure (Mclville and Maly 1981; Leibold 1990), water temperature (Sameoto 1986; Castro *et al.* 1991) and dissolved oxygen (Weikert 1982; Sameoto 1986; Horppila 1997). Discrepancy among these studies suggests that dominant factor affecting vertical distribution differs among zooplankton species and study sites. It is not yet clear when and where specific factors are important in determining vertical distribution of zooplankton species. In addition, no studies have examined how changes in vertical distribution relate with population dynamics of zooplankton species.

Most of previous studies examined relationship between vertical distribution of zooplankton

and environmental factors such as temperature, food abundance and predation pressures. Among these, food is most important factors, because without food they can not grow and reproduce. It should be noted, however, that food abundance is not necessarily reflect nutritional status of zooplankton because they can store energy and nutrients as reserve materials. This implies that present nutrient status of zooplankton is an integration of food environment from the near past to the present. Therefore, internal nutritional status should be examined to assess effects of food environments on distribution and migration of zooplankton. Regardless of the present food environment, zooplankton may behave to avoid risk such as predation in expense of food gain when their internal nutritional status is high, while they may behave to gain food in expense of risk when nutritional status is low. These possibilities suggest that vertical migration and distribution of zooplankton relate with not only present environmental factors but also internal condition of zooplankton.

In the present study, I focused on *Daphnia galeata* Sars (Cladocera: Crustacea) because this species is common zooplankton in many Japanese lakes. I examined how nutritional status as well as other environmental factors relates with diel vertical migration and seasonal changes in their distribution. In addition, I examined how changes in vertical migration and distribution contribute to population dynamics of this species. In chapter 2, relationship between internal nutritional status and diel vertical migration was examined. The results show the possibility that nutritional status of *D. galeata* change according to position of reproduction cycle, which in turn induce different migration behaviors among individuals. Difference in nutritional status among individuals is quantitatively examined more precisely in chapter 3, by analyzing individually composition of fatty acids, major energy reserve materials of this species. Why vertical migration behavior change according to internal nutritional status is discussed in chapter 4 using a mathematical model. How internal nutritional status affects on seasonal

changes in vertical distribution of *D. galeata* is discussed in chapter 5, by showing that vertical distribution is determined by seasonal migration and vertical difference in reproduction. In chapter 6, results presented in the previous chapters are summarized and integrated, where importance of internal nutritional status and its individual variance are remarked in relation with migration, distribution and population dynamics of *D. galeata*.

Chapter 2. The relationship between nutritional status and diel vertical migration of *Daphnia galeata*

Abstract

The relationship between nutritional status, indicated by storage lipid content, and diel vertical migration of *Daphnia galeata* in Lake Kizaki was investigated in July 1991. The *D. galeata* population was divided into two groups, i.e., upper and deep layer groups during daytime. The upper layer group consisted of juveniles and adults of poor nutritional status, while the deep layer group consisted only of adults of better nutritional status. Diel changes in the vertical distribution of *D. galeata* revealed that adults in the deep layer performed diel vertical migration, whereas juveniles and adults in the upper layer did not migrate vertically. These result implied that adult *D. galeata* with better nutritional status performed vertical migration in the context of their life cycle.

The predation avoidance hypothesis (Zaret and Suffern 1976) as an explanation for the significance of diel vertical migration of zooplankton has been widely supported. However, Huntley and Brooks (1982) found that *Calanus pacificus* did not migrate vertically when food was limited regardless of the presence of predators. Therefore, it has been speculated that there is a trade-off between food abundance and predation avoidance (Kerfoot, 1985; Lampert 1989). The trade-off, however, can not sufficiently explain the mechanism of diel vertical migration because the nutritional status of zooplankters seems to be a more important factor than food abundance in its effect on their migration behavior.

In mesotrophic Lake Kizaki in central Japan, the *Daphnia galeata* population distributes only in the epilimnion during daytime from May to June, and in both the epilimnion and hypolimnion from late June to August every year (Sekino, unpublished). The seasonal change in vertical distribution of *D. galeata* during daytime is due to the change in migration behavior, which may relate not only to changes in food abundance and predation pressure, but also to changes in the nutritional status of *D. galeata*. In the present study, I investigated the relationship between nutritional status and diel vertical migration of the *D. galeata* population in Lake Kizaki in July 1991, when the population was vertically divided into two groups.

Methods

Diel vertical migration of *D. galeata* was investigated from 19 July to 20 July 1991 at the center of Lake Kizaki (36°33′N, 137°50′E, 29 m deep). The sampling was conducted at four hour intervals from 10:00 on 19 July to 10:00 on 20 July. For quantitative zooplankton analyses, twenty liters of lake water were collected with a 6.7-liter Van Dorn water sampler at 2 m-intervals from 0 m to 28 m deep. *Daphnia* at each depth were collected by filtering the sampled water through a 40-μm mesh net, and then fixed with 5% sugar formalin (Haney and Hall 1973). The clutch sizes, developmental stages of eggs in the brood pouches and carapace lengths of *D. galeata* individuals collected at 10:00 on 19 July and 02:00 on 20 July were measured. Animals larger or smaller than the minimum carapace length of animals with eggs (500 μm) were counted as adults or juveniles. The egg developmental stage was determined according to Threlkeld (1979).

The storage lipid content in *Daphnia* was evaluated using a lipid index (LI) based on the number of oil droplets in the animals (Goulden and Hornig 1980). The animals for measurement of LI were collected by towing a closing plankton net (23.5 cm diameter, 100-

μm mesh) from the surface down to 27m at 4 or 3 m intervals. The animals were carried back to a cabin at the lake shore. For all or a part of the animals in the samples, the measurement of LI was immediately performed after collection in order to minimize fading of the oil droplets.

Results and Discussion

During the daytime (10:00-18:00 on 19 July), the population density of *D. galeata* had two peaks in the upper layer (above 16 m) and the deep layer (below 24 m). And the average population densities in those layers during daytime were 10.8 and 11.8 individuals · L^{-1}, respectively (Fig. 1). At 10:00 on 19 July, 59% of individuals distributed in the upper layer were adults, whereas adults comprised 98% of individuals distributed in the deep layer. At night (22:00 on 19 July ; 02:00 on 20 July), most *D. galeata* individuals were distributed in the upper layer. The average population density in that layer at night was 11.4 individuals · L^{-1}. On the other hand, the average population density in the deep layer was 3.6 individuals · L^{-1}. At 02:00 on 20 July, adults comprised occupied 66% of individuals distributed in the upper layer, and 99% distributed in the deep layer. On the next morning (10:00 on 20 July), two vertical density peaks of *D. galeata* appeared again. And the average population densities in the upper and deep layers were 9.1 and 6.8 individuals · L^{-1}, respectively. These observations indicated that the *D. galeata* population in the deep layer consisted mainly of adults both day and night, while juveniles were hardly distributed in the deep layer at any time. Thus, diel changes in vertical distribution of *D. galeata* suggest that only adults distributed in the deep layer in daytime may perform diel vertical migration, which is true of neither juveniles nor adults distributed in the upper layer in daytime.

High predation pressure by fish and the presence of sufficient food in the upper layer are the factors inducing diel vertical migration of zooplankton as a predator avoidance mechanism

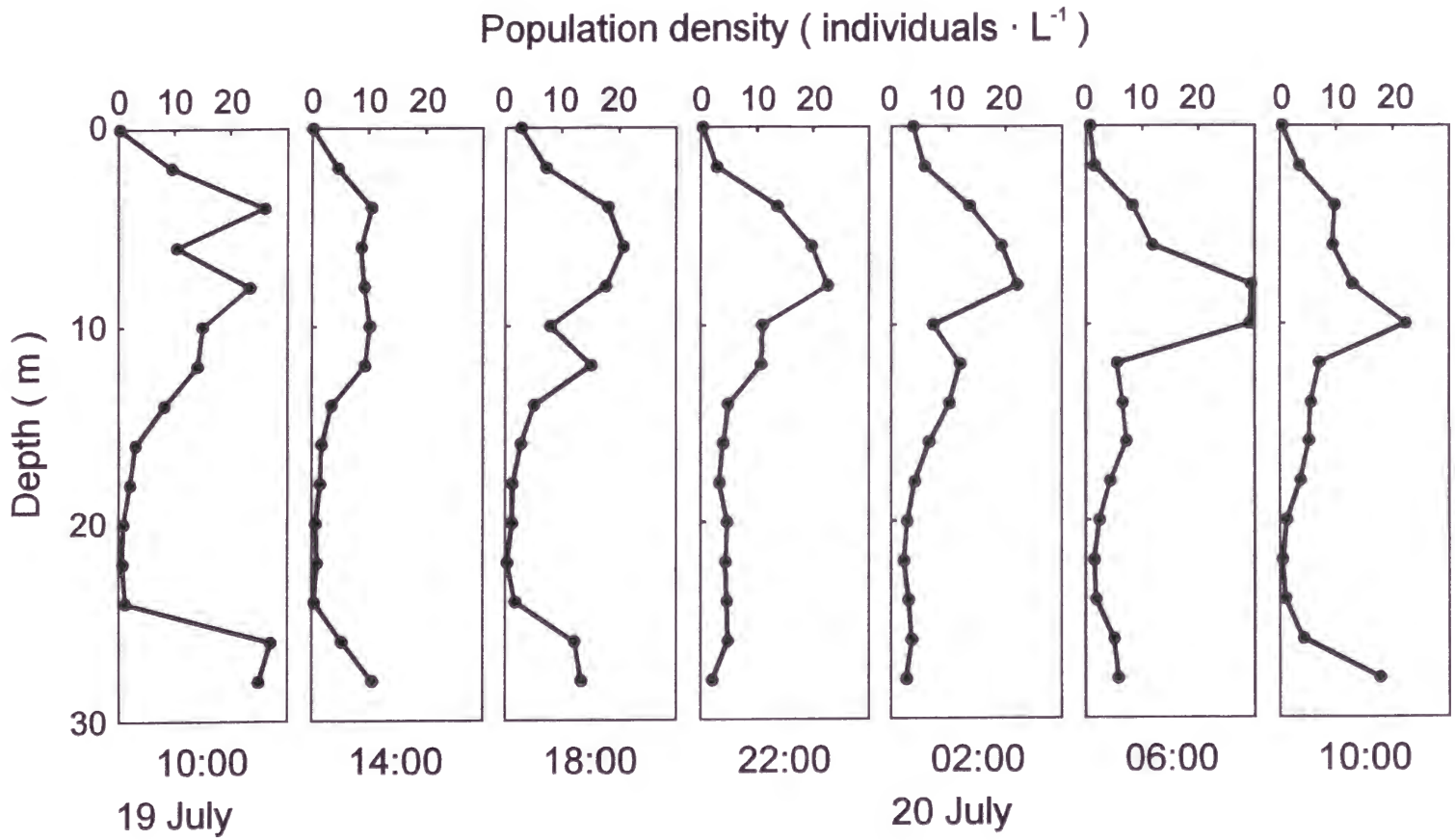

Fig. 1 Vertical distribution and age structure (10:00 on July 19 and 02:00 on 20 July) of *Daphnia galeata* in Lake Kizaki on 19-20 July in 1991. Hatched area: juveniles; dotted area: adults.

(Kremer and Kremer 1988). A cyprinid planktivorous fish (*Zacco platypus*) and a pond smelt (*Hypomesus transpacificus*), which prefer feeding on *Daphnia* (Shiraishi 1960; Urabe and Maruyama 1986), are abundant in Lake Kizaki. The mean chlorophyll a concentration two days before the investigation (17 July) was 12.4 $\mu g \cdot L^{-1}$ in the upper layer and 4.1 $\mu g \cdot L^{-1}$ in the deep layer (Sekino, unpublished), suggesting that food available to *Daphnia* was relatively abundant in the upper layer. Thus, the diel vertical migration of the adult *D. galeata* distributed in the deep layer was probably a predation avoidance mechanism. However, food in the upper layer may no be sufficient for all *D. galeata* to make the migration.

The migration behavior of the *D. galeata* population of Lake Kizaki, observed here, was similar to that of the *Daphnia* population in Lake Constance which consisted of migrating *D. hyalina* and non-migrating *D. galeata* (Stich and Lampert 1981). However, the *Daphnia* population of Lake Kizaki consisted of only one species, within which there were differences in migration behavior. Therefore, the *D. galeata* population in Lake Kizaki differed in behavior from either population of the two *Daphnia* species in Lake Constance. On the other hand, in some lakes, the zooplankton populations consisted of one species which changed their migration behavior seasonally (Forsyth *et al.* 1990; Ringelberg *et al.* 1991a). However, the *D. galeata* population in Lake Kizaki exhibits two different migration behaviors concurrently, and thus the behavior is unique. The difference in behavior of the adult *D. galeata* may reflect the difference in the physiological condition of each individual.

Vertical distribution of the lipid index (LI) of *D. galeata* showed diel changes (Table 1). In the daytime, the percentage of individuals with high LI (LI 2 and 3) was significantly higher in the deep layer than in the upper layer. The percentage of individuals with LI 0 was significantly lower in the deep layer than in the upper layer. From daytime to nighttime in the deep layer, individuals with LI 2 and 3 decreased from 11% to 2%, and individuals with LI 1 decreased

Table 1

Spatial and temporal changes in population density (individuals · L^{-1}) and percentage (in parenthesis) of *Daphnia galeata* individuals with different lipid indices in Lake Kizaki during 19-20 July, 1991. Each percentage is a mean for each layer at a given time. Significantly different values from those in deep layer (24-28 m) are marked with asterisks (χ^2-test: * = $p \leq 0.05$, ** = $p \leq 0.001$).

Time	Layer(m)	Lipid indices LI 0	LI 1	LI 2, 3
Daytime	0-16	6.3 (58)**	4.4 (41)	0.2 (1)**
(10:00-18:00	16-24	0.8 (62)	0.5 (38)	0.0 (1)
on 19 July	24-28	4.9 (42)	5.6 (48)	1.3 (11)
Nighttime	0-16	6.4 (56)*	4.6 (40)*	0.4 (3)
(22:00 on 19 July	16-24	2.4 (65)	1.3 (35)	0.0 (0)
- 02:00 on 20 July)	24-28	2.6 (72)	0.9 (26)	0.1 (2)
Next morning	0-16	5.9 (65)**	3.0 (33)	0.2 (2)**
(10:00 on 20 July)	16-24	1.0 (49)	1.1 (51)	0.0 (0)
	24-28	2.2 (32)	3.8 (56)	0.8 (12)

from 48% to 26%. By contrast, individuals in the upper layer with LI 2 and 3 increased slightly from 1% to 3%. The percentage of individuals with LI 0 distributed in the deep layer increased from 42% to 72% from daytime to nighttime, while that in the upper layer did not change. Thus, the percentage of individuals with lipid indices 1, 2 and 3 distributed in the deep layer was lower than in the upper layer at night, while that with LI 0 was higher in the deep layer than in the upper layer. However, this does not inevitably indicate a downward migration of individuals with low LI from the upper layer to the deep layer, because the population density in the deep layer considerably decreased from day to night (Fig. 1). On the next morning, the percentage of individuals with LI 1, 2 and 3 was again higher in the deep layer than in the upper layer, and that with LI 0 was again low in the deep layer.

The LI data presented in Table 1 included the values for both adults and juveniles. Since almost all individuals distributed in the deep layer (about 98% of the deep population) were adults, the LI data in the deep layer would represent those for adults in the layer. The LI value for adults in the upper layer in the daytime was estimated from LI (Table 1), population density (Fig. 1) and the ratio of adults and juveniles at 10:00 on 19 July. Assuming all juveniles in the layer preferentially occupy the high LI classes, the LI for most adults would be zero, since the population density with LI of 1, 2 and 3 (4.6 individuals $\cdot$ L^{-1}) is almost equal to the juvenile density (4.4 individuals $\cdot$ L^{-1}). Assuming all juveniles in the upper layer have LI of 0, the average LI for adults in the layer is calculated to be 0.7. In both cases, the estimated LI values for adults distributed in the upper layer in daytime are not higher than the average LI value in the deep layer (0.7). Thus, I considered that adults distributed in the deep layer had more storage lipid than those in the upper layer, regardless of whatever LI juveniles in the upper layer had. The relative abundance of individuals with each LI in the deep layer changed greatly in a single day (Table 1). The rapid changes in storage lipid content seem not to be explained

by assimilation, respiration and so on. Therefore, the changes in the vertical distribution of LI might be due to the vertical migration of adults with high storage lipid contents. The results, obtained here, indicated that migrating adults were in better nutritional states (high LI) than non-migrating adults which spent any time in the upper layer.

From the relationship between migration behavior and protein content of *D. hyalina-galeata*, Guisande *et al.* (1991) has suggested that individuals in better nutritional status stay in the upper layer all day, while individuals in poor nutritional status make diel vertical migrations. They concluded that the difference in individual nutritional status was the result of different migrating strategies. The results obtained in the present study contradict the results of Guisande *et al.* (1991). In the present study, I have demonstrated that individuals with large energy reserves and high tolerance to starvation will migrate vertically to avoid predation, whereas individuals in poor nutritional status with low tolerance to starvation do not migrate but spend any time in the upper layer where food is abundant. Thus, individual nutritional status should be regarded as a cause of the strategies of migration behavior rather than the result of those strategies. In other words, *D. galeata* individuals chose their migrating strategy depending on their individual nutritional status. This conclusion implies the existence of a trade-off between individual nutritional status and predation avoidance.

Why does nutritional status differ among adults within a population? When food becomes scarce, *Daphnia* stops reproduction and conserves its energy for metabolism (Goulden and Hornig 1980). From 60 to 70% of adults in a whole water column did not have eggs, and the average clutch size was lower than 2 during the observation period (data not shown). These observations indicated that food was not sufficient for all adult *Daphnia* to continuously produce eggs. Therefore, changes in the food supply and difference in length of time after the last egg production for each individual might have caused the difference in nutritional status

Table 2.

The population density (individuals · L^{-1}) and percentage (in parenthesis) of *Daphnia galeata* individuals with deferent development stage of eggs (Threlkeld, 1979) in each layer at the daytime (10:00 on 19 July) and the nighttime (02:00 on July).

Time	Layer (m)	Egg stages 1	2	3	4	5
10:00	0-16	1.6 (61)	0.6 (23)	0.1 (3)	0.2 (8)	0.2 (6)
on	16-24	0.3 (68)	0.1 (21)	0.0 (2)	0.0 (5)	0.0 (5)
19 July	24-28	1.2 (43)	0.6 (20)	0.2 (7)	0.7 (24)	0.2 (6)
02:00	0-16	1.7 (61)	0.6 (22)	0.1 (3)	0.2 (6)	0.2 (8)
on	16-24	0.7 (58)	0.3 (27)	0.0 (2)	0.1 (6)	0.1 (6)
20 July	24-28	0.7 (54)	0.3 (23)	0.0 (3)	0.2 (16)	0.1 (5)

among adults which bore no eggs. This was also the case for egg-bearing adults. Since egg development and lipid accumulation in the maternal body occur at the same time, adult *D. galeata* with more developing eggs have more lipid than those with stage 1 eggs. In the upper layer, among all *D. galeata* bearing eggs at 10:00 on 19 July and at 2:00 on 20 July, the percentages of adults which bore eggs in the stage 3 or higher were 16% and 17%, respectively (Table 2). On the other hand, the percentage in the deep layer during daytime and nighttime were 37% and 24%, respectively. The difference in the percentage between the upper and deep layers was not significant at night. However, during the daytime, the percentages of adults which bore eggs in stage 3 or higher was significantly higher in the deep layer than in the upper layer (χ^2-test: $p \leq 0.001$). From the diel changes in population density (Fig. 1) and in LI (Table 1), the adults with more developing eggs would have higher lipid levels and would make the vertical migration. Since egg production occurs periodically according to molt, it is thought that migration behavior of *Daphnia* individuals changes alternately with periodical changes in their nutritional status, or with the amount of lipid storage, in relation to their life cycle.

Chapter 3. Inter- and intra-specific differences in fatty acid composition of freshwater crustacean zooplankton

Abstruct

The composition of fatty acids from C14 to C18 was measured for edible seston and for individual *Daphnia galeata*, *Diaphanosoma brachyurum* and *Eodiaptomus japonicus* from Lake Biwa using a Pyrolysis-Gas Chromatography (Py-GC) method. Based on the relative abundance of the fatty acids, inter- and intra-specific difference in composition were examined. Among the fatty acids, C16:0 was the most abundant, both in the three zooplankton species and in edible seston smaller than 20 μm, suggesting that the composition of the zooplankton was roughly reflected by their diet. However, the abundance of C18:1 relative to C16:1 and C18:0 was much higher in each zooplankton species than in the diet. Comparison of the relative abundance of fatty acids among the three zooplankton species revealed that intraspecific differences in fatty acid composition are greater than interspecific differences. These results indicate that variability in fatty acids composition is not necessarily species-specific, and can be obscured by variation in composition between individuals.

Differences in fatty acid composition of zooplankton have been associated with differences in the fatty acid composition in their food (Bourdier and Amblard 1989). Therefore, the fatty acid composition of zooplankton has been used as a marker for tracing food web structure (Lee *et al.* 1971; Falk-Petersen *et al.* 1987). If the fatty acid composition of zooplankton is determined only by the food, interspecific differences in fatty acid composition of zooplankton

should reflect differences in diet. However, the fatty acid composition is also affected by a number of external and internal factors other than diet, including water temperature (Farkas 1979) and developmental stage (Kattner and Krause 1987). Hence, interspecific differences in fatty acid composition do not necessarily reflect the interspecific differences in diet. Furthermore, since sensitivity to both external and internal factors is expected to depend on the physiological condition of each individual, fatty acids composition could differ even within the same species. If the intraspecific difference in fatty acid composition caused by the physiological condition of each individual is larger than the interspecific difference in the composition depending on food, it would be difficult to estimate the interspecific differences in diet among zooplankton species. Unfortunately, no study has examined intraspecific differences in the fatty acid composition of zooplankton species.

Analysis of the fatty acid composition of zooplankton has traditionally used gas chromatography (GC). In this case, pretreatments, including extraction, saponification and esterification are required before the GC analysis. Unfortunately, GC lacks the sensitivity necessary for analyzing the fatty acid composition of individual zooplankters. A flame ionization detector (FID), which is a typical detector for a GC, can detect approximately 20 pg of each compound (McMinn and Hill 1990). When fatty acid composition in zooplankton is analyzed, the sample pretreated in solvent has to contain at least 1 ng / μL of each fatty acid species. This means that zooplankton samples for analysis of fatty acid composition have to contain at least 1 μg of each fatty acid species, because the sample pretreatments usually require more than 1 ml of solvent (Kattner and Fricke 1986). On the other hand, an individual *Daphnia pulex* has about 5μg of total lipid (Goulden and Place 1990), and the relative abundance of most individual fatty acids in crustaceans is less than 1 % of total lipid (Farkas 1979). Thus, the content of most individual fatty acid in a *Daphnia* individual is less than 0.05

μg, less than 5 % of the required amount.

Recently, I have developed a new method for analyzing the fatty acid composition of zooplankton at the individual level. This technique is based on a pyrolysis gas chromatography (Py-GC) method which has been successfully applied for measuring the fatty acid composition of cultured *Daphnia galeata* (Ishida *et al.* 1996). In this Py-GC method, pretreatments of a sample are done by reactive pyrolysis in the presence of tetramethylammonium hydroxide (THMA) on line. The Py-GC method makes it possible to examine differences in the fatty acid composition of individual zooplankton. Using this approach, I measured the relative abundance of fatty acids for three dominant zooplankton species in Lake Biwa in order to characterize the pattern of variability at both the species, and among species and among individuals levels.

Methods

Zooplankton were collected at the Wani station of Lake Biwa (35°10'30"N, 135°56'30"E, 53 m deep) from 10:00 to 13:00 on 13 September 1994. Sampling was conducted with a closing 100 μm plankton net from the 0-10 m, 10-20 m and 20-50 m layers. The samples were not fixed, and the dominant species (*Daphnia galeata*, *Diaphanosoma brachyurum* and *Eodiaptomus japonicus*) were individually picked up with tweezers, dried in a vacuum desiccator and used for analysis of fatty acid composition.

Lake water was also collected from 0, 5, 10, 15, 20, 30, 40 and 50 m using a Van Dorn water sampler. Since crustacean zooplankton in Lake Biwa mainly ingest food particles smaller than 20 μm (Okamoto 1984a and Urabe *et al.* 1996), water samples were prefiltered through a 20 μm plankton net. Chlorophyll *a* concentrations in the < 20 μm size fraction and in the total phytoplankton community were determined by filtering subsamples onto GF/F filters. Chlorophyll *a* concentrations were determined fluorometrically using the method of Lorenzen

(1967). Aliquots for filtered (< 20 μm) subsamples were concentrated on precombusted Whatman GF/F filters for analysis of the fatty acids on edible seston. Filters were subsequently dried in a vacuum desiccator. Water temperature and chlorophyll *a* fluorescence were measured vertically with a conductivity temperature depth profiler (SBE 25, Sea-Bird Electronics, Inc.) equipped with a fluorometer (Sea Tech, Inc.).

The fatty acid composition of each individual zooplankter and the concentrated seston was analyzed qualitatively by the Py-GC method. Dry weights of zooplankton individuals used for the analysis were 2-5 μg for *D. galeata*, 1-3 μg for *D. brachyurum* and 1-5 μg for *E. japonicus*, respectively. The concentrated seston was 196-217 μg.

Details of the Py-GC method for fatty acid analysis have been discussed in Ishida *et al.* (1996). In short, 2 μl of a methanol solution (25 wt. %) of tetramethylammonium hydroxide (THMA) were added to a sample of seston or a single individual zooplankter in a small platinum cup. The cup with the sample was introduced into the pyrolyzer (Yanaco GP1018) of the Py-GC (HP 5890). The pyrolysis temperature was set at 400 °C. The temperature of the GC column was set initially at 50 °C, then programmed up to 190 °C at a rate of 5 °C/min and finally from 190 °C to 240 °C at a rate of 1 °C/min. The column was an HP INNOWAX (50 m × 0.02 mm ID) coated with a crosslinked PEG (polyethyleneglycol) film (0.20 μm). Identification of the peaks on the pyrograms was carried out by a GC-MS system (JEOL AUTOMASS 150) with an electron impact ionization source to which the pyrolyzer was directly attached. The relative abundance of each fatty acid was calculated from the peak area on the pyrogram.

Data for statistical analysis were normalized using log(n+1) transformations. Interspecific differences in the fatty acid composition of zooplankton were examined by a two-level nested ANOVA. Differences in the fatty acid composition of each zooplankton species among

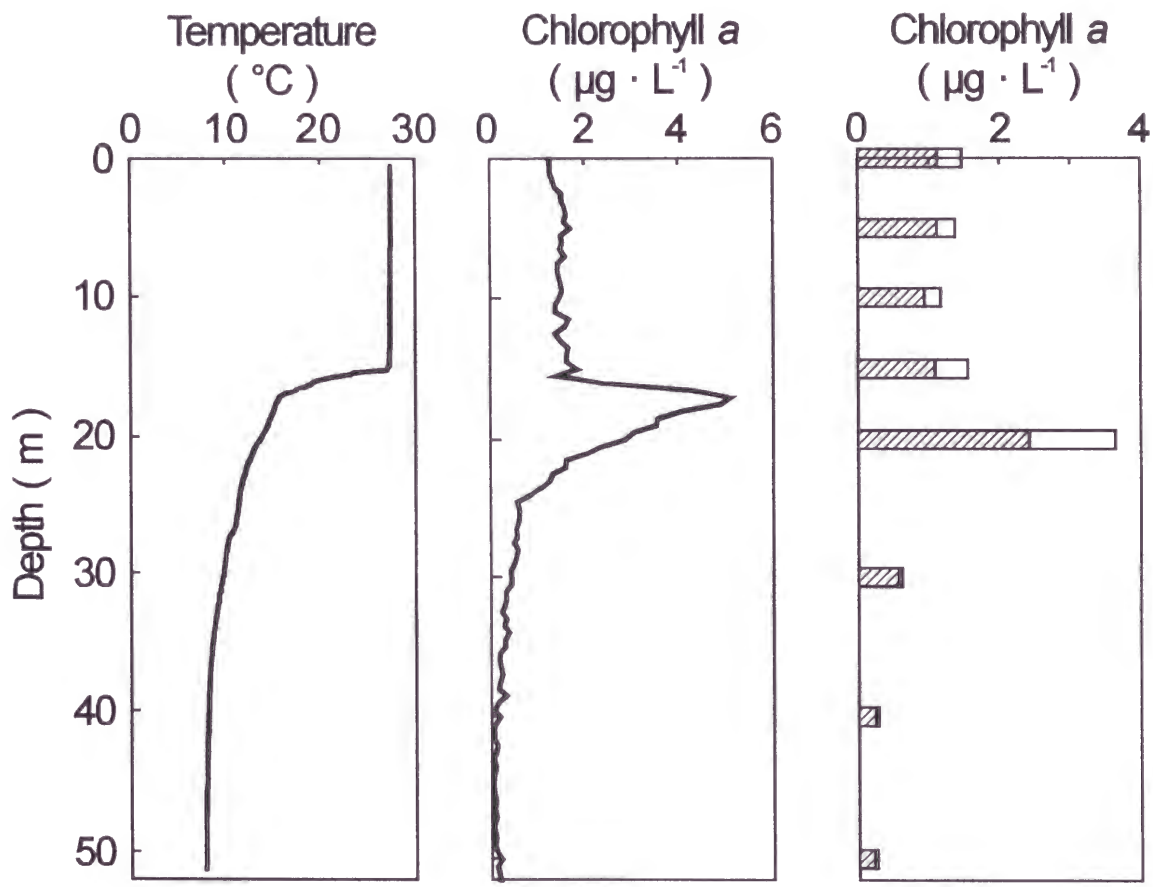

Fig. 2 Vertical profiles of water temperature, chlorophyll a fluorescence and size fractionated chlorophyll *a* < 20 μm (hatched area) and ≥ 20 μm (open area). Chlorophyll *a* fluorescence was converted to chlorophyll *a* concentration.

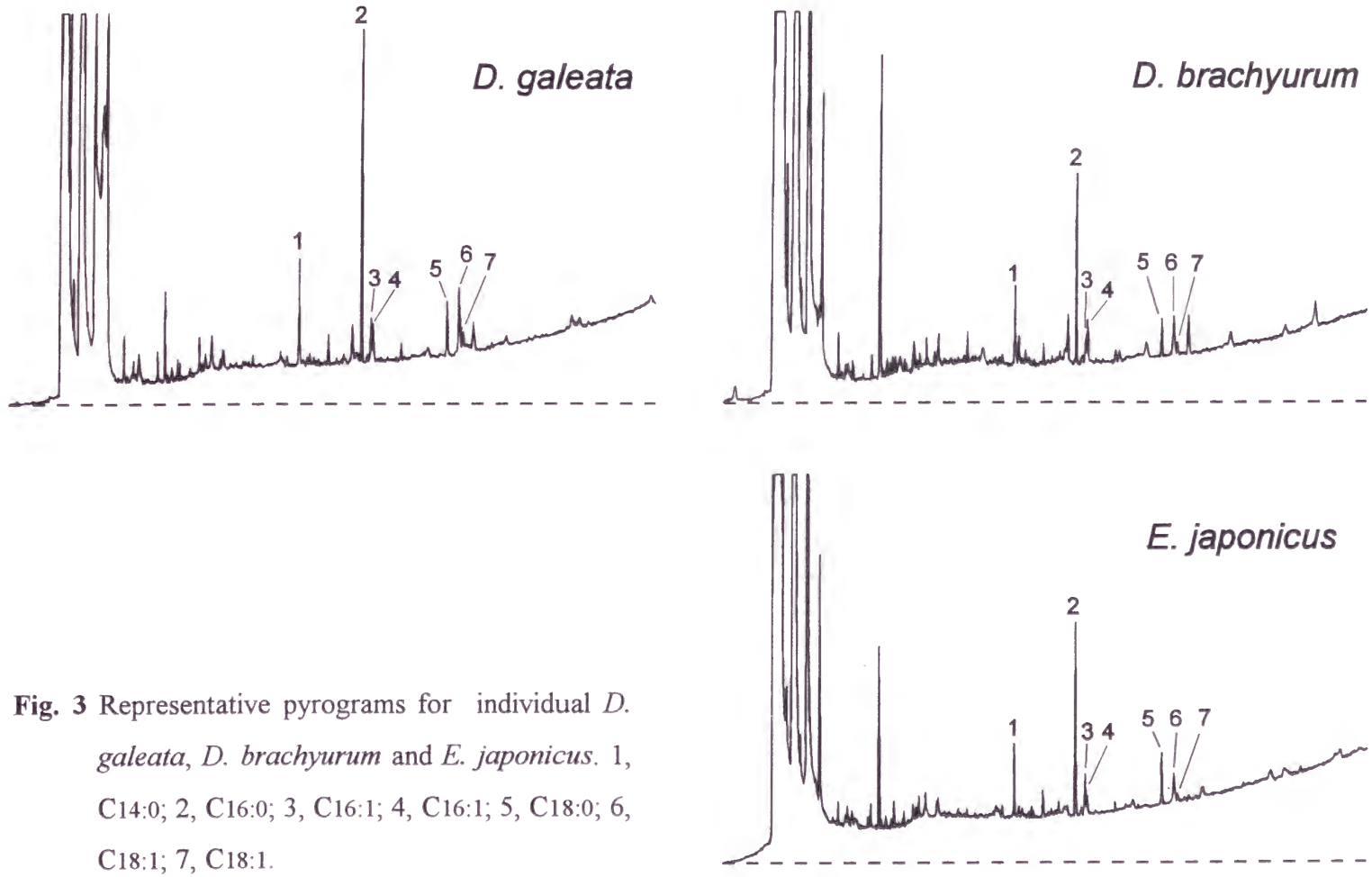

Fig. 3 Representative pyrograms for individual *D. galeata*, *D. brachyurum* and *E. japonicus*. 1, C14:0; 2, C16:0; 3, C16:1; 4, C16:1; 5, C18:0; 6, C18:1; 7, C18:1.

distribution layers were examined by a single-classification ANOVA (Sokal and Rohlf 1981).

Results

On the sampling date, the thermocline was at 15 m, and water temperature in the epilimnion and hypolimnion were 27 °C and 8 °C, respectively (Fig. 2). A maximum of chlorophyll *a* fluorescence 5.1 $\mu g \cdot L^{-1}$ was found slightly below the thermocline. Chlorophyll *a* fluorescence in epi- and hypolimnion were about 1.5 $\mu g \cdot L^{-1}$ and 0.2 $\mu g \cdot L^{-1}$, respectively. Throughout the water column more than 70% of chlorophyll *a* was found in the edible fraction (< 20 μm).

The three crustacean plankton, *Daphnia galeata*, *Diaphanosoma brachyurum* and *Eodiaptomus japonicus* were the most numerous species. Saturated fatty acids from C14 to C18 and two isomers of monoenoic C16 and C18 fatty acids were identified for each species (Fig. 3). The reactive pyrolysis with TMAH often isomerizes fatty acids (Ishida *et al.* 1996). The two isomers of monoenoic C16 and C18 fatty acids may be produced by the isomerization, and I could not distinguish between isomerized and non-isomerized fatty acids. Thus, these isomers of each monoenoic fatty acid were treated as one fatty acid species for the following analysis. The fatty acid composition of the edible seston showed depth-dependent changes with the C16:0 being significantly more abundant above the thermocline (<15m deep) while C16:0 and C18:0 dominated in the seston below the thermocline (Fig. 4). The C16:0 was also the most abundant fatty acid in the three zooplankton species except for *D. galeata* collected from the 10-20 m and 20-50 m layers. C18:0 did not significantly increase with depth in zooplankton (Fig. 4). The relative abundance of C18:1 in zooplankton species was significantly larger than that in the edible seston except for *E. japonicus* collected from the 20-50 m layer. However, the relative abundance of other fatty acids did not significantly differ between zooplankton and edible seston.

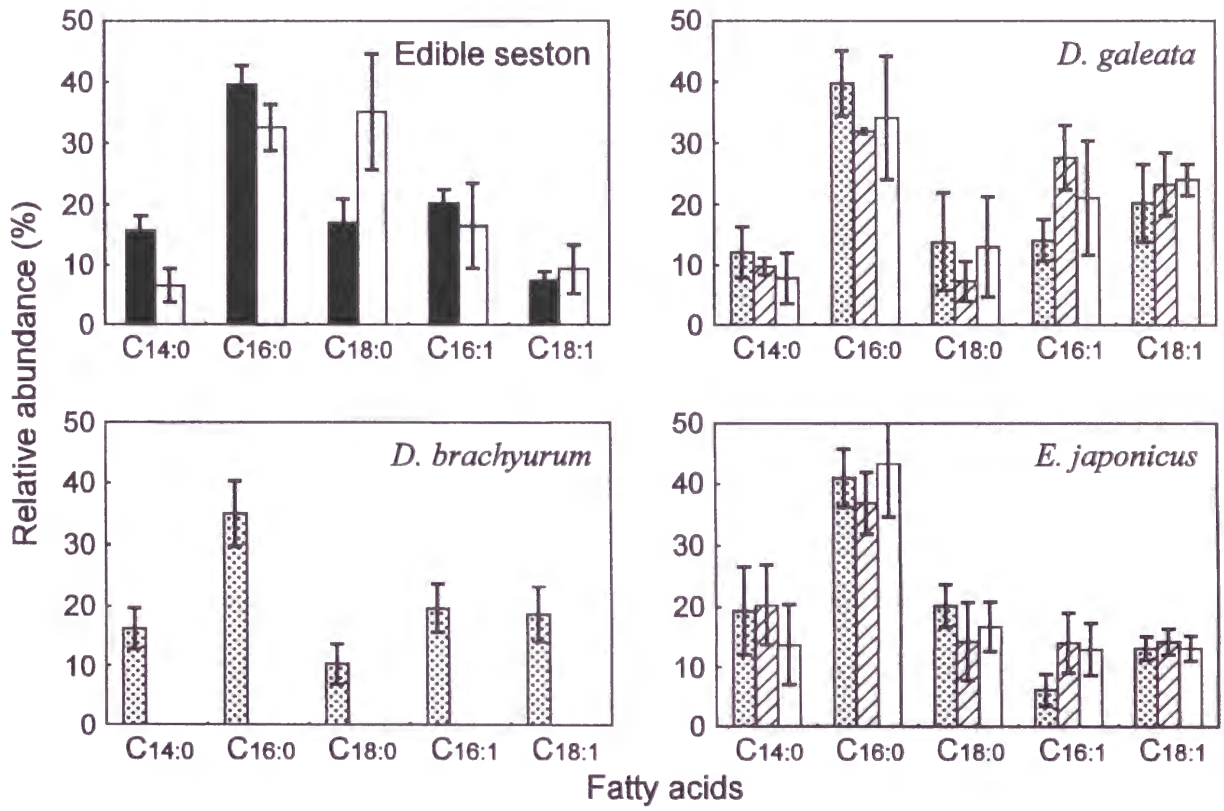

Fig. 4 Relative abundance for each fatty acid in edible seston and zooplankters. Error bars show the 95 % confidence intervals. Edible seston: solid bars, seston collected from 0-15m deep (n = 4); open bars, seston collected from 20-50 m deep (n = 4). Zooplankton: dotted bars, individuals collected from the 0-10 m layer (*D. galeata*, n = 4; *D. brachyurum*, n = 7; *E. japonicus*, n = 4); hatched bars, individuals collected from the 10-20 m layer (*D. galeata*, n = 3; *D. brachyurum*, no data; *E. japonicus*, n = 4); open bars, individuals collected from the 20-30 m layer (*D. galeata*, n = 3; *D. brachyurum*, no data; *E. japonicus*, n = 4)

Table 3

Two-level nested ANOVA calculation for difference in the ratios of fatty acids among zooplankton species with depth.

	C18:1 / C18:0				C18:1 / C16:1			
Source of variation	*df*	*MS*	*F*	percentage of variance	*df*	*MS*	*F*	percentage of variance
Among species within depths	2	0.170	5.32	53	2	0.014	0.32	0
Among depths within species	4	0.032	3.14	17	4	0.044	8.01*	66
Within species	22	0.008		30	22	0.004		34

* Significant difference at 5% level.

To examine the relationship among the relative abundance of fatty acids between the zooplankton and edible seston, the C18:1/C18:0 ratio for zooplankton was plotted against C18:1/C16:1 ratio for the edible seston (Fig. 5). In all species, both ratios were much higher than in the edible seston (< 20 μm). *E. japonicus* tended to have a smaller C18:1/C18:0 ratio than the other species. However, according to the two-way nested ANOVA, the differences in both the C18:1/C16:1 and the C18:1/C18:0 ratios were not significant among the species (Table 3). This implies that differences within species exceeded differences between species. Within *D. galeata* and *E. japonicus*, individuals collected from the 0-10 m and 10-20 m layers tended to have smaller C18:1/C18:0 ratios and larger C18:1/C16:1 ratios than those from the 20-50 m layers. However, significant differences among depth layers were detected only for the C18:1/C16:1 ratio among *E. japonicus* individuals (Table 4). The relative abundance of fatty acids in individuals of *D. brachyurum* was not tested, because not enough individuals were collected from the 10-20 m and 20-50 m layers.

Discussion

The fatty acid composition of each zooplankton species was roughly similar to that of the edible seston (< 20 μm) (Fig. 4). Indeed, C16:0 was the most abundant fatty acid in both the zooplankton and the edible seston. This result suggests that the fatty acid composition of zooplankton was basically reflected by the composition of their food. However, the zooplankton's fatty acid composition did not reflect food sources alone. The relative abundance of C18:0 did not increase with depth in zooplankton, while the relative abundance of C18:0 increased with depth in edible seston. Furthermore, both the C18:1/C16:1 and C18:1/C18:0 ratios were higher in zooplankton than in the edible seston, suggesting that the relative abundance of C18:1 in zooplankton was higher than in the edible seston, and/or the relative

Table 4

Single-classification ANOVA for differences in the ratios of fatty acids among depth layers within *Daphnia* and *Eodiaptomus*.

	C18:1 / C18:0			C18:1 / C16:1		
Source of variation	*df*	*MS*	*F*	*df*	*MS*	*F*
Among depths within *Daphnia*	2	0.824	4.12	2	0.395	3.99
Within *Daphnia*	7	0.200		7	0.099	
Among depths within *Eodiaptomus*	2	0.009	2.80	2	0.054	16.22**
Within *Eodiaptomus*	9	0.003		7	0.003	

** Significant difference at 0.005 level.

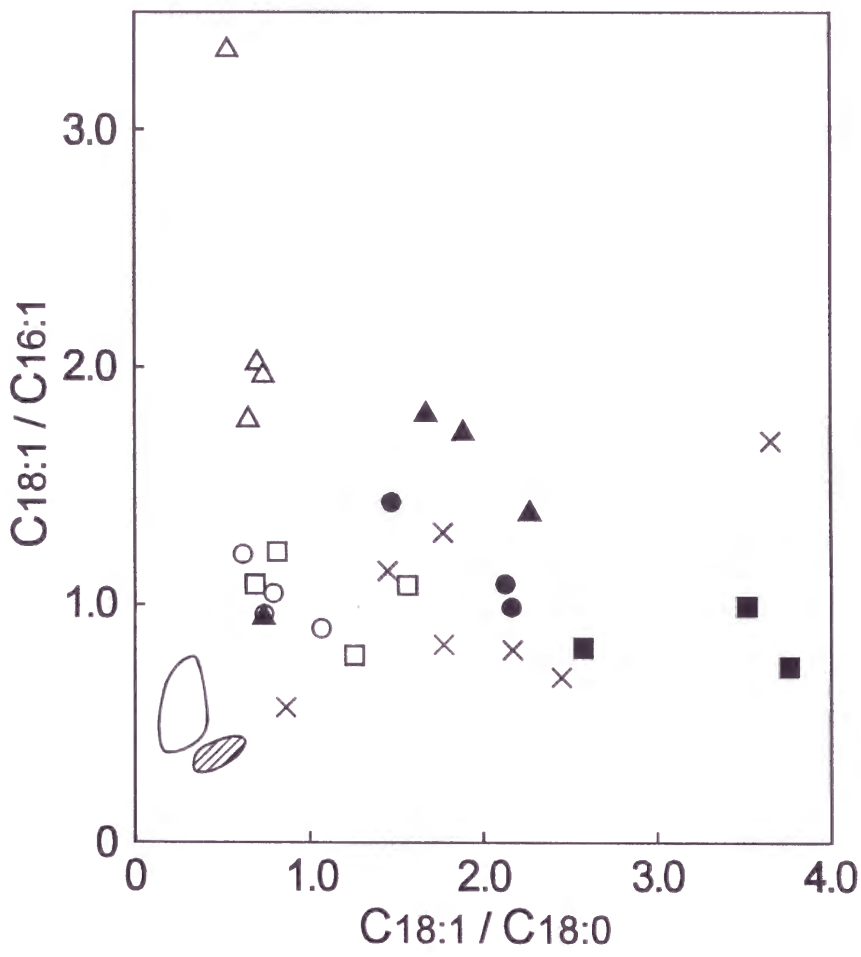

Fig. 5 Ratios of C18:1 fatty acid to C18:0 and C16:1 fatty acid in zooplankters and edible seston. *D. galeata* in 0-10 m (▲), 10-20 m (■), 20-50 m (●); *E. japonicus* in 0-10 m (△), 10-20 m (□), 20-30 m (○) layers; *D. brachyurum* in 0-10 m (×). Open area, edible seston in 0-15 m; hatched area, edible seston in 20-50 m.

abundance of C16:1 and C18:0 in zooplankton were lower than in the edible seston (Fig. 5). The higher relative abundance of C18:1 and the lower relative abundance of C16:1 and C18:0 in zooplankton could be due to selective feeding or differences in the assimilation, catabolism, synthesis, or modification of fatty acid by the zooplankton. Investigations of fatty acid budgets are required to understand these factors, but these investigations were not carried out in the present study. However, the cladocerans *D. galeata* and *D. brachyurum* do not have the ability to select food on the basis of its chemical composition (Butler *et al.* 1989; DeMott 1990). Thus, it is suggested that the differences in the C18:1/C16:1 and C18:1/C18:0 ratios between these cladocerans and the edible seston might be influenced by processes occurring after ingestion, such as excretion, respiration and the modification of fatty acids. On the other hand, since calanoid copepods have the ability of selective feeding to discriminate between foods on the basis of chemical composition (Butler *et al.* 1989), I cannot reject the possibility that *E. japonicus* selectively ingested food with high C18:1 content and/or food with low C16:1 and C18:0 contents. Thus, in addition to potential internal physiological differences, selective feeding may influence the differences in the C18:1/C16:1 and C18:1/C18:0 ratios between *E. japonicus* and edible seston.

A significant difference was detected in the C18:1/C16:1 ratio of *E. japonicus* from different depth layers within (Table 4). This result implies that depth-related factors have contributed to the differences in physiological status and/or the diet. Water temperature and food abundance expressed as chlorophyll *a* differed in the water column (Fig. 2). These factors are known to influence fatty acid composition of zooplankton (Lee *et al.* 1971; Farkas 1979). In the present study, food abundance was highest in the thermocline (10-20 m layer), and lowest in the hypolimnion (20-50 m layer). However, the C18:1/C16:1 ratios of *E. japonicus* in the food-rich 10-20 m layer and food-poor 20-50 m layer were similar to each other. Thus, a vertical

difference in the C18:1/C16:1 ratio of *E. japonicus* can not be explained by food abundance. However, in accord with the difference in the C18:1/C16:1 ratio, water temperature was higher in 0-10m layer than the deeper layer. Thus, differences in the C18:1/C16:1 ratio of *E. japonicus* could be explained by changes in temperature with depth. On the other hand, significant differences in the C18:1/C16:1 and C18:1/C18:0 ratios within *D. galeata* were not detected among depths (Table 4). This result implies that temperature and food abundance changes with depth did not influence the C18:1/C16:1 and C18:1/C18:0 ratios of *D. galeata*.

It should be noted that the fatty acid composition can differ among developmental stages within a species (Kattner and Krause 1987). Therefore, intraspecific differences in the C18:1/C16:1 and C18:1/C18:0 ratios of zooplankton could be related to differences in developmental stage. Furthermore, the vertical distributions of the various developmental stage of *E. japonicus* differ in Lake Biwa (Kawabata 1987). Hence, the vertical difference in the C18:1/C16:1 ratio may have been due to differences in the developmental stage. Unfortunately, since neither the body size nor developmental stage of individuals for fatty acids measurement were recorded, the influence of the developmental stage on the C18:1/C16:1 ratios can not be assessed.

Interspecific differences in the fatty acid composition of zooplankton have been associated with differences in the fatty acid composition of their food (Bourdier and Amblard 1989). In the present study, the fatty acid composition of each zooplankton species was roughly similar to that of edible seston (Fig. 4). Thus, our results support the hypothesis that the fatty acid composition of zooplankton is basically reflected by the fatty acid composition in their food. However, a significant interspecific difference was not found in the C18:1/C16:1 and C18:1/C18:0 ratios due to intraspecific differences (Table 3). Assuming that the fatty acid composition of zooplankton is food specific and is not influenced by other factors, lack of

interspecific differences in fatty acid composition implies that differences in the fatty acid composition of food ingested by each zooplankton individual within the same species were greater than the difference among species. However, as noted above, it is suggested that the difference in fatty acid composition does not reflect food source alone, but water temperature and developmental stage also influence fatty acid composition. Thus, in the present study, the lack of interspecific differences in the fatty acid composition of zooplankton implies that the interspecific difference in fatty acid composition in their food are smaller than the intraspecific difference due to the influence of water temperature and developmental stage.

In the present study, fatty acids longer than C_{20} and poly unsaturated fatty acids could not be detected due to the pyrolysis conditions in the analysis (Ishida *et al.* 1996). However, the present study suggests that intraspecific differences in the fatty acid composition can exceeds the interspecific differences among crustacean plankton. These results suggest that large intraspecific differences in HUFA composition could also occur. Since traditional GC methods require a large number of individual zooplankton to measure fatty acid composition, differences among individuals cannot be detected. Due to this technical problem, previous studies have analyzed the fatty acid composition of zooplankton species without considering intraspecific difference. However, to examine if changes in fatty acid composition are species-specific, the intraspecific difference should now be considered.

Chapter 4. Diel vertical migration of *Daphnia*: Optimum migration schedule based on energy accumulation

Abstract

Zooplankton perform diel vertical migration (DVM) to avoid predators at the upper layer, while often stay at the upper layer throughout a day in spite of presence of the predators. This difference in migration behavior has been explained by difference in environmental condition or genetic difference. Here, I theoretically examined how internal conditions of zooplankton individuals relate to determining different migration behavior based on their reproductive success. A simple optimization model demonstrates that zooplankton individuals change their migration behavior depending on energy accumulation for reproductive investment. Such energy accumulation and its investment for reproduction are repeated every reproduction cycle. Therefore, if the reproduction cycle is not synchronized among individuals, different migration behaviors will be observed within a population regardless of neither difference in environmental condition nor genetic difference. Our model demonstrates that such coexistent of the two migration behaviors is possible in natural *Daphnia* populations, and suggests that internal conditions of zooplankton individuals can not be neglected as a factor for determining migration behavior of zooplankton.

Diel vertical migration (DVM) is general phenomenon in marine and fresh water zooplankton. Usually, zooplankton ascend at dusk after staying at the deeper layer during daytime, and then descend at dawn after staying at the upper layer during nighttime (Cushing

1951; Hutchinson 1967). Several hypotheses have been proposed for the reason of DVM (Kerfoot 1985; Lampert 1989, 1993). Among these, 'predator avoidance hypothesis' has been most supported by many researchers (Zaret and Suffern 1976; Stich and Lampert 1981; Wright *et al.* 1980). This hypothesis states that zooplankton stay at the deeper layer with dark and low food condition to avoid visually searching predators such as fish during daytime, but stay at the food-rich upper layer to gain food during nighttime.

Huntley and Brooks (1982) reported that when food supply was limited, zooplankton stayed at the upper layer throughout a day in spite of presence of predators. This fact suggests that whether zooplankton perform DVM or stay at the upper layer throughout a day is a result of a trade-off between avoidance from visually searching predators and food gain (Johnsen and Jakobsen 1987; Lampert 1989). Therefore, it has been explained that seasonal changes in balance of the trade-off between predation pressure and food abundance cause seasonal changes between the two migration behaviors. Apart from these seasonal changes, two inter- or intraspecific groups with the different migration behaviors are observed within lakes (Stich and Lampert 1981; Weider 1984; Guisande *et al.* 1991). Since prey body size influences on visibility for predator (Zaret and Kerfoot 1975; Brooks and Dodson 1965; Write *et al.* 1980), it is supposed that genetic difference in body size causes difference in vulnerability of predation, resulting differentiation of the migration behavior (DeMeester *et al.* 1995). However, Sekino and Yoshioka (1995) reported that the different migration behaviors, which cannot be explained by the body size, coexist within a population. They suggested that difference in nutritional status is important to explain migration behavior of zooplankton.

Zooplankton invest most of accumulated energy through feeding for reproduction after maturation (Tessier and Goulden 1982). Thus, zooplankton with more accumulated energy can produce more offsprings and have higher potential of reproductive fitness. In this respect,

zooplankton which stay at the upper layer throughout a day have more advantages than those which perform DVM. Ingested zooplankton by predators, however, lose whole accumulated energy otherwise used for the reproduction. If the expected loss of accumulated energy due to predation is larger than the net energy gain, zooplankton may move to the deeper layer to avoid predator at the expense of energy gain. Assuming that environmental conditions are constant, the net energy gain is proportional to time period when they stay at the upper layer. Whereas the expected loss of accumulated energy due to predation increases with increasing amount of accumulated energy. Therefore, it is expected that zooplankton begins to perform DVM when the amount of accumulated energy exceeds a certain threshold. Since energy accumulation and reproductive investment are repeated (Tessier and Goulden 1982), zooplankton may alter their migration behavior according to reproduction cycle. If the reproduction cycle is not synchronized in a population, different migration behavior will be observed within the population because amount of accumulated energy exceeds the threshold in some individuals but not in the others.

Several theoretical models about DVM of zooplankton have been proposed in previous studies (demographic simulation model, Wright *et al.* 1980; game model, Iwasa 1982; ESS model, Gabriel and Thomas 1988a, b; life history model, Fiksen 1997). However, there were no studies which consider a fact that the amount of accumulated energy changed according to reproduction cycle. In the present study, I investigate an optimum migration schedule within a reproduction cycle to maximize reproductive fitness using a simple mathematical model. I further examine how such a schedule change according to food abundance and predation pressure. Here, I define the ratio and order of the two migration behaviors within a certain period as a migration schedule. The present study is carried out using *Daphnia* (Cladocera: Crustacea) as a model animal, because they are dominant zooplankton in most lakes.

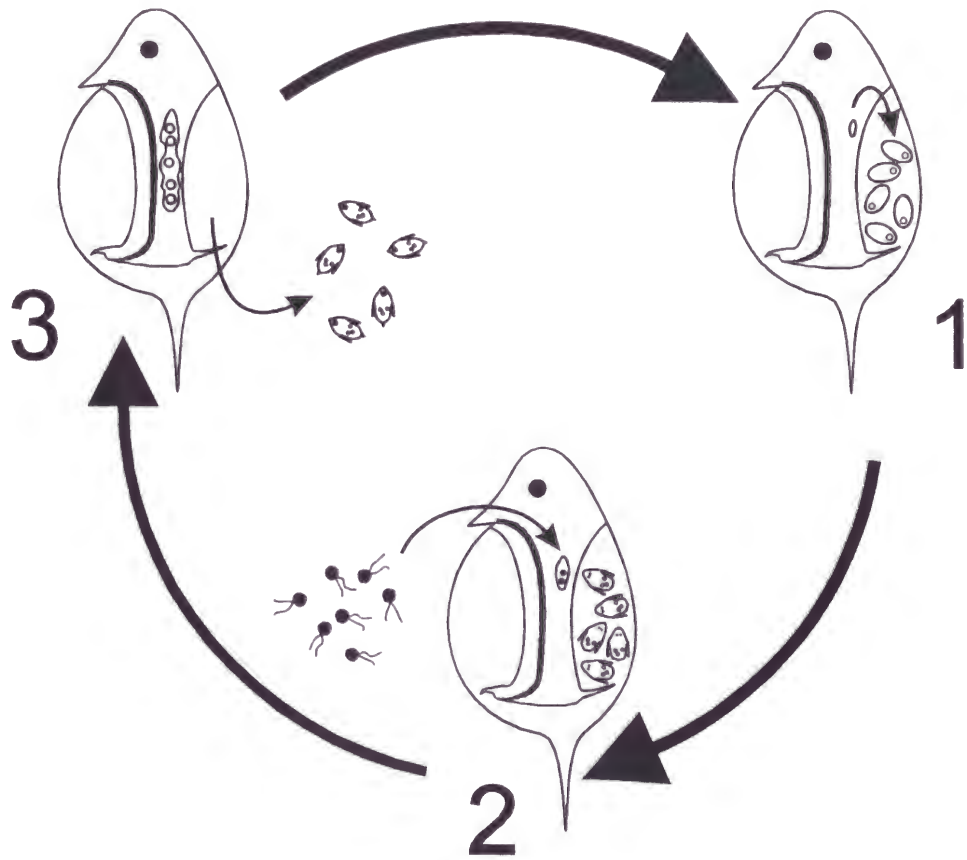

Fig. 6. A reproduction cycle of *Daphnia*. 1. A maternal *Daphnia* produces eggs from accumulated energy, and moves the newly produced eggs into the brood porch in its back. 2. The *Daphnia* develops the eggs in the brood porch, and accumulates energy for the next reproductive investment. 3. The *Daphnia* releases eggs at the brood porch.

Model

Since *Daphnia* produce eggs at each molt after maturation, a reproduction cycle corresponds to molt cycle (Fig. 6). Within each molt, *Daphnia* accumulate energy and invest them to egg production at the end of inter molt. The produced eggs are released into brood porch where they develop until next maternal molt when the juveniles leave from the maternal individuals (Tessier and Goulden 1982). Since these eggs in the brood porch obtain no nutrition from maternal individual, duration of egg development depends only on water temperature. Since egg development time roughly equal to inter molt duration (Tessier and Goulden 1982), our model assumes that a reproduction cycle corresponds to egg development time.

The reproductive fitness over a given reproduction cycle (W) can be expressed as

$$W = S(\alpha + \beta \cdot pR) \ , \tag{1}$$

where S and R are the survival probability ($cycle^{-1}$) and the amount of accumulated energy ($J \cdot cycle^{-1}$) during the reproduction cycle, and p is the number of eggs produced by unit energy ($eggs \cdot J^{-1}$). Here, the expected clutch size in next reproduction cycle is expressed as pR. α is the reproductive value of maternal individual including eggs in its brood porch, and it is expressed as

$$\alpha = 1 + NM \ , \tag{2}$$

where 1 means reproductive value of a given maternal individual; N is the number of eggs in brood porch; These eggs are produced in previous reproduction cycle and develop in the brood porch; M is the survival probability of a juvenile from leaving the brood porch to maturation. β is the reproductive value of a newly produced egg and is expressed as

$$\beta = S' \cdot M \ , \tag{3}$$

where S' is the expected value of survival probability of an egg during development in the next reproduction cycle until leaving the brood porch.

When an individual stays at the upper layer for the former i days ($0 \leq i \leq T$) and performs DVM for the latter T - i days in T days of a reproduction cycle, the survival probability (S) and the amount of accumulated energy during the reproduction cycle (R) are expressed as

$$S = s_s^{\,i} \cdot s_m^{\,(T-i)} \tag{4}$$

$$R = r_s i + r_m (T - i) \ , \tag{5}$$

where s_s and r_s are the daily survival rates (day^{-1}) and the daily amounts of accumulated energy ($J \cdot day^{-1}$), when the individual stays at the upper layer throughout a day; s_m and r_m are the daily survival rate and the daily amount of accumulated energy, when the individual performs DVM. Here, I assume $s_s < s_m$, because the death rate due to predation is lower for the migrating individuals than for the staying individuals. On the other hand, I assume $r_s > r_m$, because the migrating individuals stay at the food-poor deeper layer during daytime. It should be noted that there is a trade-off between S and R because S decreases with increasing i while R increases, and *vice versa*. Applying equation (4) and (5) for equation (1), I can represent the fitness when an individual stays at the upper layer for i days (W_i) as

$$W_i = s_s^{\,i} \cdot s_m^{\,(T-i)} \{\alpha + \beta \cdot p(r_s i + r_m (T - i))\} \quad (i = 0, 1, 2, \cdots, T) \ . \tag{6}$$

I consider that migration schedule evolves so that the fitness (W_i) is maximized. The optimal solution can be obtained by the following procedure.

I define w_i as the ratio of W_{i+1} to W_i:

$$w_i = \frac{W_{i+1}}{W_i} = \frac{s_s}{s_m} \cdot \frac{\alpha + \beta \cdot p((r_s - r_m)(i+1) + r_m T)}{\alpha + \beta \cdot p((r_s - r_m) i + r_m T)} \quad (i = 0, 1, 2, \cdots, T-1) \tag{7}$$

Since $r_s > r_m$, w_i decrease monotonously with increasing i. Neglecting special cases with $w_i = 1$, this means that W_i has a unique maximum value at a certain i under any condition.

If $w_0 < 1$, W_0 is maximum because $w_i < 1$ for any i so that

$$W_o > W_1 > W_2 > \cdots > W_T \ .$$

If $w_{i-1} > 1$ and $w_i < 1$, W_i is maximum $(i = 1,2,\cdots,T-1)$ because

$$W_0 < \cdots < W_{i-1} < W_i \quad \text{and } W_i > W_{i+1} > \cdots > W_T \ .$$

Finally, if $w_{T-1} > 1$, W_T is maximum because $w_i > 1$ for any i so that

$$W_o < W_1 < W_2 < \cdots < W_T \ .$$

Applying Equation (7), the above inequalities for w_i can be rewritten as follows:

W_0 is maximum when

$$\frac{s_m}{s_s} > \frac{\frac{\alpha}{\beta}\cdot\frac{1}{pr_s} + 1 + \frac{r_m}{r_s}(T-1)}{\frac{\alpha}{\beta}\cdot\frac{1}{pr_s} + \frac{r_m}{r_s}T} \ ; \tag{8}$$

W_i is maximum for $i = 1,2,\cdots,T-1$ when

$$\frac{\frac{\alpha}{\beta}\cdot\frac{1}{pr_s} + i + \frac{r_m}{r_s}(T-i)}{\frac{\alpha}{\beta}\cdot\frac{1}{pr_s} + i - 1 + \frac{r_m}{r_s}(T-i+1)} < \frac{s_m}{s_s} < \frac{\frac{\alpha}{\beta}\cdot\frac{1}{pr_s} + i + 1 + \frac{r_m}{r_s}(T-i-1)}{\frac{\alpha}{\beta}\cdot\frac{1}{pr_s} + i + \frac{r_m}{r_s}(T-i)} \ ; \tag{9}$$

W_T is maximum when

$$\frac{s_m}{s_s} < \frac{\frac{\alpha}{\beta}\cdot\frac{1}{pr_s}}{\frac{\alpha}{\beta}\cdot\frac{1}{pr_s} + T - 1 + \frac{r_m}{r_s}} \ . \tag{10}$$

Inequality (10) is the condition where staying at the upper layer during a whole reproduction cycle (non-DVM) is optimum migration schedule. Inequality (8) is the condition where DVM everyday during a whole reproduction cycle (continuous-DVM) is optimum migration schedule. Inequality (9) is the condition where it is optimum migration schedule that the individual stays at the upper layer for i days, and then performs DVM for T-i days (occasional-

Table 5

Glossary of symbols.

Symbol	Interpretation	Unit
(Variables)		
i	number of days for staying at the upper layer	days
W	reproductive fitness over a given reproduction cycle	
W_i	reproductive fitness for i days staying at the upper layer during T days of reproduction cycle	
S	survival rate over a given reproduction cycle	$cycle^{-1}$
R	amount of accumulated energy over a given reproduction cycle	$J \cdot cycle^{-1}$
(Parameters)		
s_s	daily survival rate for staying at the upper layer	day^{-1}
s_m	daily survival rate for DVM	day^{-1}
r_s	daily amount of accumulated energy for staying at the upper layer	$J \cdot day^{-1}$
r_m	daily amount of accumulated energy for DVM	$J \cdot day^{-1}$
T	number of days for a reproduction cycle	days
α	reproductive value of a given maternal individual	
β	reproductive value of a newly produced egg	
N	number of eggs in brood porch	eggs
M	survival probability of a juvenile during maturation	
S'	survival probability of an egg in brood porch during development	
p	number of eggs produced by unit energy	$eggs \cdot J^{-1}$

DVM). When parameters T, pr_s and α / β are given, conditions for these optimum migration schedules can be identified as regions in the space of the ratios of s_m / s_s and r_m / r_s according to inequalities (8), (9) and (10).

Results

To examine relationship between optimum migration schedule and environmental conditions, I used literature values on *Daphnia* species for standard values of parameters T, pr_s and α / β. I also examined effect of variation from these standard values. Since duration of egg development for *Daphnia* species is about 5 days at 15 °C (Bottrell *et al.* 1976), I set T at 5 days. If individuals continue to stay at the upper layer during a whole T days of reproduction cycle, the number of produced eggs within the reproduction cycle (i.e. the clutch size) is expressed as pr_sT. Assuming this egg number as clutch size for the next reproduction cycle (pr_sT), it was set at 15 which fall within usual clutch size for *Daphnia* species (Hall 1964). Therefore, pr_s was set at 3. The number of eggs in brood porch in this reproduction cycle (N) was set at 15 from usual clutch size for *Daphnia* species. Survival probabilities of M and S' were tentatively assumed as 0.2 and 0.8, though they may vary in the natural population. Therefore, α / β was set at 25 from assumed N, M through S' and equations (2) and (3)

Using values mentioned above, I illustrated the number of days for the staying at the upper layer (i) to maximize the fitness (W_i) (Fig. 7). When the value of s_m / s_s is low, the fitness is maximum at $i = T$. This means that the optimum migration schedule is staying at the upper layer during the whole reproduction cycle (non-DVM). As s_m / s_s increases, the optimum value of i, the number of days for the staying at the upper layer, is decreased. This implies that the increase in s_m / s_s enhances DVM. When optimum value of i is 0, the individual performs DVM everyday during the whole reproduction cycle (continuous-DVM). Whereas, when optimum

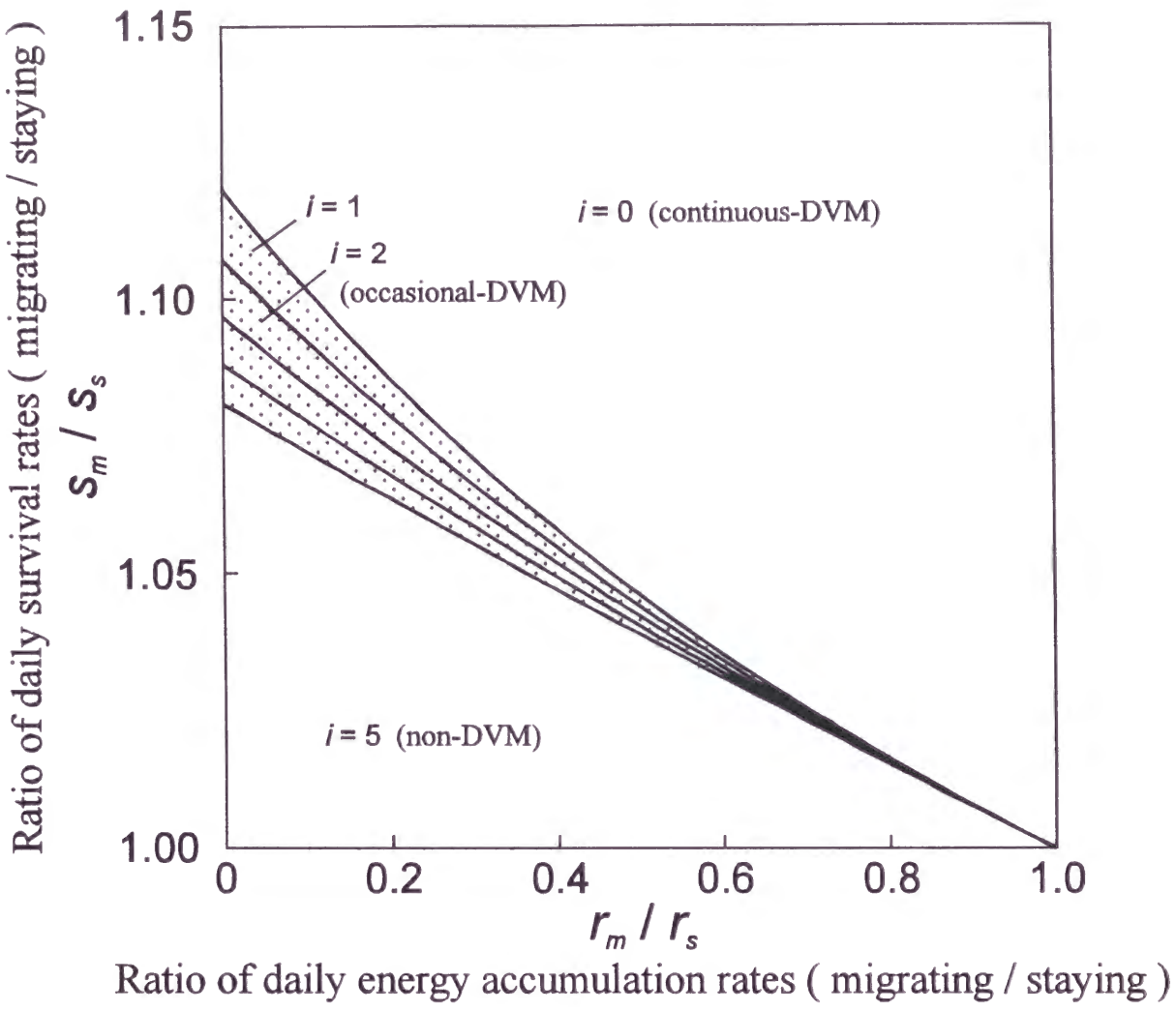

Fig. 7. The number of days for the staying at upper layer (i) to maximize the fitness (W_i) illustrated as regions in the space of the ratio of survival rates (s_m / s_s) and the ratio of daily amounts of energy accumulation (r_m / r_s). T, pr_s and α / β are set at 5, 3 and 25, respectively.

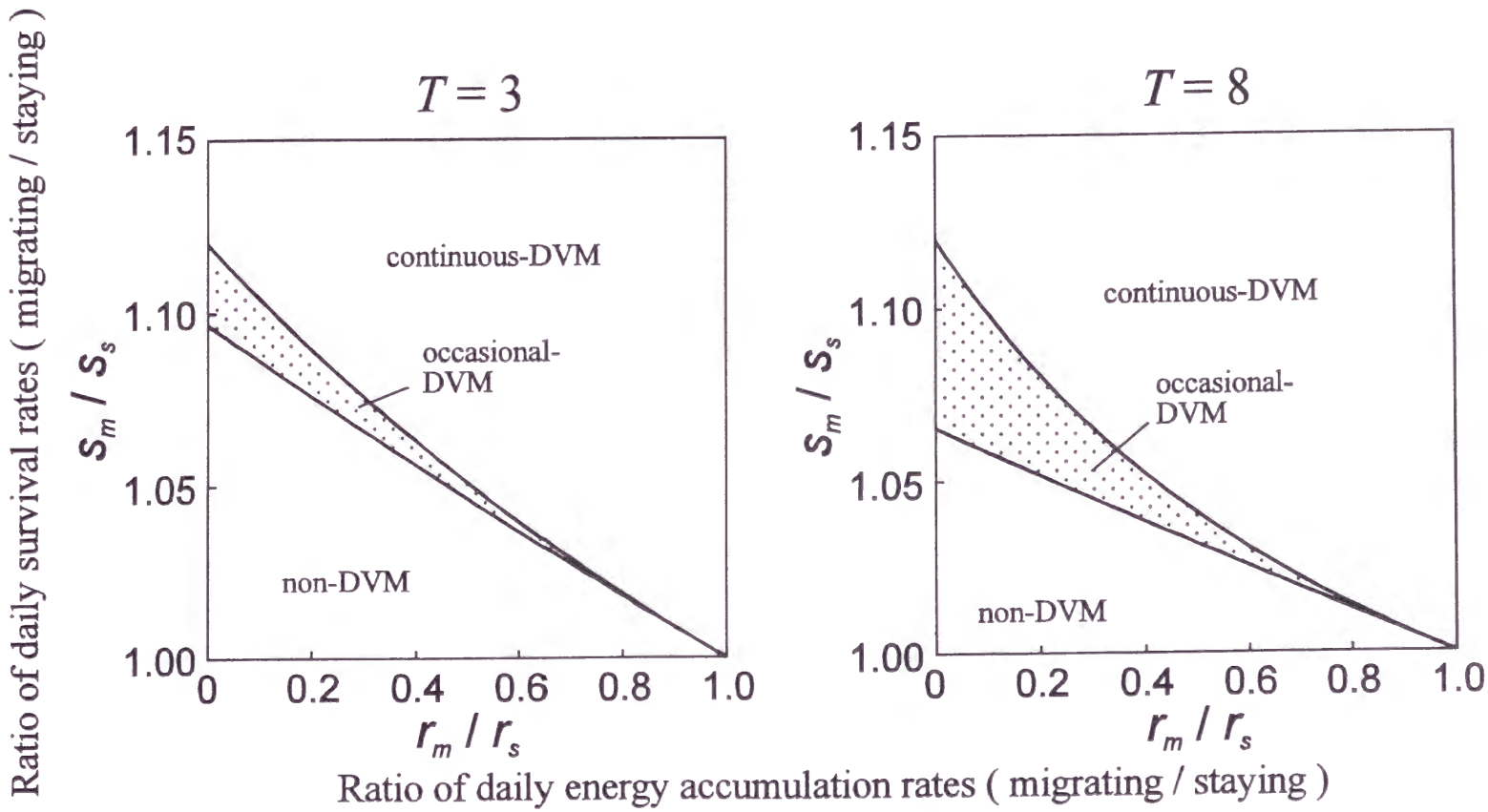

Fig. 8a, b Optimum migration schedule in the space of the ratios of s_m / s_s and r_m / r_s . Left panel (a), $T = 3$; Right panel (b), $T = 8$. pr_s and α / β are set at 3 and 25, respectively.

value of i is between 1 and 4 (i.e. T-1), the individual continues to stay at the upper layer for i days, and then performs DVM for T-i days (occasional-DVM). The result indicates that it is advantageous for *Daphnia* to change their migration behavior depending on the ratio of survival rates of the two migration behaviors within a reproduction cycle. As r_m / r_s increase at a low value of s_m / s_s, the optimum value of i gradually decreases. This means that with decreasing the daily amount of energy accumulation at the upper layer, the optimum migration schedule change from non-DVM to continuous-DVM through occasional-DVM. However, when s_m / s_s takes a higher value, non-DVM never becomes the optimum migration schedule for any r_m / r_s. As s_m / s_s increases further, optimum migration behavior is only continuous-DVM for any r_m / r_s. In this sense, I can say that s_m / s_s affects the optimal behavior more strongly than r_m / r_s.

Since the period of a reproduction cycle (T) is affected by water temperature (Hall 1964; Bottrell *et al.* 1976), I examined the effect of water temperature on optimum migration schedule. The optimum migration schedules in the space of s_m / s_s and r_m / r_s were calculated at 20 and 10 °C, where reproduction cycle of *Daphnia* (T) is 3 and 8 days (Fig. 8). In the calculations, the other parameters were fixed to those when $T = 5$ in Fig. 7. The regions of continuous-DVM for $T = 3$ and $T = 8$ is scarcely different from that for $T = 5$, implying that water temperature does not greatly influence on the condition where continuous-DVM is optimum. On the other hand, the region of occasional-DVM is larger for larger T values, while the region of non-DVM is smaller for larger T values, implying that when T increases due to low water temperature, the condition where occasional-DVM is an optimum is expanded, while the condition where non-DVM is optimum is restricted.

To examine the effect of changes in parameter α / β , the optimum migration schedules when α / β is 10 and 50 were calculated (Fig. 9). In the calculations, the other parameters

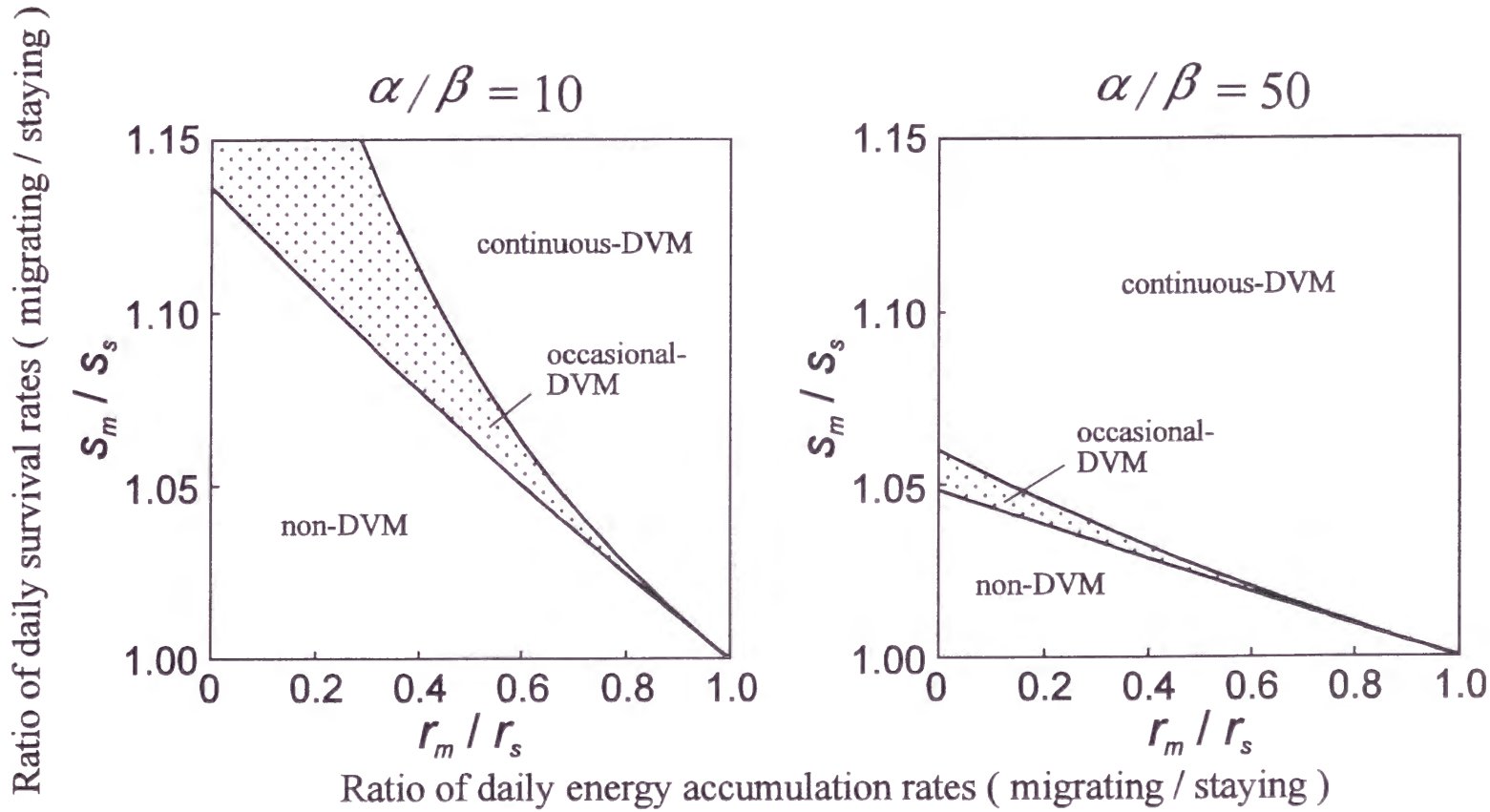

Fig. 9a, b Optimum migration schedule in the space of the ratios of s_m / s_s and r_m / r_s . Left panel (a), $\alpha / \beta = 10$; Right panel (b), $\alpha / \beta = 50$. T and pr_s are set at 5 and 3, respectively.

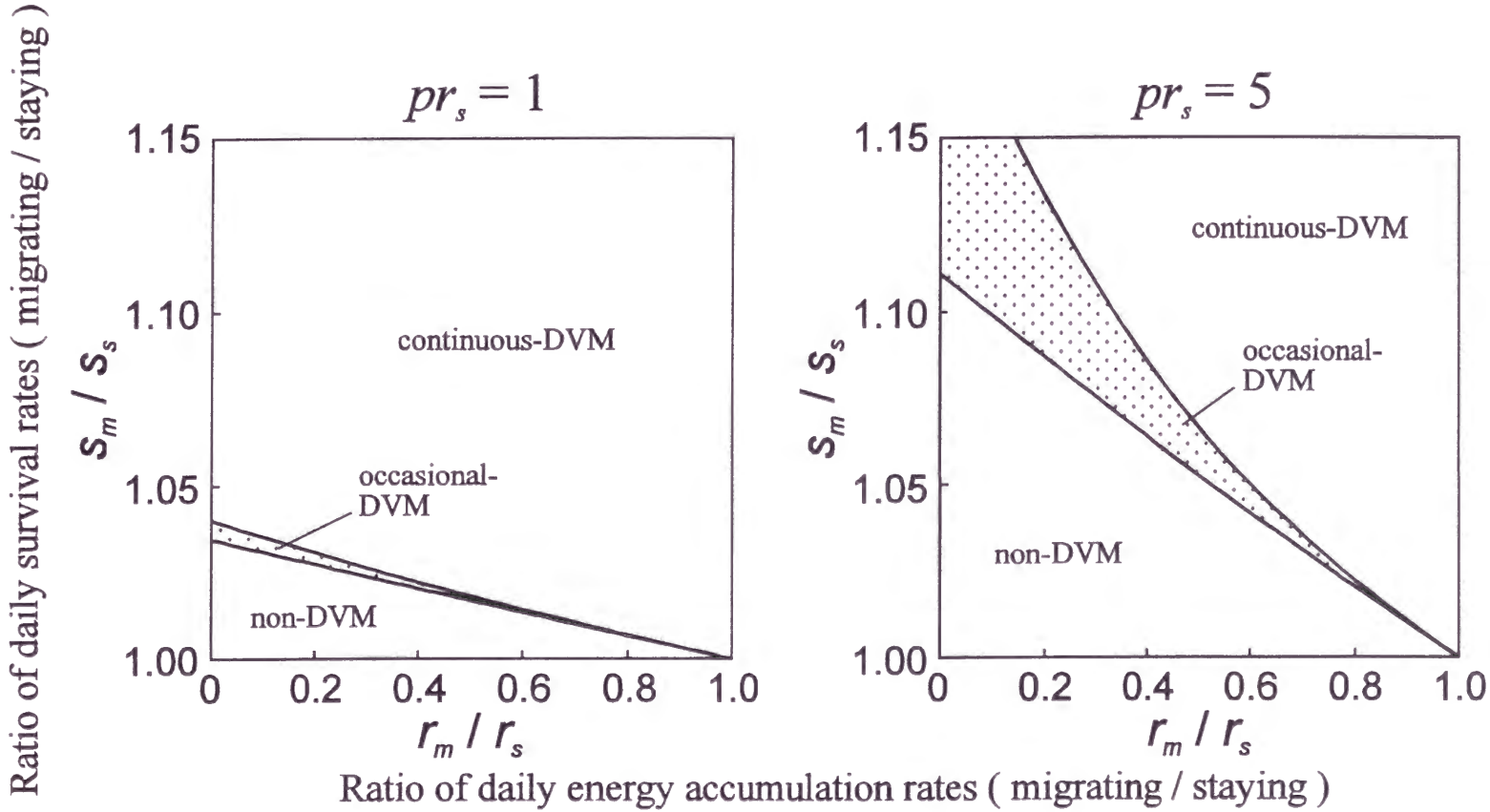

Fig. 10a, b Optimum migration schedule in the space of the ratios of s_m / s_s and r_m / r_s . Left panel (a) , $pr_s = 1$; Right panel (b) , $pr_s = 5$. T and α / β are set at 5 and 25, respectively.

were fixed to those when $\alpha / \beta = 25$ in Fig. 7. The region of continuous-DVM is larger for larger α / β values, whereas the regions of non-DVM and occasional-DVM are smaller for larger α / β values. These results imply that increase in α / β enhances DVM. According to equation (2) and (3), α / β increases when M and / or S' decrease and when N increases. Thus, the condition for each migration schedule to be advantageous is connected with juvenile survival probability (M), egg survival probability in the next reproduction cycle (S') and the number of eggs in the brood porch (N).

The optimum migration schedules when pr_s is 1 and 5 were calculated (Fig. 10). In the calculation, the other parameters were fixed to those when $pr_s = 3$ in Fig. 7. Since T is set at 5, the expected clutch size in next reproduction cycle for an individual of non-DVM (pr_sT) is 5 eggs for $pr_s = 1$ and 25 eggs for $pr_s = 5$. The region of continuous-DVM is smaller for larger pr_s values, whereas those of occasional-DVM and non-DVM are larger for larger pr_s values. It should be noted that p is inversely proportional to the egg mass, and r_s is proportional to food abundance at the upper layer. Since fluctuation of egg mass is smaller than that of food abundance (Trubetskova and Lampert 1995), pr_s depends mainly on food abundance at the upper layer in actual cases. Thus, the effect of changes in parameter pr_s on the optimum migration schedule indicates that the increase in food abundance at the upper layer under a constant ratio of r_m / r_s enhances staying at the upper layer throughout a day.

Discussion

The present study suggests that *Daphnia* may change its migration behavior during a reproduction cycle depending on the amount of accumulated energy. Since the reproduction cycle of *Daphnia* is not generally synchronized among individuals (Hall 1964), I may observe two individual groups with different migration behaviors within a population: one individual

group stays at the upper layer throughout a day and the other individual group performs DVM. In the previous studies, coexistence of different migration behaviors has been explained by genetic differences (Guisande et al. 1991; DeMeester *et al.* 1995). In support with this explanation, some genotypes are known to coexist simultaneously in a lake (Weider 1984; Müller and Seitz 1993; Spaak and Hoekstra 1993). However, our result suggests that difference in migration behavior can be also explained simply by difference in the amount of the accumulated energy among individuals within the same population. Some previous studies showed that changes in migration behavior of zooplankton can be explained by phenotypical polymorphism because they were induced by increasing predators (Ringelberg 1991; DeMeester 1993), suggesting that phenotypical changes in migration behavior may also be induced by changes in the amount of the accumulated energy.

The survival rate for each migration behavior (s_m and s_s) depends on predation pressure at different depths. When predators do not exist at both layers, s_m / s_s is 1. As predation pressure at the upper layer increases, s_m / s_s increases due to decreasing s_s. As less than 0.5 of survival rate per day for *Daphnia* is not usually observed in a natural population (Hall 1964; Byron *et al.*, 1986; Lueck *et al.* , 1990; Walters *et al.*, 1990; Lampert 1991), the maximum s_m / s_s is not larger than 2. Thus, most of s_m / s_s value for natural population is likely in the range between 1 and 2. In addition, Lampert (1987) reported that daily death rates of migrating individuals and of staying individuals at the upper layer were 0.019 and 0.100 in a natural population of *Daphnia*, respectively, in Lake Constance. In this case, since $s_m = 0.981$ and $s_s = 0.900$, s_m / s_s is 1.090. Therefore, the range of s_m / s_s examined in the present study is realistic in natural populations.

Daily amount of energy accumulation (r_m and r_s) depends on food abundance at the upper and the deeper layers. The vertical difference in food abundance changes between seasons

(Huntley and Brooks 1982). When the vertical difference in food abundance is small, r_m / r_s approaches 1. On the other hand, when food abundance at the deeper layer is smaller than that at the upper layer, r_m / r_s is less than 1. If vertical difference in food abundance is great, r_m / r_s is roughly equal to ratio of nighttime length per day because nighttime length determines the period that migrating individuals can gain food at the food-rich upper layer. Thus, r_m / r_s can be much less than 0.5 in summer except for low latitude areas. In addition, since migrating individuals require time for migration between the deeper and the upper layer, the period that migrating individuals gain food at the upper layer is shorter than nighttime length. Therefore, r_m / r_s may often approach 0. Consequently, it is supposed that the value of r_m / r_s in natural population may take various values between 0 and 1 in which I examined the optimal migration behavior.

As shown in figure 7, occasional-DVM appears generally in region where $s_m / s_s \cong 1.1$ and $r_m / r_s < 0.5$ when other parameters take standard values for *Daphnia* species. The above discussion implies that these values are not unrealistic in natural populations, and therefore, the occasional-DVM may be realistic as an optimum migration schedule.

Occasional-DVM was defined as the migration schedule that zooplankton perform DVM after staying at the upper layer within a reproduction cycle. In our model, however, the fitness does not change between individuals performing DVM before and after staying at the upper layer, if number of days when they stay at the upper layer is the same (see equation (6)). Nevertheless, in natural populations, it is supposed that *Daphnia* perform DVM after staying at the upper layer in occasional-DVM, by the following reason which was not included in the model assumption. *Daphnia* accumulate lipid as energy for reproductive investment (Tessier and Goulden 1982). These lipids are combined with carotenoids, and therefore are colored (Green 1957). In addition, colored eyes appear in eggs in the brood porch with egg

development (Threlkeld 1979). Therefore, individuals with colored lipid and developed eggs during the latter period of a reproduction cycle are more visible than individuals during the earlier period of the reproduction cycle. Generally, transparency and color of zooplankton body affect on visibility for predator (Zaret and Kerfoot 1975; Kerfoot 1985). Thus, predation pressure for individuals are larger during latter period than earlier period of the reproduction cycle. This difference in predation pressure between the periods implies that individuals should stay at the upper layer during earlier period of the reproduction cycle in occasional-DVM. This is consistent with an observation by Sekino and Yoshioka (1995) that *Daphnia* with early stage eggs stayed at the upper layer throughout a day, while *Daphnia* with late stage eggs performed DVM. It is also easily expected that if the model is modified so that the survival probability decreases more strongly in s_s than s_m as reproduction cycle proceeds, the above order of migration behavior becomes optimal.

In our model, the period of a reproduction cycle (T) is assumed to be constant, and does not change depending on migration behaviors. Decrease in water temperature actually causes an increase in the period of a reproduction cycle. Therefore, migrating zooplankton which stay at the cool deep layer during daytime have longer period of a reproduction cycle than zooplankton which stay at the warm upper layer throughout a day (Orcutt and Porter 1983; Ringelberg *et al.* 1991). How does the result of our mode change if this factor is incorporated? Suppose that the reproduction cycle of migrating *Daphnia* is 8 days and that of staying *Daphnia* at the upper layer is 3 days. In this case, the boundary between the region where continuous-DVM and occasional-DVM are optimal will be almost the same as the boundary in Fig. 8b, because *Daphnia* with occasional-DVM, near the boundary, have also a reproduction period near $T = 8$. By the same reason, the boundary between the regions where non-DVM and occasional-DVM are optimal will be almost the same as the boundary in Fig. 8a where $T =$

3. Since, as I have already noted, the former boundary scarcely changes between Fig. 8a and Fig. 8b, the pattern of optimal migration behavior in the (r_m / r_s, s_m / s_s) space in this case is almost the same as that in Fig. 8a. Thus, I can approximately apply the result of our model with constant reproduction cycle for the case where migrating *Daphnia* require a longer reproduction period.

Ratio of reproductive values of the given maternal individual to a newly produced egg (α / β) strongly affected the range of condition where each migration schedule is optimum: as seen in equation (2) and (3), decrease in the survival probability of a juvenile during maturation (M) and the survival probability of an egg in the brood porch (S') enhances DVM (Fig. 9). Zaret and Suffern (1976) suggested that zooplankton perform DVM when the survival rate decreases with increasing predation pressure. This suggestion referred to the direct predation pressure on individuals which are represented by s_m and s_s in our model. Since M and S' are the expected survival probabilities after this reproduction cycle, the optimal migration behavior is also affected by future predation pressure. Thus, zooplankton may behave more adaptively if they can estimate future predation pressure.

Effect of α / β on migration schedule suggests that increase in the number of eggs in the brood porch (N) also enhances DVM (see equation (2) and (3)). It is known that *Daphnia* with more eggs in brood porch is more vulnerable to predation by fish (Tucker and Woolpy 1984; O'Brien 1987). Therefore, it is considered that increasing egg number in brood porch enhance DVM according to increasing predation pressure. The present model, however, showed that changes in N affects on the migration behavior without affecting directly on survival rates of *Daphnia*. They can obtain more fitness through DVM when they carry more eggs because zooplankton protect their existent eggs from predators.

In the previous studies, migration behavior of zooplankton has been explained by the trade-

off between avoidance from visually searching predators and food gain (Johnsen and Jakobsen 1987; Lampert 1989). Our model explains this mechanism by changes in s_m / s_s and r_m / r_s which relate to vertical difference in predation pressure and food abundance, respectively. In addition, our model demonstrates that not only relative food abundance at the upper layer but also absolute food abundance under constant r_m / r_s enhances staying at the upper layer throughout a day (Fig. 10). However, some previous studies demonstrate contrarily that decreasing in absolute food abundance reduces DVM (Huntley and Brooks 1982; Johnsen and Jakobsen 1987). According to these studies, zooplankton cease DVM at low food abundance to avoid starvation even under a high predation pressure. However, our model assumed that *Daphnia* are not starved, and invest net energy gain for reproductive investment. Therefore, at high food abundance, energy gain by staying at the upper layer exceeds the mortality loss. The difference in the present and previous studies suggests that the influence of absolute food abundance on migration behavior differs between starved and unstarved conditions.

In conclusion, difference in migration behavior of *Daphnia* can be explained by behavioral changes depending on the amount of accumulated energy. This conclusion implies that nutritional status of zooplankton individuals are important to determine their migration behavior. Nutritional status has scarcely been studied as the factor of migration behavior, though nutritional status as a result of the migration behavior has been studied (Guisande *et al.* 1991; Duncan *et al.* 1993). Our model considers such internal condition, and therefore, can provides a theoretical base which explains various types of migration behavior with neither genetic difference nor fluctuation of environmental conditions.

Chapter 5. Seasonal changes in vertical distribution of *Daphnia galeata*: Effects of migration and food conditions

Abstract

Daphnia galeata population in Lake Biwa was examined to clarify factors inducing seasonal changes in the vertical distribution. Birth and death rates of *D. galeata* were estimated at each depth and compared with vertical changes in individual density. The analyses revealed that vertical distribution of *D. galeata* was changed by vertical difference in birth rate when their nutritional status was high, while that was changed by migration when their nutritional status was low. Thus, seasonal change in vertical distribution of zooplankton is caused by migration and vertical difference in birth rate. *Daphnia* increase their density at depths where food is abundant, but they migrate and disperse to search for food vertically, when food is depleted.

Zooplankton change their vertical distribution diurnally and seasonally. Diurnal changes in vertical distribution of zooplankton, which is called diel vertical migration, are explained by avoidance from visually searching predators at daytime in the deeper layer and migration to the upper layer to obtain food at nighttime (Zaret and Suffern 1976; Stich and Lampert 1981; Wright *et al.* 1980). Compared to diurnal changes, seasonal changes in vertical distribution of zooplankton have been less focused. A limited number of studies, however, suggested that these are related with various environmental factors such as food abundance (Leibold 1990; Cummigel 1983; George 1983; Sameoto 1984; Paffenhöfer 1983), predation pressure (Leibold

1990; Mclville and Maly 1981), water temperature (Sameoto 1986; Castro *et al.* 1991) and dissolved oxygen (Horppila 1997; Sameoto 1986; Weikert 1982). These studies were made by comparing simply patterns of vertical distribution of zooplankton with environmental factors.

However, it should be noted that vertical distribution of zooplankton is determined by two immediate causes. These are, 1) immigration to and emigration from certain depths; 2) vertical differences in birth and death rates. Seasonal changes in vertical distribution induced by immigration and emigration are a response to present environmental condition, while those induced by vertical difference in birth and death rates are a result of past environmental condition. Thus, the two causes should be examined separately to clarify environmental factors inducing seasonal changes in vertical distribution of zooplankton . However, previous studies have not separated these two causes, and therefore not been sufficient to examine the relationship between the vertical distribution and environmental factors.

In the present study, I estimated depth-specific birth and death rates of *Daphnia* population and their vertical migration in Lake Biwa. The estimated rates were used to clarify why they seasonally change their vertical distribution.

Methods

Lake Biwa is located in the center of Honshu Island in Japan at an altitude of 85 m, and is 674.4 km^2 in surface area and 104.0 m in maximum depth (Horie 1984). Samplings were carried out biweekly except for winter during the period from April 1994 to March 1995 at the station in the north basin (35°10'30"N, 135°56'30"E, 43m deep). In winter time (form January to March), samplings were made monthly.

At sampling, zooplankton were collected in the morning (from 10:00 to 12:00) from 0m to 42m deep at 3 m intervals with a Schindler zooplankton sampler (10L, 100μm mesh). The

samples were fixed with 5% sugar formalin (Haney and Hall 1973), and were counted under a microscope. At the same time of zooplankton sampling, lake water for measurement of chlorophyll *a* concentration were collected from 0, 5, 10, 15, 20, 30 and 40 m depth with a modified 10 L Van-Dorn water sampler. Since crustacean zooplankton in Lake Biwa ingest food particles smaller than 20μm (Okamoto 1984a and Urabe *et al.* 1996), water samples were passed through a 20-μm plankton net, and aliquots of its filtrate were filtered onto Whatman GF/F filters for determining chlorophyll *a* concentration in the < 20 μm size fraction. Chlorophyll *a* concentration were determined fluorometrically by the method of Lorenzen (1967). Vertical profile of water temperature was measured with a thermistor thermometer (Tohodentan, RB-2). A part from these sampling, *Daphnia* individuals werer collected by vertical tows from bottom to surface with a plankton-net (200 μm mesh; 30 cm diameter) These individuals were used to measure lipid index (Goulden and Hornig 1980). When more than 50 individuals of *Daphnia* could be collected for this measurement, an average of lipid index was used for the following analysis.

To analyze dynamics of the *Daphnia* population, average population density in the water column was calculated from population density at each depth by the following formula.

$$X = \frac{1}{42}\sum_{i=0}^{39}\frac{(x_i + x_{i+3}) \times 3}{2} \qquad (i = 0, 3, \cdots, 39), \tag{1}$$

where X is the average population density in the water column, and x_i is the population density at i m deep. Using this value, instantaneous change rate of population density in the water column (r, day^{-1}) between two sampling days (t_1 and t_2) was calculated according to Edomondson (1960):

$$r = \frac{\ln(X_{t_2}) - \ln(X_{t_1})}{t_2 - t_1} \tag{2}$$

where X_{t_1} and X_{t_2} are average population density in the water column at time t_1 and t_2. Instantaneous birth rate in the water column (b, day^{-1}) was calculated from instantaneous birth rate at each 3 m intervals layer . Egg development time of the layer from i to i+3 m depth (D_{i-i+3}, days) was estimated from average water temperature between i and i+3 m depth (T_{i-i+3}) by

$$\ln(D_{i-i+3}) = 3.3956 + 0.2193\ln(T_{i-i+3}) - 0.3414(\ln(T_{i-i+3}))^2$$

$$(i = 0, 3, \cdots, 39) \qquad (3)$$

(Bottrell *et al.* 1976). Instantaneous birth rate of the layer from i to i+3 m deep (b_{i-i+3}, day^{-1}) was calculated from average of egg ratio between i and i+3 m depth (E_{i-i+3}, eggs · individual^{-1}) and the estimated egg development time of the layer (D_{i-i+3}) according to the egg ratio method:

$$b_{i-i+3} = \frac{\ln(E_{i-i+3} + 1)}{D_{i-i+3}} \qquad (i = 0, 3, \cdots, 39) \qquad (4)$$

(Edmondson 1968, 1971; Paloheimo 1974). If no mortality is assumed, population density of the layer between i and i+3 m at t_2 ($x_{t_2, i-i+3}$) is estimated as

$$x_{t_2, i-i+3} = x_{t_1, i-i+3} \times \exp(b_{t_1, i-i+3} \cdot (t_2 - t_1)) \qquad (i = 0, 3, \cdots, 39), \qquad (5)$$

where $x_{t_1, i-i+3}$ and $b_{t_1, i-i+3}$ are population density and instantaneous birth rate at time t_1 estimated by formula (4). Therefore, average population density in the water column at t_2 ($\hat{X}_{t_2}$) is expected as

$$\hat{X}_{t_2} = \frac{1}{14}\sum_{i=0}^{39} x_{t_2, i-i+3} \qquad (i = 0, 3, \cdots, 39), \qquad (6)$$

Using this value, instantaneous birth rate in the water column (b, day^{-1}) between two sampling days (t_1 and t_2) was estimated as

$$b = \frac{\ln(\hat{X}_{t_2}) - \ln(X_{t_1})}{t_2 - t_1}, \quad (7)$$

where X_{t_1} is average population density in the water column at t_1 calculated by formula (1). Average death rate in the water column (d, day^{-1}) between two sampling days is estimated by:

$$d = b - r \quad (8)$$

(Edmondson 1968, 1971).

Instantaneous birth and death rates of *Daphnia* at each depth were also calculated for analyzing vertical difference in demographic parameters. Moving average of population density and egg ratio were used for the calculation to reduce influence of random error. Instantaneous birth rate at each depth is calculated from egg ratio and egg development time estimated from water temperature at each depth (Bottrell *et al.* 1976) according to egg ratio method (Edmondson 1968, 1971; Paloheimo 1974). Instantaneous growth rate at each depth was calculated from population density at each depth according to Edomondson 1960. Using calculated instantaneous birth and growth rates at each depth, instantaneous death rate at each depth was estimated (Edmondson 1968, 1971). This analysis was carried out for data series when population density was more than 0.2 individuals · L^{-1} in the average in the water column.

The immigration and emigration within water column were determined from the death rate at each depth. When the estimated death rate is less than 0, the growth rate of the population is larger than estimated the birth rate. The negative death rate is caused by immigration from the different depth or recruitment from resting eggs (DeBernardi 1974; Lampert 1978). If recruitment from resting eggs can be neglected, the negative death ratecan be used as an index of immigration from the different depth.

Influence of water temperature on vertical distribution of *Daphnia* was tested statistically. If *Daphnia* is distributed according to water temperature, *Daphnia* should be distributed at the

depths with the same water temperature between two sampling days, even though distribution depth is changed. In the present study, I also estimated *Daphnia* density in the layers which are divided vertically according to water temperature of distributed depth at 2 °C intervals. Distributions of *Daphnia* in each layer with the same water temperature were compared for successive sampling date by Kolmogorv-Smirnov Test (Sokal and Rohlf 1995).

Results

Environmental condition

During the study period, thermocline was formed around 10-15 m depth from June to September (Fig. 11). Thereafter, the thermocline gradually shifted toward the deeper layer, and then, disappeared in December. Chlorophyll *a* concentration in the < 20 μm size fraction showed three peaks during the investigation period (Fig. 11). Chlorophyll *a* increased in early July, and reached to peak with density of 5.0 $\mu g \cdot L^{-1}$ at 15 m depth in late June. The next peak was at the thermocline in August with density of 5.3 $\mu g \cdot L^{-1}$. This second chlorophyll *a* peak gradually declined toward the deeper layer, and disappeared in October. The third peak of chlorophyll *a* formed above the thermocline from September to December, and extended to deeper layer along the thermocline. Comparing the first and the second chlorophyll *a* peak, the third chlorophyll *a* peak was small, and was less than 2.5 $\mu g \cdot L^{-1}$.

Population dynamics of *Daphnia* population

Daphnia population density showed three peaks during the study period (Fig. 12). *Daphnia* population density increased from mid June but decreased immediately after the

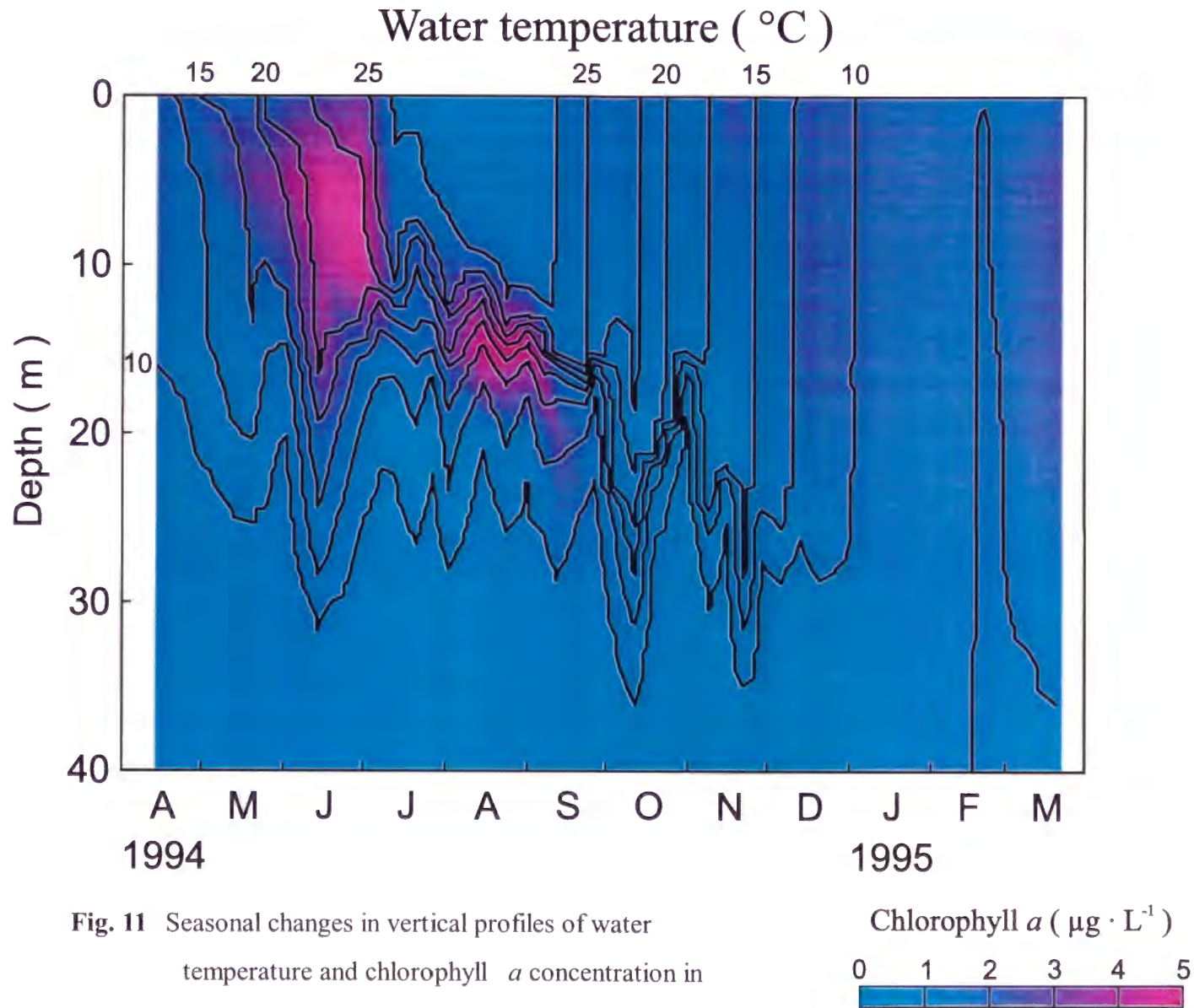

Fig. 11 Seasonal changes in vertical profiles of water temperature and chlorophyll *a* concentration in the < 20 µm size fraction.

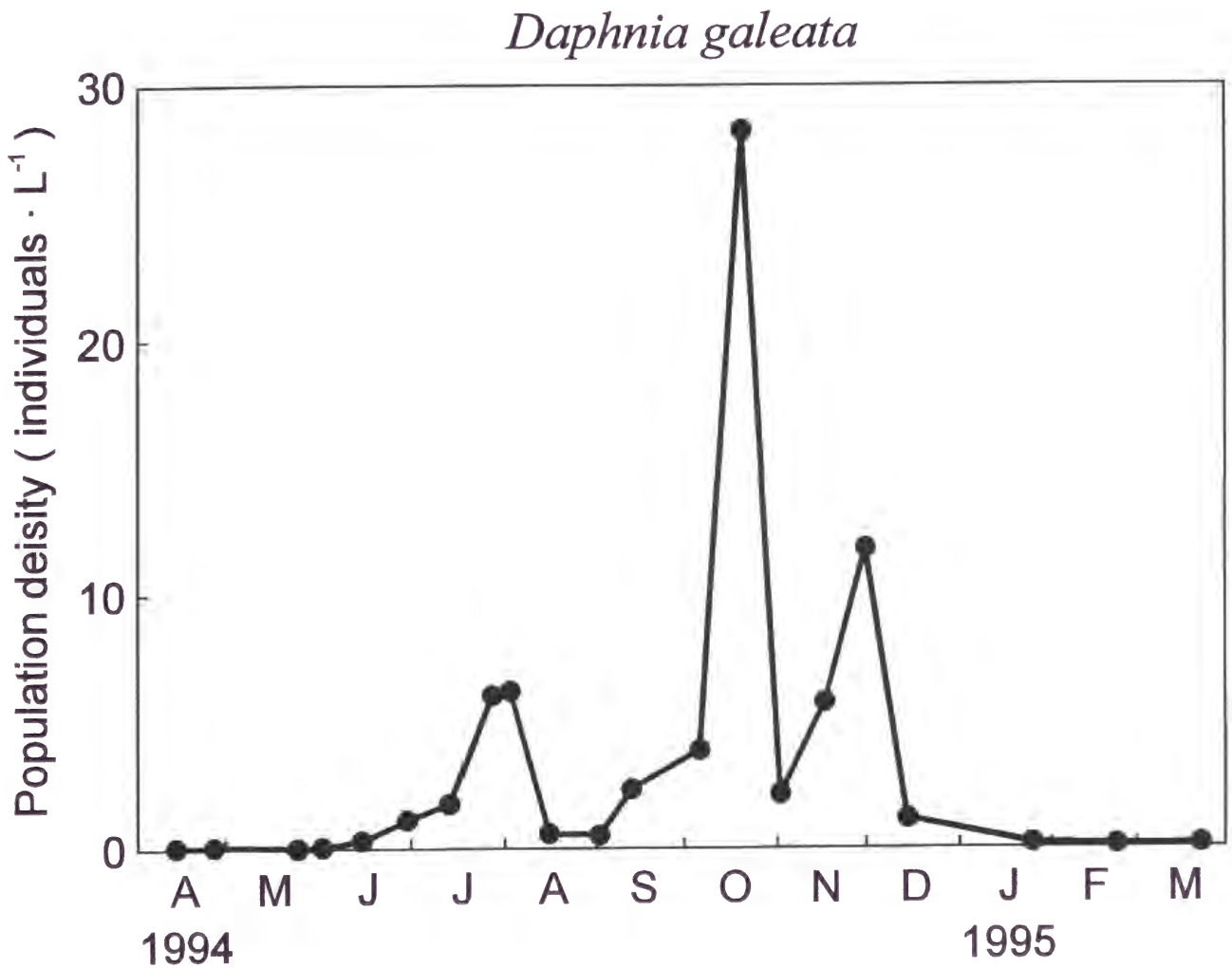

Fig. 12 Seasonal changes in population density of *D. galeata* within water column.

population density reached to a peak with density of 6.2 individuals · L^{-1} in early August. In mid September, *Daphnia* population density increased and formed second peak with 28.2 individuals · L^{-1} in late October. Thereafter, the population density once decreased. However, it recovered again in early November, and reached to 11.8 individuals · L^{-1} in early December. The *Daphnia* population was low in winter (form December to March).

Seasonal changes in average instantaneous birth and death rates with through water column are shown in figure 3. The birth rate increased rapidly from early June, and reached to the maximum, being 0.22. After rapid decrease, the birth rate increased again from early July and reached to 0.09 in late August. After September, the birth rate decreased gradually, and was less than 0.01 in December. Similar seasonal trends were found in lipid index (Fig. 14). Indeed, birth rate of *Daphnia* population significantly correlated with the lipid index (Kendall's rank correlation, $p < 0.01$). However, the birth rate did not correlate the average water temperature which was weighted according to density of *Daphnia* at each depth (Kendall's rank correlation, $p > 0.05$).

Average of instantaneous death rate of *Daphnia* population through water column showed three peaks during the study period (Fig. 13). The death rate was higher than 0.10 in mid June, and late July, and decreased from August to September. In November and December, the death rate was also high but less than 0.10. The high death rate in June was apparently not due to food limitation, because lipid index showed high values. However, second (July) and third (November) peaks in death rate were appeared when lipid index was low.

Seasonal change in vertical distribution

Corresponding to the seasonal changes in population density (Fig. 12), *Daphnia* formed several assemblages with distinct vertical distribution (Fig. 15). From June to August, *Daphnia*

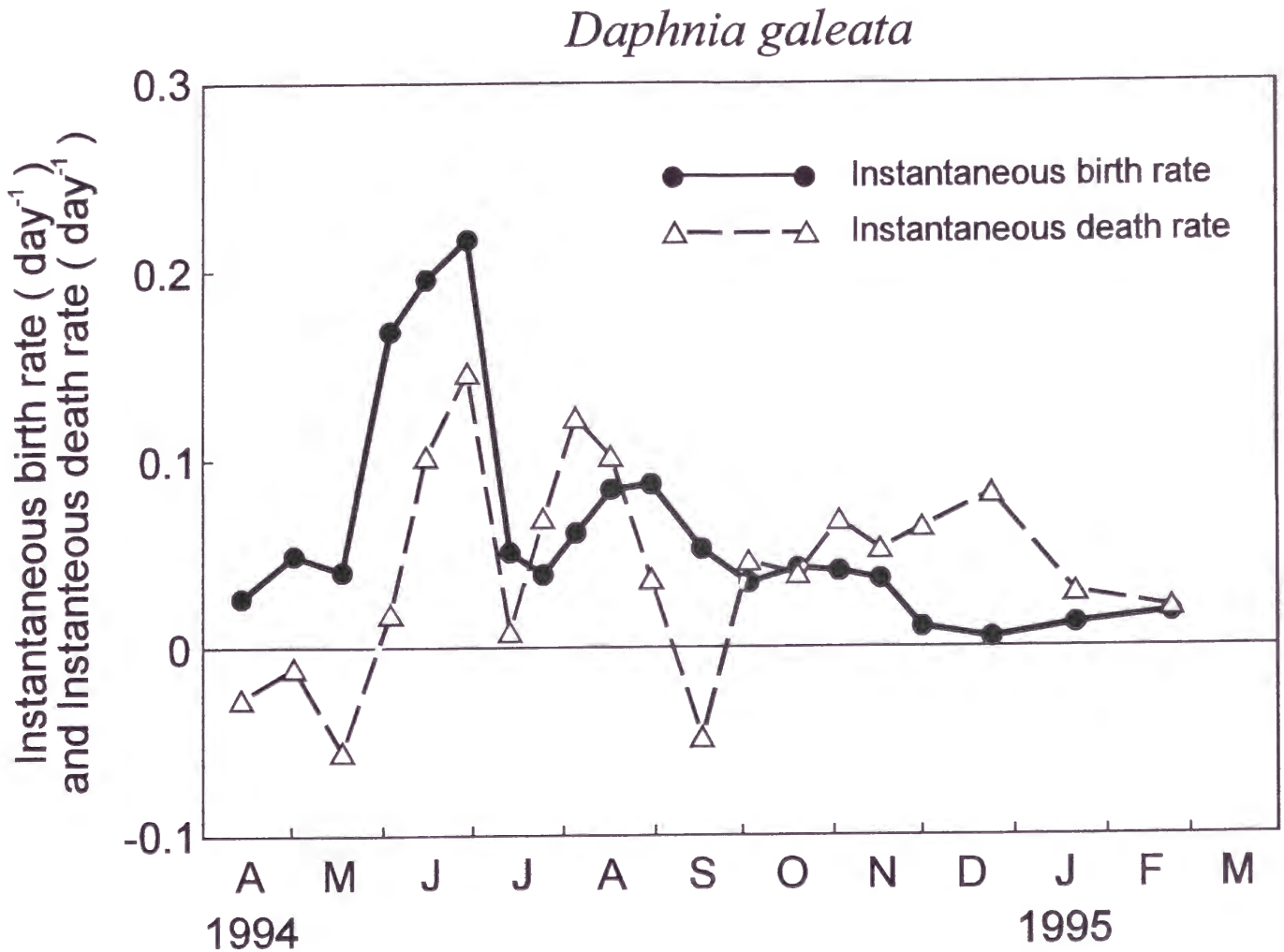

Fig. 13 Seasonal changes in instantaneous birth and death rate of *D. galeata* population. The rates are average over water column (see text).

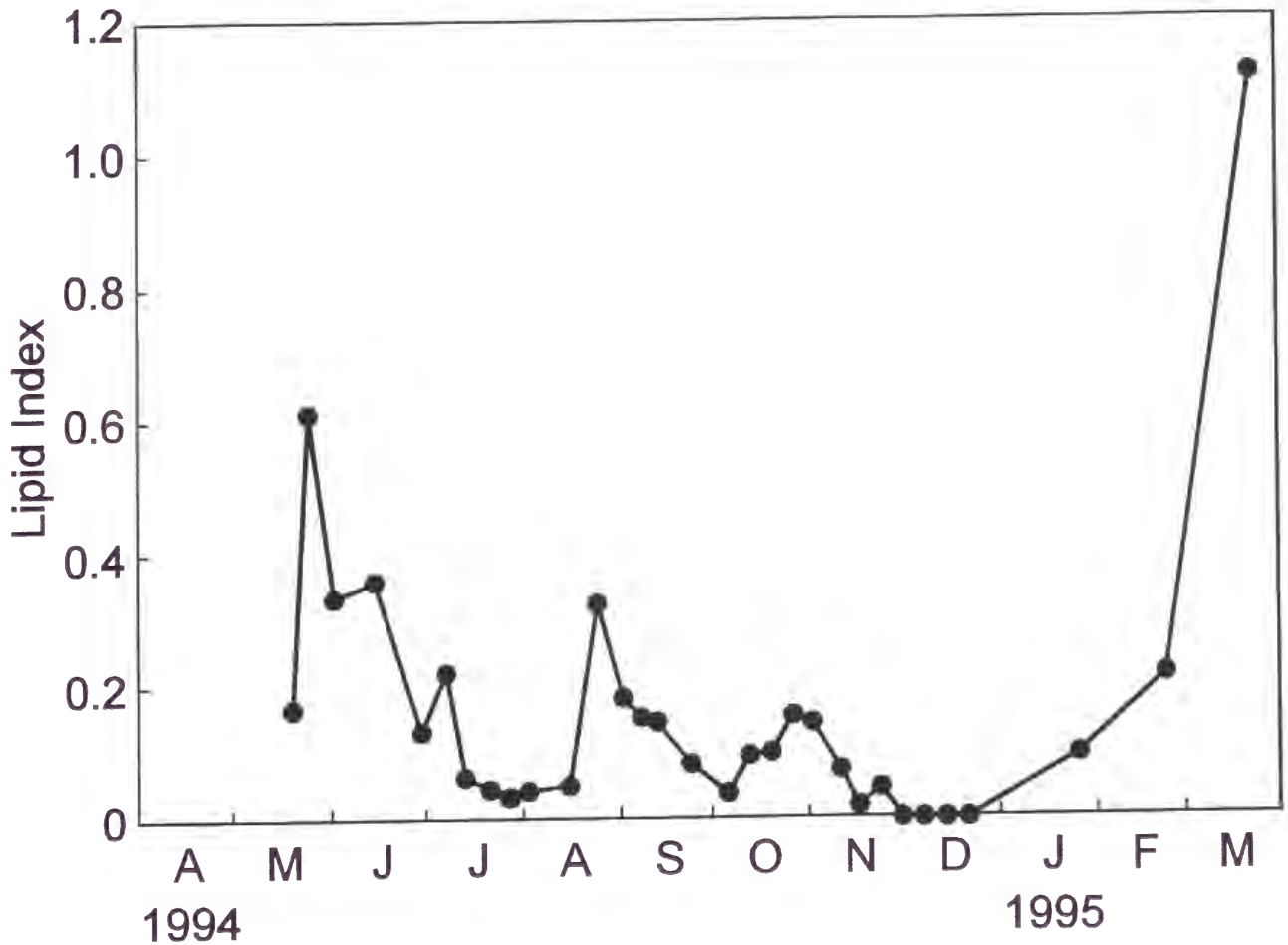

Fig. 14 Seasonal changes in mean lipid index of *D. galeata* population.

assemblage was found in layer from 10 m to 20 m depth (Fig. 12). The maximum density of this assemblage was 30.6 individuals · L^{-1} at 15 m deep in late July. At this time, other assemblages of *Daphnia* were found at 25 m deep and at near the surface in early August. In late August, *Daphnia* seemed to assemble around 20 m depth. This assemblage moved toward the deeper layer during September, and then disappeared in late September. Corresponding to the increase in population density in October (Fig. 12), *Daphnia* formed two different assemblages above and below 15 m depth. The maximum densities were 176.0 individuals · L^{-1} in the upper assemblage and 24.4 individuals · L^{-1} in the lower assemblages. In November, another assemblage was appeared at 10 m depth, moved toward deeper depth in late November and disappeared in late December .

Water temperature did not correlate with seasonal change in vertical distribution of *Daphnia* after late June when the thermocline was developed (Kolmpogorv-Smirnov test, $p < 0.05$). However, vertical changes in *Daphnia* density correlated with chlorophyll *a* concentration in summer (Table 6).

Causes for seasonal changes in vertical distribution

Instantaneous birth and death rates at each depth were estimated during the period from mid June to December when average population density of *Daphnia* through water column was more than 0.2 individuals · L^{-1} (Fig. 16). The birth rate was high at the depth from 10 m to 25 m in June, and correlated with individual density at each depth in next sampling date (Table 7). Similarly, the death rate was also high at depths where *Daphnia* was abundant. Thus, relatively high density of *Daphnia* around 15 m was caused by high birth rate. In mid July, the death rate was smaller than 0 at depth from 10 m to 30 m. This negative value means that *Daphnia* immigrated to these depths during the period from mid to late July. In the same way,

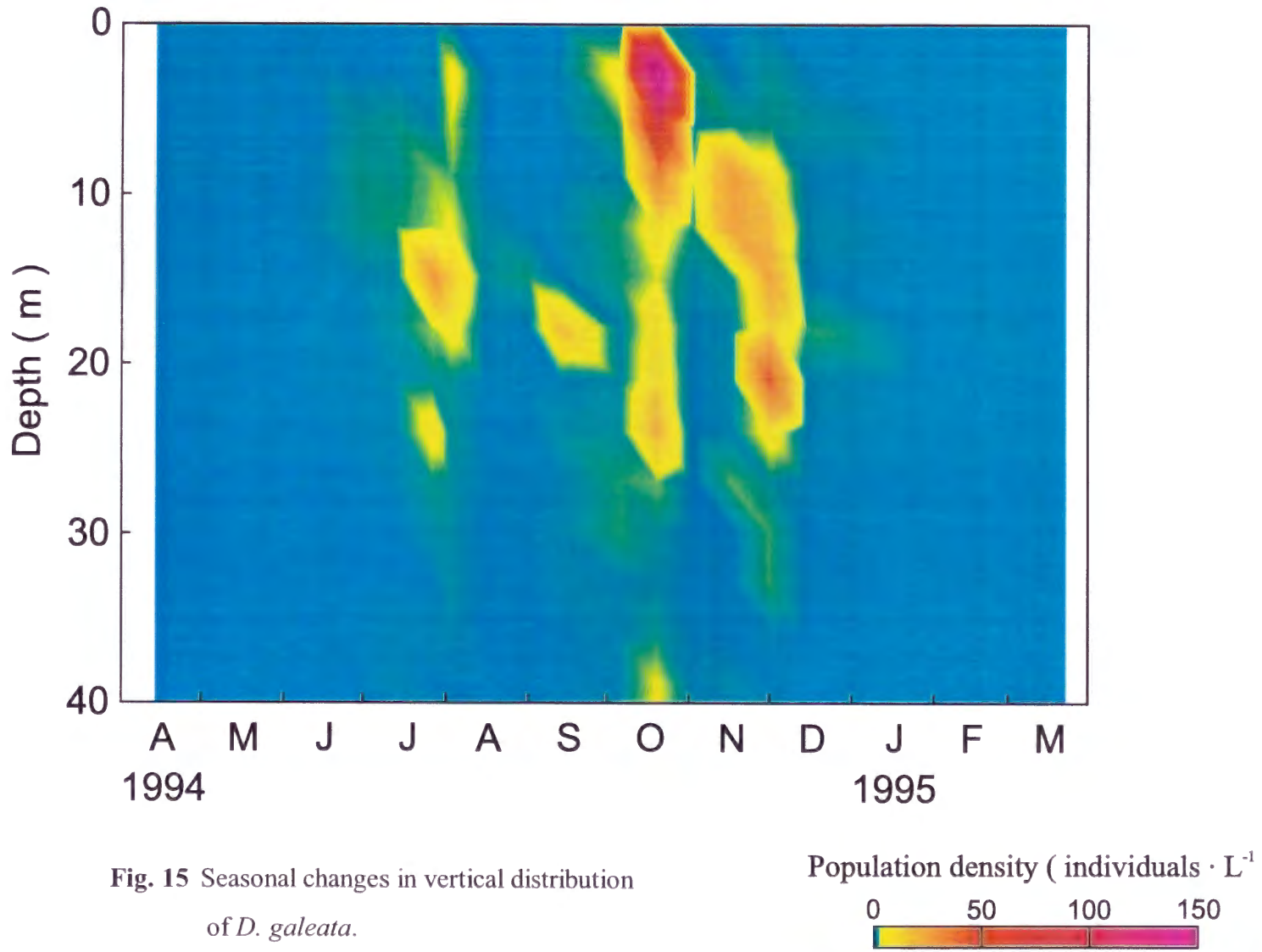

Fig. 15 Seasonal changes in vertical distribution of *D. galeata*.

Table 6

Correlation coefficient between daphnia density and Chlorophyll a concentration in < 20 μm size fraction at different depths.

Date	Jun 15	Jun 30	Jul 14	Jul 28	Aug 3	Aug 16	Sep 2	Sep 13	Oct 6	Oct 20	Nov 2	Nov 17	Dec 1	Dec 15
r	0.488	0.787*	0.776*	0.699	0.892*	0.911**	0.968**	0.826*	0.265	0.402	0.616	0.212	0.429	0.403

the negative death rate at depths from 3 m and 10 m deep in late July means that *Daphnia* immigrated to these depths during the period from late July to early August. During the period from mid July to early August, most *Daphnia* individual moved from 10 m to 15 m, but some individuals moved to near the surface. These results indicate that changes in vertical distribution were due to immigration of *Daphnia*.

In early August, the birth rate was high around 15 m, and correlated with individual density in next sampling date (Table 7). However, the death rate had small vertical change, and was not smaller than 0 in August. This result indicates that high density of *Daphnia* individuals around 15 m in mid August was caused by vertical changes birth rate, but was not related with vertical changes in death rate and immigration. In the term from early to mid September, most *Daphnia* individuals moved to about 20m when the negative death rate was found around 20 m. This result indicates that vertical distribution of *Daphnia* was changed by immigration to 20 m.

In early October, the birth rate was not high through water column, and did not correlate with *Daphnia* density on next sampling date (Table 7). However, the negative death rate was found above 15 m deep in late September and above 30m deep in early October. These results indicate that the high density of *Daphnia* individuals above and below 15 m in early October was not caused by vertical difference in the birth rate, but was caused by vertical migration.

During the term from late October to early November, the birth rate was high above 20 m, and correlated with vertical distribution of *Daphnia* on next sampling date (Table 7). Nevertheless, the death rate was not smaller than 0 in whole of water column in late October. These results indicate that high density of *Daphnia* individuals around 10 m in November was caused by vertical difference in birth rate, but was not caused by vertical difference in the death rate and immigration. However, in mid November, the negative death rate was found from 20

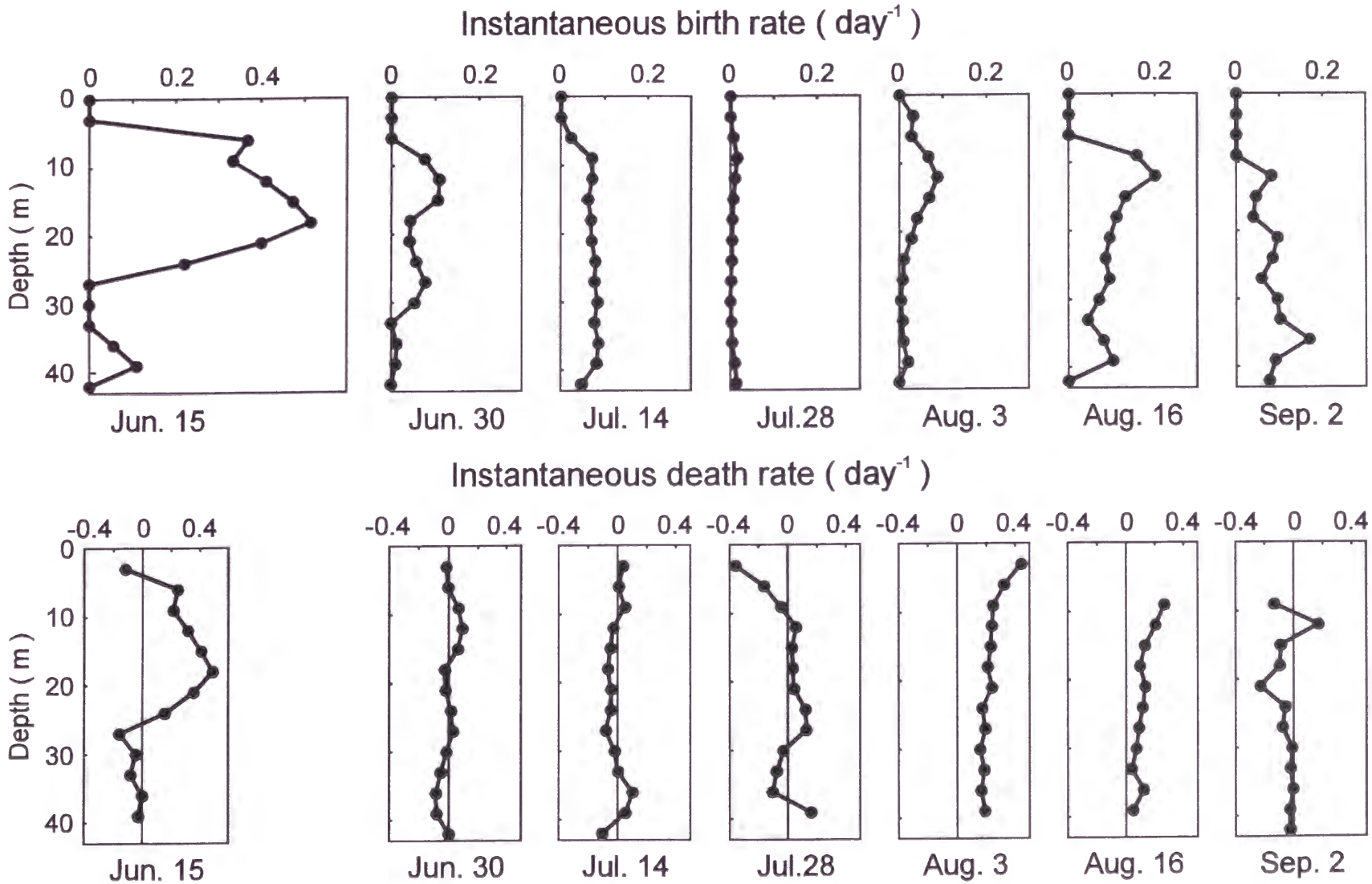

Fig. 16 Vertical changes in instantaneous birth and death rate of *D. galeata*.

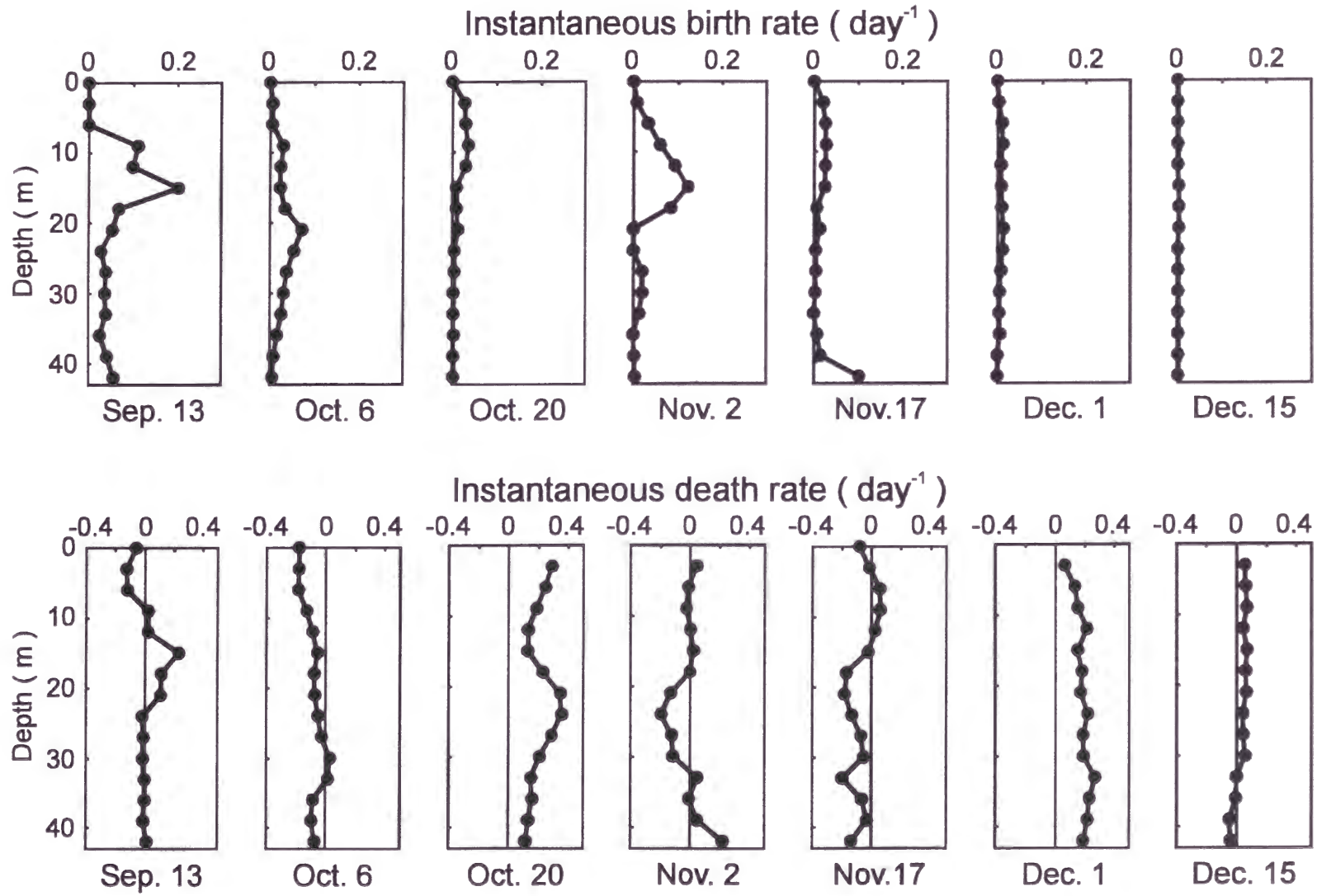

Fig. 16 Continued.

Table 7

Correlation coefficient between instantaneous birth rate at each depth and individual density at next sampling date.

Date	Jun 15	Jun 30	Jul 14	Jul 28	Aug 3	Aug 16	Sep 2	Sep 13	Oct 6	Oct 20	Nov 2	Nov 17	Dec 1	Dec 15
τ	0.495*	0.419*	0.086	0.105	0.429*	0.238	0.219	0.000	-0.010	0.457*	0.410*	0.114	0.495*	0.086

m to 30 m. This result indicates that extending of *Daphnia* toward to deeper layer in late November was caused by immigration to the deeper layer.

Discussion

In the present study, cause of seasonal changes in vertical distribution was vertical difference in birth rate and vertical migration. Assuming that *Daphnia* emigrated always from depth with the maximum population density of that, seasonal changes in vertical distribution of *Daphnia* can be illustrated in Fig. 17. The figure shows that vertical difference in birth rate and vertical migration alternately influence on vertical distribution of *Daphnia*.

Although migrations of *Daphnia* between depths were estimated based on immigration, individuals may immigrate from not only different depth but also by horizontally different site. However, most of the average death rate of *Daphnia* through water column was more than 0 during the term from June to December (Fig. 13). This result implies that population dynamics of *Daphnia* can be explained without immigration from horizontally different site. Present analysis also assumed that recruitment originated from resting eggs was negligible. Since no *Daphnia* with resting eggs was found during the study period, this assumption seems to be appropriate.

Zooplankton can vertically migrate either by swimming or by water movement. If migration of *Daphnia* is due to water movement, changes in vertical distribution of *Daphnia* should correlate with vertical profile of water temperature, because water movement relates closely with vertical profile of water temperature (Wetzel 1983; Goldman and Horne 1983). However, change in vertical distribution of *Daphnia* was not explained by vertical profile of water temperature during the period from June to December. Therefore, this implies that seasonal vertical migrations found in the present study were due to swimming of *Daphnia*

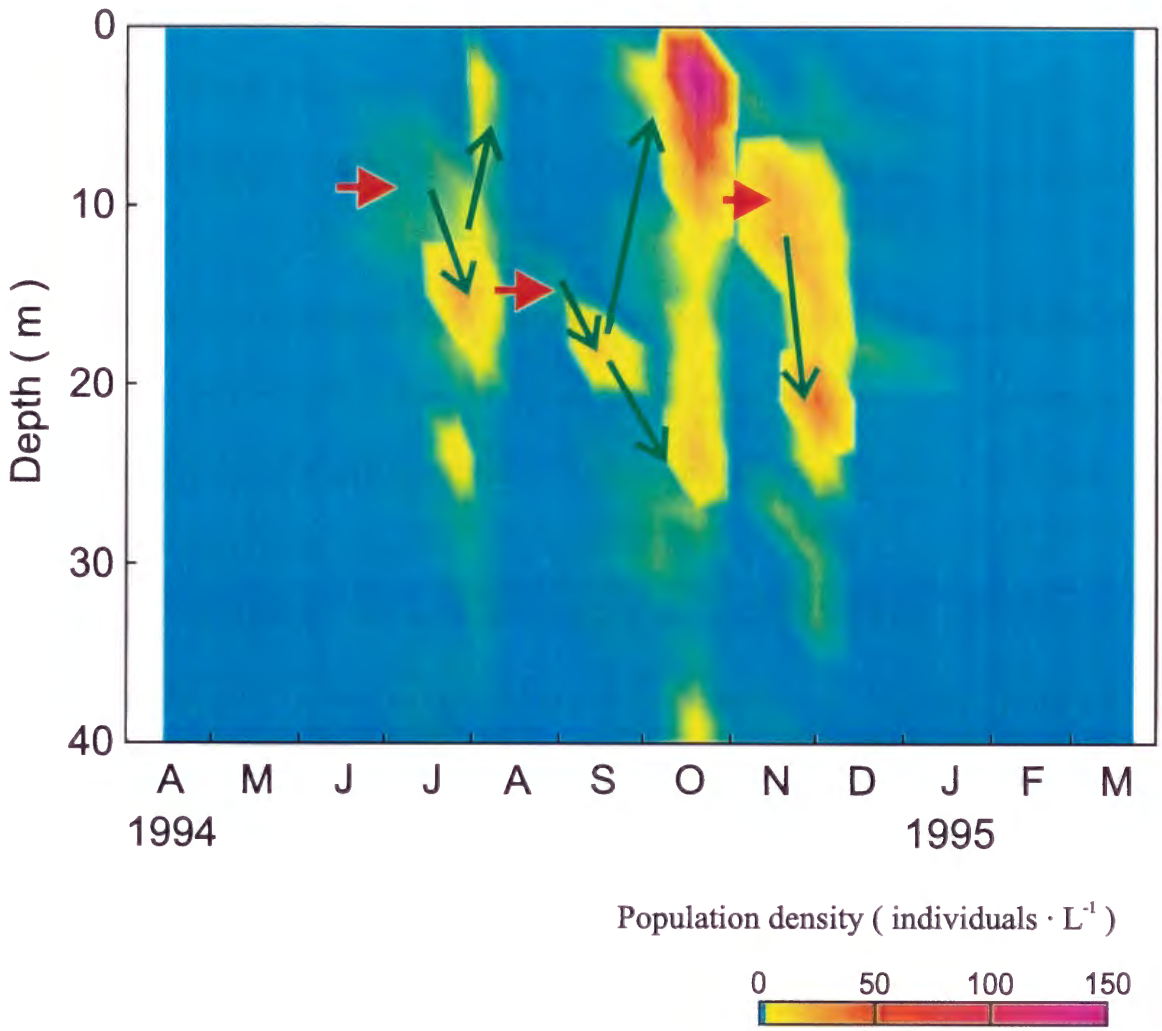

Fig. 17 Schematic diagram showing changes in vertical distribution of *D. galeata* in Lake Biwa. Red arrows, multiplication; Green arrows, migration. Vertical distribution was changed by vertical difference in birth rate when the red arrows were shown, and was changed by migration when the green arrows were shown.

themselves.

Several factors can be considered to induce seasonal vertical migration of *Daphnia*. Many researchers examined influence of harmful UV light on vertical distribution of zooplankton (Bollons and Frost 1990; Williamson *et al.* 1994; Hessen 1994). Unfortunately, measurements on UV light was not made out in the present study. Nevertheless, although harmful UV light influence on zooplankton distributed shallower than at most 20 cm deep in natural lake (Zagarese *et al.* 1994), *Daphnia* did not distribute such a shallow layer in Lake Biwa. Thus, effect of harmful UV light was not a factor to the vertical migration of *Daphnia*.

As diel vertical migration is caused by avoidance from predators (Zaret and Suffen 1976), the seasonal vertical migration of *Daphnia* may be also caused by the avoidance from predators. Although several species of fish in Lake Biwa prey on zooplankton (Nagoshi 1982), most of the species are not abundant except for sweetfish (*Plecoglossus altivelis altivelis*) (Nakanishi and Sekino 1996). Sweetfishes ingest zooplankton at the surface layer in pelagic area from late spring to summer (Azuma 1964). Therefore, predation by the sweetfishes may have influenced on population dynamics of *Daphnia* in the summer, when the death rate was high (Fig. 14) even though lipid index was high during the term from June to July (Fig. 15). However, *Daphnia* distributed in the surface layer above 10 m in June, and migrated vertically to the surface layer in August (Fig. 17). Thus, it is difficult that avoidance from predators was a factor of the seasonal vertical migration of *Daphnia*. In addition, it is known that most of crustacean zooplankton in Lake Biwa did not show diel vertical migration (Nagoshi 1982; Okamoto 1984b and Kawabata 1987). This tendency implies that influence of predation pressure on zooplankton is little in Lake Biwa.

Vertical changes in individual density of *Daphnia* correlated significantly with chlorophyll *a* concentration in most date during the period from June to September (Table 6). In addition,

distribution of *Daphnia* extended toward the deeper layer when chlorophyll *a* was high even in the deeper layer in December. Such relationships between of *Daphnia* and chlorophyll *a* were found when vertical migrations were occurred (Fig. 17). Furthermore, lipid index of *Daphnia* was low when vertical distribution of *Daphnia* was changed by vertical migration (Fig. 13 and 17). Thus, poor food condition for *Daphnia* may stimulate changes in vertical migration. Swimming or migrating behavior of zooplankton are known to be changed according to food condition (Cuddington and McCauley 1994; VanDuren, Videler 1995; Johnsen and Jacobsen 1987). It is supposed that when food condition deteriorated, *Daphnia* migrate vertically and dispersed in the water column to search food.

It is known that abundance of zooplankton influence on their own food condition, and often cause food limitation (Tessier *et al.* 1992; Mitchell and Wiliams 1982). The maximum population density was found when the lipid index was low (Fig. 12 and 14). Thus, it is likely that increase in *Daphnia* caused food limitation for themselves.

In summary process of seasonal changes in vertical distribution of the *Daphnia* population is considered as follows. Birth rate of *Daphnia* increased at the depth where food patch was formed, so that *Daphnia* was assembled at that depth. However, as population density increase, food is depleted. Under such situation, *Daphnia* migrate vertically and dispersed in the water column to search for food. Among the dispersed *Daphnia*, a part of the individuals distributed at the depth where next food patch was formed could obtain enough food to colonize there. Birth rate of *Daphnia* increased again, so that a new assemblage of *Daphnia* was formed at that depth.

Increase in population density after dispersion implied that a part of population is selected. Thus, this selection may induce changes in not only vertical distribution but also genetic structure of *Daphnia* population. However, such a process can not be noted at all by the

previous studies which examine correlation between environmental factors and vertical distribution of zooplankton. To understand more deeply the cause and effect of genetic and population structure of zooplankton, it should be need to examine seasonal changes in vertical distribution.

Chapter 6. Conclusion

A large number of studies have addressed short term (diurnal time scale) and long term (seasonal time scale) changes in vertical distribution of zooplankton species. Although several studies pointed out importance of food condition to vertical distribution of zooplankton, no studies examined relationship between the vertical distribution and internal nutritional status which reflects integrated food condition form the near past to present. Results in the previous chapters showed that *Daphnia galeata* individuals alter their diel migration behavior according to changes in their internal nutritional status along reproduction cycles. In addition, when the nutritional status is decreased due to food depletion, they vertically migrate to search for new depth with abundant food. These results indicate that nutritional status is a key factor determining vertical distribution of *D. galeata* both in diurnal and seasonal time scales.

In Lake Kizaki, young individuals of *D. galeata* stayed at upper layer throughout a day. However, adults of *D. galeata* with high nutritional status performed diel vertical migration, although those with low nutritional status tended to stay at upper layer throughout a day (chapter 1). An analysis of egg age distribution indicated that migrating individuals have eggs with late development stage, while non-migrating individuals have eggs with early development stage. Generally, nutritional status of adult with late stage eggs is high because they already accumulated much energy and materials for next egg production, while that of adults with early stage eggs is low because they just consumed energy and materials accumulated during previous reproductive cycle for present egg production. These facts imply that adults of *D. galeata* can change their migration behavior according to nutritional status determined by position of reproduction cycle.

Within a habitat, nutritional status of *D. galeata* was, in deed, largely different among

individuals (chapter 3). Since *Daphnia* store energy as fatty acids (triglyceride), fatty acids composition was examined to quantify nutritional status. To do, a pyrolysis gas chromatography method for analyzing fatty acids was developed, which enable to quantify fatty acids composition at the individual level. The analysis revealed that the fatty acids composition differed largely among individuals. In addition, difference in the fatty acids composition among individuals did not necessarily relate with food condition when *D. galeata* was collected. These results suggest that their nutritional status is affected by not only the present food condition but also internal condition such as reproduction cycles.

Why do *D. galeata* individuals change migrating behavior according to their internal nutritional status? This question was theoretically examined using an optimization model (chapter 4). The model indicated that it is advantageous for *Daphnia* to change their behavior from non-migrating to migratimg at a position of the reproduction cycle under certain environmental condition. The results imply that migration behavior of *Daphnia* individuals is not necessarily genetically fixed but flexible (phenotypic plasticity), and is affected by amount of energy and nutrients accumulated for next reproduction relative to mortality loss in the upper layer.

In Lake Biwa, *D. galeata* changed seasonally their vertical distribution, although they did not show marked diel vertical migration due to poor food environment in average. Demographic analysis indicated that these seasonal changes were caused by migration and vertical difference in reproduction rate. *Daphnia* tended to colonize at a certain layer where food was abundant. As long as their nutritional status was high, *D. galeata* stayed at that layer. However when their nutritional status was decreased due to food depletion, *D. galeata* dispersed through water column. There was a trend that phytoplankton increased in a layer where *D. galeata* density was low. Therefore, by dispersion, *D. galeata* seemed to search the

layer where phytoplankton was increasing. This evidences implies that *D. galeata* changes their vertical distribution according to their internal nutritional status.

Population dynamics of *Daphnia* species has been well analyzed in many lakes (Hall 1964; George and Edwards 1974; Edmondson 1982; Tessier *et al.* 1992). However, no study investigated how vertical migration and distribution contribute to population dynamics or persistence of the species in a given lake. The present evidence indicates that aquatic habitats are not homogeneous environments for zooplankton. Food conditions as well as physical and chemical conditions vary spatially and temporally. *Daphnia* species seems to persist such a dynamic environment by using heterogeneous space efficiently.

In the present studies, I focused internal nutritional status as a factor affecting vertical migration and distribution of *D. galeata*. As indicated by the present studies, internal nutritional status of zooplankton reflected not only the present but also the near past food condition. Therefore, the nutritional status can be regarded as integrated information on their food condition. It serves to be a useful parameter to examine the various aspects on ecological processes mediated by zooplankton from species interactions to material cycling in aquatic ecosystems.

Acknowledgments

I wish to thank Dr. Takahito Yoshioka for his helpful advice, invaluable discussion and support on earlier period of the study. I am also grateful to Dr. Hidetake Hayashi and Mr. Masaki Funakoshi for their encouragement and helpful coments on earlier period of the study.

I am grateful to my advisers, Dr. Masami Nakanishi, Dr. Jotaro Urabe and Dr. Norio Yamamura for their for helpful advice, invaluable discussion and reviewing the manuscript. I am also grateful to my advisers, Eitaro Wada and Tetsuya Narita for their criticisms and encouragement.

I also thank Mr. Tadatoshi Koitabashi, Mr. Takaaki Ueda, Mr. Akio Kawabata and students of Faculty of Science, Shinshu University for kind assistance with sampling.

Study in chapter 3 was carried out in collaboration with Yasuyuki Ishida, Shin-ichi. Isomura, Shin Tsuge (School of Engineering, Nagoya University), Hajime Ohtani (Center for Integrated Research in Science and Engineering, Nagoya University) and Takashi Kimoto (Research Institute of Oceano-Chemistry). I am grateful to them for their technical supports and helpful comments.

Finally, I wish to acknowledge my parents, Morio Sekino and Sachiko Sekino, for their encouragement and financial support during the study.

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添付論文1

Note

The Relationship between Nutritional Condition and Diel Vertical Migration of *Daphnia galeata*

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陸水学雑誌
56巻2号，1995，p. 145-150別刷
Reprinted from
THE JAPANESE JOURNAL OF LIMNOLOGY
Vol. 56, No. 2 p. 145-150
April 1995

Jpn. J. Limnol., *56*, 2, 145–150, 1995

Note

The Relationship between Nutritional Condition and Diel Vertical Migration of *Daphnia galeata*

Tatsuki SEKINO and Takahito YOSHIOKA

Abstract

The relationship between nutritional condition, indicated by storage lipid content, and diel vertical migration of *Daphnia galeata* in Lake Kizaki was investigated in July 1991. The *D. galeata* population was divided into two groups, *i. e.*, upper and deep layer groups during daytime. The upper layer group consisted of juveniles and adults of poor nutritional condition, while the deep layer group consisted only of adults of better nutritional condition. Diel changes in the vertical distribution of *D. galeata* revealed that adults in the deep layer performed diel vertical migration, whereas juveniles and adults in the upper layer did not migrate vertically. These result implied that adult *D. galeata* with better nutritional condition performed vertical migration in the context of their life cycle.

Key words: vertical migration, *Daphnia galeata*, lipid, energy reserve

The predation avoidance hypothesis (ZARET and SUFFERN, 1976) as an explanation for the significance of diel vertical migration of zooplankton has been widely supported. However, HUNTLEY and BROOKS (1982) found that *Calanus pacificus* did not migrate vertically when food was limited regardless of the presence of predators. Therefore, it has been speculated that there is a trade-off between food abundance and predation avoidance (KERFOOT, 1985; LAMPERT, 1989). The trade-off, however, can not sufficiently explain the mechanism of diel vertical migration because the nutritional condition of zooplankters seems to be a more important factor than food abundance in its effect on their migrating behavior.

In mesotrophic Lake Kizaki in central Japan, the *Daphnia galeata* population distributes only in the epilimnion during daytime from May to June, and in both the epilimnion and hypolimnion from late June to August every year (SEKINO, unpublished). The seasonal change in vertical distribution of *D. galeata* during daytime is due to the change in migrating behavior, which may relate not only to changes in food abundance and predation pressure, but also to changes in the nutritional condition of *D. galeata*. In the present study, we investigated the relationship between nutritional condition and diel vertical migration of the *D. galeata* population in Lake Kizaki in July 1991, when the population was vertically divided into two groups.

Diel vertical migration of *D. galeata* was investigated from 19 July to 20 July 1991 at the center of Lake Kizaki (36°33′N, 137°50′E, 29m deep). The sampling was conducted at four hour intervals from 10:00 on 19 July to 10:00 on 20 July. For quantitative zooplankton analyses, twenty liters of lake water were collected with a 6.7-liter Van Dorn water sampler at 2m-intervals from 0 m to 28m deep. *Daphnia* at each depth were collected by filtering the sampled water through a 40-μm mesh net, and then fixed with 5% sugar formalin (HANEY and HALL, 1973). The clutch sizes, developmental stages of eggs in the brood pouches and carapace lengths of *D. galeata* individuals collected at 10:00 on 19

July and 02:00 on 20 July were measured. Animals larger or smaller than the minimum carapace length of animals with eggs (500μm) were counted as adults or juveniles. The egg developmental stage was determined according to THRELKELD (1979).

The storage lipid content in *Daphnia* was evaluated using a lipid index (LI) based on the number of oil droplets in the animals (GOULDEN and HORNIG, 1980). The animals for measurement of LI were collected by towing a closing plankton net (23.5cm diameter, 100-μm mesh) from the surface down to 27m at 4 or 3m intervals. The animals were carried back to a cabin at the lake shore. For all or a part of the animals in the samples, the measurement of LI was immediately performed after collection in order to minimize fading of the oil droplets.

During the daytime (10:00-18:00 on 19 July), the population density of *D. galeata* had two peaks in the upper layer (above 16m) and the deep layer (below 24m). And the average population densities in those layers during daytime were 10.8 and 11.8 individuals • l^{-1}, respectively (Fig. 1). At 10:00 on 19 July, 59% of individuals distributed in the upper layer were adults, whereas adults comprised 98% of individuals distributed in the deep layer. At night (22:00 on 19 July ; 02:00 on 20 July), most *D. galeata* individuals were distributed in the upper layer. The average population density in that layer at night was 11.4 individuals • l^{-1}. On the other hand, the average population density in the deep layer was 3.6 individuals • l^{-1}. At 02:00 on 20 July, adults comprised occupied 66% of individuals distributed in the upper layer, and 99% distributed in the deep layer. On the next morning (10:00 on 20 July), two vertical density peaks of *D. galeata* appeared again. And the average population densities in the upper and deep layers were 9.1 and 6.8 individuals • l^{-1}, respectively. These observations indicated that the *D. galeata* population in the deep layer consisted mainly of adults both day and night, while juveniles were hardly distributed in the deep layer at any time. Thus, diel changes in vertical distribution of *D. galeata* suggest that only adults distributed in the deep layer in daytime may perform diel vertical migration, which is true of neither juveniles nor adults distributed in the upper layer in daytime.

High predation pressure by fish and the pres-

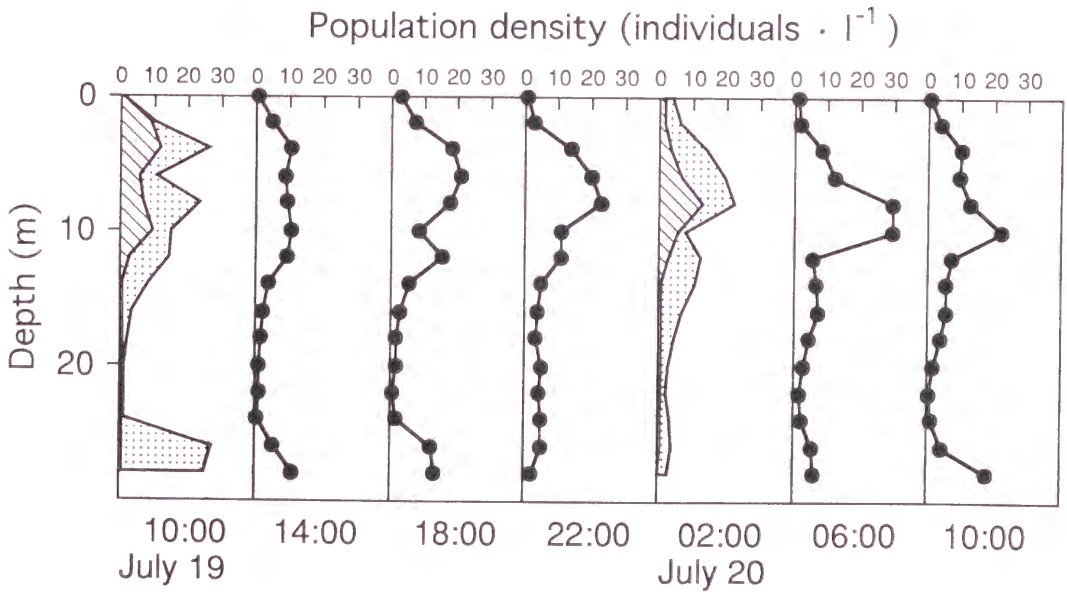

Fig. 1. Vertical distribution and age structure (10:00 on 19 July and 02:00 on 20 July) of *Daphnia galeata* in Lake Kizaki on 19 20 July in 1991. Hatched area : juveniles ; dotted area : adults.

ence of sufficient food in the upper layer are the factors inducing diel vertical migration of zooplankton as a predator avoidance mechanism (KREMER and KREMER, 1988). A cyprinid planktivorous fish (*Zacco platypus*) and a pond smelt (*Hypomesus transpacificus*), which prefer feeding on *Daphnia* (SHIRAISHI, 1960; URABE and MARUYAMA, 1986), are abundant in Lake Kizaki. The mean chlorophyll *a* concentration two days before the investigation (17 July) was 12.4μg・l^{-1} in the upper layer and 4.1μg・l^{-1} in the deep layer (SEKINO, unpublished), suggesting that food available to *Daphnia* was relatively abundant in the upper layer. Thus, the diel vertical migration of the adult *D. galeata* distributed in the deep layer was probably a predation avoidance mechanism. However, food in the upper layer may not be sufficient for all *D. galeata* to make the migration.

The migrating behavior of the *D. galeata* population of Lake Kizaki, observed here, was similar to that of the *Daphnia* population in Lake Constance which consisted of migrating *D. hyalina* and non-migrating *D. galeata* (STICH and LAMPERT, 1981). However, the *Daphnia* population of Lake Kizaki consisted of only one species, within which there were differences in migrating behavior. Therefore, the *D. galeata* population in Lake Kizaki differed in behavior from either population of the two *Daphnia* species in Lake Constance. On the other hand, in some lakes, the zooplankton populations consisted of one species which changed their migrating behavior seasonally (FORSYTH *et al*., 1990; RINGELBERG *et al*., 1991). However, the *D. galeata* population in Lake Kizaki exhibits two different migrating behaviors concurrently, and thus the behavior is unique. The difference in behavior of the adult *D. galeata* may reflect the difference in the physiological condition of each individual.

Vertical distribution of the lipid index (LI) of *D. galeata* showed diel changes (Table1). In the daytime, the percentage of individuals with high LI (LI 2 and 3) was significantly higher in the deep layer than in the upper layer. The percentage of individuals with LI 0 was significantly lower in the deep layer than in the upper layer. From daytime to night time in the deep layer, individuals with LI 2 and 3 decreased from 11% to 2%, and individuals with LI 1 decreased from 48% to 26%. By contrast, individuals in the upper layer with LI 2 and 3 increased slightly from 1% to 3%. The percentage of individuals with LI 0 distributed in the deep layer increased from 42% to 72% from daytime to night time, while that in the upper layer did not change. Thus, the percentage of individuals with lipid indices 1,2 and 3 distributed in the deep layer was lower than in the upper layer at night, while that with LI 0 was higher in the deep layer than in the upper layer.

Table 1. Spatial and temporal changes in population density (individuals・l^{-1}) and percentage (in parenthesis) of *Daphnia galeata* individuals with different lipid indices in Lake Kizaki during 19–20 July, 1991. Each percentage is a mean for each layer at a given time. Significantly different values from those in deep layer (24–28m) are marked with asterisks (χ^2-test: *=$P \leq 0.05$, **=$P \leq 0.001$).

Time	Layer(m)	Lipid indices LI 0	LI 1	LI 2, 3
Daytime	0–16	6.3 (58)**	4.4 (41)	0.2 (1)**
(10:00–18:00 on 19 July)	16–24	0.8 (62)	0.5 (38)	0.0 (1)
	24–28	4.9 (42)	5.6 (48)	1.3 (11)
Night time	0–16	6.4 (56)*	4.6 (40)*	0.4 (3)
(22:00 on 19 July–02:00 on 20 July)	16–24	2.4 (65)	1.3 (35)	0.0 (0)
	24–28	2.6 (72)	0.9 (26)	0.1 (2)
Next morning	0–16	5.9 (65)**	3.0 (33)	0.2 (2)**
(10:00 on 20 July)	16–24	1.0 (49)	1.1 (51)	0.0 (0)
	24–28	2.2 (32)	3.8 (56)	0.8 (12)

However, this does not inevitably indicate a downward migration of individuals with low LI from the upper layer to the deep layer, because the population density in the deep layer considerably decreased from day to night (Fig. 1). On the next morning, the percentage of individuals with LI 1, 2 and 3 was again higher in the deep layer than in the upper layer, and that with LI0 was again low in the deep layer.

The LI data presented in Table 1 included the values for both adults and juveniles. Since almost all individuals distributed in the deep layer (about 98% of the deep population) were adults, the LI data in the deep layer would represent those for adults in the layer. The LI value for adults in the upper layer in the daytime was estimated from LI (Table 1), population density (Fig. 1) and the ratio of adults and juveniles at 10:00 on 19 July. Assuming all juveniles in the layer preferentially occupy the high LI classes, the LI for most adults would be zero, since the population density with LI of 1, 2 and 3 (4.6 individuals $\cdot$ l^{-1}) is almost equal to the juvenile density (4.4 individuals $\cdot$ l^{-1}). Assuming all juveniles in the upper layer have LI of 0, the average LI for adults in the layer is calculated to be 0.7. In both cases, the estimated LI values for adults distributed in the upper layer in daytime are not higher than the average LI value in the deep layer (0.7). Thus, we considered that adults distributed in the deep layer had more storage lipid than those in the upper layer, regardless of whatever LI juveniles in the upper layer had. The relative abundance of individuals with each LI in the deep layer changed greatly in a single day (Table 1). The rapid changes in storage lipid content seem not to be explained by assimilation, respiration and so on. Therefore, the changes in the vertical distribution of LI might be due to the vertical migration of adults with high storage lipid contents. The results, obtained here, indicated that migrating adults were in better nutritional states (high LI) than non-migrating adults which spent any time in the upper layer.

From the relationship between migrating behavior and protein content of *D. hyalina-galeata*, GUISANDE *et al.* (1991) has suggested that individuals in better nutritional condition stay in the upper layer all day, while individuals in poor nutritional condition make diel vertical migrations. They concluded that the difference in individual nutritional condition was the result of different migrating strategies. The results obtained in the present study contradict the results of GUISANDE *et al.* (1991). In the present study, we have demonstrated that individuals with large energy reserves and high tolerance to starvation will migrate vertically to avoid predation, whereas individuals in poor nutritional condition with low tolerance to starvation do not migrate but spend any time in the upper layer where food is abundant. Thus, individual nutritional condition should be regarded as a cause of the strategies of migrating behavior rather than the result of those strategies. In other words, *D. galeata* individuals chose their migrating strategy depending on their individual nutritional condition. This

Table 2. The population density (individuals $\cdot$ l^{-1}) and percentage (in parenthesis) of *Daphnia galeata* individuals with different development stage of eggs (THRELKELD, 1979) in each layer at the daytime (10:00 on 19 July) and the night time (02:00 on 20 July).

Time	Layer(m)	Egg stages 1	2	3	4	5
10 : 00	0 16	1.6 (61)	0.6 (23)	0.1 (3)	0.2 (8)	0.2 (6)
on	16-24	0.3 (68)	0.1 (21)	0.0 (2)	0.0 (5)	0.0 (5)
19 July	24 28	1.2 (43)	0.6 (20)	0.2 (7)	0.7 (24)	0.2 (6)
02 : 00	0 16	1.7 (61)	0.6 (22)	0.1 (3)	0.2 (6)	0.2 (8)
on	16 24	0.7 (58)	0.3 (27)	0.0 (2)	0.1 (6)	0.1 (6)
20 July	24-28	0.7 (54)	0.3 (23)	0.0 (3)	0.2 (16)	0.1 (5)

conclusion implies the existence of a trade-off between individual nutritional condition and predation avoidance.

Why does nutritional condition differ among adults within a population? When food becomes scarce, *Daphnia* stops reproduction and conserves its energy for metabolism (Goulden and Hornig, 1983). From 60 to 70% of adults in a whole water column did not have eggs, and the average clutch size was lower than 2 during the observation period (data not shown). These observations indicated that food was not sufficient for all adult *Daphnia* to continuously produce eggs. Therefore, changes in the food supply and difference in length of time after the last egg production for each individual might have caused the difference in nutritional condition among adults which bore no eggs. This was also the case for egg-bearing adults. Since egg development and lipid accumulation in the maternal body occur at the same time, adult *D. galeata* with more developing eggs have more lipid than those with stage 1 eggs. In the upper layer, among all *D. galeata* bearing eggs at 10:00 on 19 July and at 2:00 on 20 July, the percentages of adults which bore eggs in the stage 3 or higher were 16% and 17%, respectively (Table 2). On the other hand, the percentage in the deep layer during daytime and night time were 37% and 24%, respectively. The difference in the percentage between the upper and deep layers was not significant at night. However, during the daytime, the percentages of adults which bore eggs in stage 3 or higher was significantly higher in the deep layer than in the upper layer ($\chi 2$-test: $P \leq 0.001$). From the diel changes in population density (Fig. 1) and in LI (Table 1), the adults with more developing eggs would have higher lipid levels and would make the vertical migration. Since egg production occurs periodically accoring to molt, it is thought that migrating behavior of *Daphnia* individuals changes alternately with periodical changes in their nutritional condition, or with the amount of lipid storage, in relation to their life cycle.

Acknowledgments

We are grateful to Dr. H. Hayashi for his encouragement and kind support during this study. We also thank Drs. M. Nakanishi, T. Narita and J. Urabe for their comments on this manuscript. This study was partly supported by Grant-in-Aid for Encouragement of Young Scientists, No. 02854079 from the Ministry of Education, Science and Culture, Japan.

摘　　要

Daphnia galeata の栄養状態と
日周鉛直移動の関係

1991年7月，木崎湖において，*Daphnia galeata* の貯蔵脂質量により評価される栄養状態と日周鉛直移動との関係を調査した。*D. galeata* 個体群は日中表層と底層の2つのグループに分かれて分布した。表層のグループは未熟個体と栄養状態の悪い成熟個体より構成されているのに対し，底層のグループは栄養状態の良い成熟個体のみで構成されていた。これら2つのグループのうち，底層の成熟個体は日周鉛直移動を行うが，表層のグループの未熟・成熟個体は終日表層に留まることが，鉛直分布の日変化から示された。これらの結果から *D.galeata* はその生活史と関連しながら，栄養状態の高い成熟個体が鉛直移動を行う性質のあることが示唆された。つまり、栄養状態と捕食者回避の間に trade-off が存在していたことが考えられる。

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Received: 17 November 1994
Accepted: 1 March 1995

Freshwater Biology (1997) **38**, 611–618

添付論文2

Inter-and intraspecific differences in fatty acid composition of freshwater crustacean zooplankton

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SUMMARY

1. The composition of fatty acids (FA) from C14 to C18 was measured for edible seston and for individual *Daphnia galeata, Diaphanosoma brachyurum* and *Eodiaptomus japonicus* from Lake Biwa using a pyrolysis–gas chromatography (Py–GC) method. Based on the relative abundance of the FA, inter-and intraspecific differences in composition were examined.

2. Among the FA, C16 : 0 was the most abundant, both in the three zooplankton species and in edible seston smaller than 20 μm, suggesting that the composition of the zooplankton was roughly reflected by their diet. However, the abundance of C18 : 1 relative to C16 : 1 and C18 : 0 was much higher in each zooplankton species than in the diet.

3. Comparison of the relative abundance of FA among the three zooplankton species revealed that intraspecific differences in FA composition are greater than interspecific differences. These results indicate that variability in FA composition is not necessarily species-specific, and can be obscured by variation in composition between individuals.

Introduction

Differences in fatty acid (FA) composition of zooplankton have been associated with differences in the FA composition in their food (Bourdier & Amblard, 1989). Therefore, the FA composition of zooplankton has been used as a marker for tracing foodweb structure (Lee, Nevenzel & Paffenhöfer, 1971; Falk-Petersen, Sargent & Tande, 1987). If the FA composition of zooplankton is determined only by the food, interspecific differences in FA composition of zooplankton should reflect differences in diet. However, the FA composition is also affected by a number of external and internal factors other than diet, including water temperature (Farkas, 1979) and developmental stage (Kattner & Krause, 1987). Hence, interspecific differences in FA composition do not necessarily reflect the interspecific differences in diet. Furthermore, since sensitivity to both external and internal factors is expected to depend on the physiological condition of each individual, FA composition could differ even within the same species. If the intraspecific difference in FA composition caused by the physiological condition of each individual were larger than the interspecific difference in the composition depending on food, it would be difficult to estimate the interspecific differences in diet among zooplankton species. Unfortunately, no study has examined intraspecific differences in the FA composition of zooplankton species.

Analysis of the FA composition of zooplankton has traditionally used gas chromatography (GC). In this case, pretreatments, including extraction, saponifica-

tion and esterification are required before the GC analysis. Unfortunately, GC lacks the sensitivity necessary for analysing the FA composition of individual zooplankters. A flame ionization detector (FID), which is a typical detector for a GC, can detect ≈ 20 pg of each compound (McMinn & Hill, 1992). When FA composition in zooplankton is analysed, the sample pretreated in solvent has to contain at least 1 ng μl^{-1} of each FA species. This means that zooplankton samples for analysis of FA composition have to contain at least 1 μg of each FA species, because the sample pretreatments usually require more than 1 ml of solvent (Kattner & Fricke, 1986). On the other hand, an individual *Daphnia pulex* has about 5 μg of total lipid (Goulden & Place, 1990), and the relative abundance of most individual FA in crustaceans is less than 1% of total lipid (Farkas, 1979). Thus, the content of most individual FA in a *Daphnia* individual is less than 0.05 μg; less than 5% of the required amount.

Recently, the authors have developed a new method for analysing the FA composition of zooplankton at the individual level. This technique is based on a pyrolysis gas chromatography (Py–GC) method which has been successfully applied for measuring the FA composition of cultured *D. galeata* (Ishida *et al.*, 1996). In this Py–GC method, pretreatments of a sample are made by reactive pyrolysis in the presence of tetramethylammonium hydroxide (THMA) on line. The Py–GC method makes it possible to examine differences in the FA composition of individual zooplankton. Using this approach, the relative abundances of FA for three dominant zooplankton species in Lake Biwa were measured in order to characterize the pattern of variability at both the species level, and among species and among individuals levels.

Methods

Zooplankton were collected at the Wani station of Lake Biwa (35°10′30′N, 135°56′30′E, 53 m deep) from 10.00 to 13.00 hours on 13 September 1994. Sampling was conducted with a closing 100-μm plankton net from the 0–10 m, 10–20 m and 20–50 m layers. The samples were not fixed, and the dominant species (*Daphnia galeata, Diaphanosoma brachyurum* and *Eodiaptomus japonicus*) were individually picked up with tweezers, dried in a vacuum desiccator and used for analysis of FA composition.

Lake water was also collected from 0, 5, 10, 15, 20, 30, 40 and 50 m using a Van Dorn water sampler. As crustacean zooplankton in Lake Biwa mainly ingest food particles smaller than 20 μm (Okamoto, 1984; Urabe *et al.*, 1996), water samples were prefiltered through a 20-μm plankton net. Chlorophyll *a* concentrations in the < 20 μm size fraction and in the total phytoplankton community were determined by filtering subsamples onto GF/F filters. Chlorophyll *a* concentrations were determined fluorometrically using the method of Lorenzen (1967). Aliquots for filtered (< 20 μm) subsamples were concentrated on precombusted Whatman GF/F filters for analysis of the FA on edible seston. Filters were subsequently dried in a vacuum desiccator. Water temperature and chlorophyll *a* fluorescence were measured vertically with a conductivity temperature depth profiler (SBE 25, Sea-Bird Electronics, Inc., USA) equipped with a fluorometer (Sea Tech, Inc., USA).

The FA composition of each individual zooplankter and the concentrated seston was analysed qualitatively by the Py–GC method. Dry weights of zooplankton individuals used for the analysis were 2–5 μg for *Daphnia galeata*, 1–3 μg for *Diaphanosoma brachyurum* and 1–5 μg for *E. japonicus*, respectively. The concentrated seston was 196–217 μg.

Details of the Py–GC method for FA analysis have been discussed in Ishida *et al.* (1996). Briefly, 2 μl of a methanol solution (25 wt.%) of tetramethylammonium hydroxide (THMA) were added to a sample of seston or a single individual zooplankter in a small platinum cup. The cup with the sample was introduced into the pyrolyser (Yanaco GP1018) of the Py–GC (HP 5890). The pyrolysis temperature was set at 400 °C. The temperature of the GC column was set initially at 50 °C, then programmed up to 190 °C at a rate of 5 °C min^{-1} and, finally, from 190 to 240 °C at a rate of 1 °C min^{-1}. The column was an HP INNOWAX (50 m × 0.02 mm ID) coated with a crosslinked polyethyleneglycol (PEG) film (0.20 μm). Identification of the peaks on the pyrograms was carried out by a gas chromatography mass spectrometer (GC–MS) system (JEOL AUTOMASS 150) with an electron impact ionization source to which the pyrolyser was directly attached. The relative abundance of each FA was calculated from the peak area on the pyrogram.

Data for statistical analysis were normalized using log (n + 1) transformations. Interspecific differences in the FA composition of zooplankton were examined by a two-level nested ANOVA. Differences in the

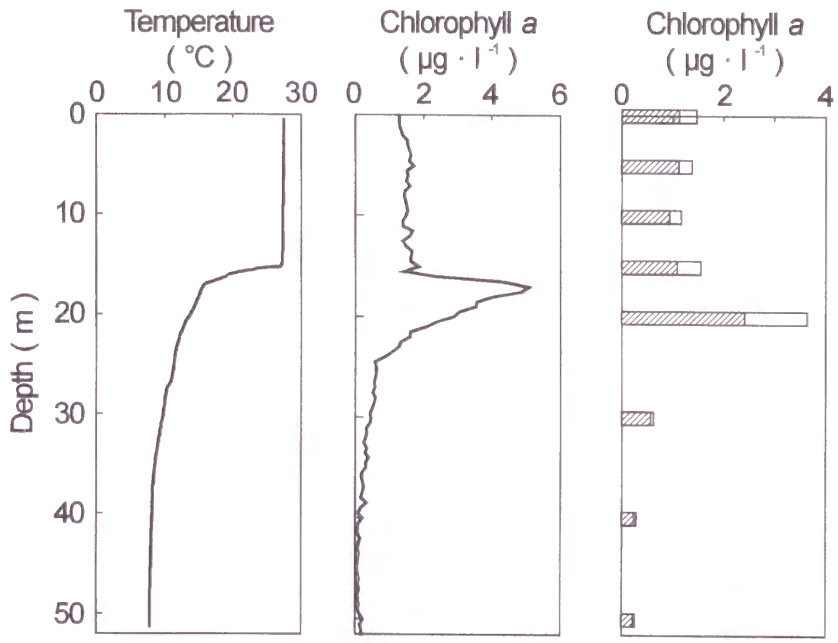

Fig. 1 Vertical profiles of water temperature, chlorophyll *a* fluorescence and size fractionated chlorophyll *a* < 20 µm (hatched area) and (>20 µm (open area). Chlorophyll *a* fluorescence was converted to chlorophyll *a* concentration.

Table 1 Two-level nested ANOVA calculation for difference in the ratios of fatty acids (FA) among zooplankton species with depth

	C18 : 1 to C18 : 0				C18 : 1 to C16 : 1			
Source of variation	d.f.	MS	*F*	Percentage of variance	d.f.	MS	*F*	Percentage of variance
Among species within depths	2	0.170	5.32	53	2	0.014	0.32	0
Among depths within species	4	0.032	3.14	17	4	0.044	8.01*	66
Within species	22	0.008		30	22	0.004		34

*Significant difference at 5% level.

Table 2 Single-classification ANOVA for differences in the ratios of fatty acids (FA) among depth layers within *Daphnia* and *Eodiaptomus*

	C18 : 1 to C18 : 0			C18 : 1 to C16 : 1		
Source of variation	d.f.	MS	*F*	d.f.	MS	*F*
Among depths within *Daphnia*	2	0.824	4.12	2	0.395	3.99
Within *Daphnia*	7	0.200		7	0.099	
Among depths within *Eodiaptomus*	2	0.009	2.80	2	0.054	16.22**
Within *Eodiaptomus*	9	0.003		7	0.003	

**Significant difference at 0.005 level.

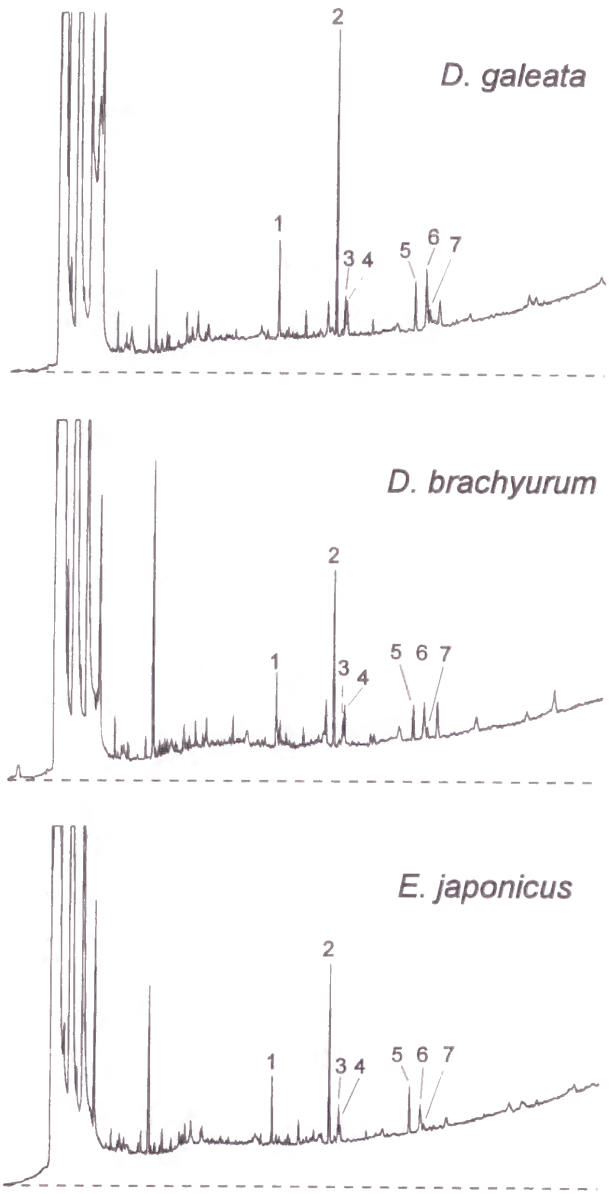

Fig. 2 Representative pyrograms for individual *Daphnia galeata*, *Diaphanosoma brachyurum* and *Eodiaptomus japonicus*. (1) C14 : 0; (2) C16 : 0; (3) C16 : 1; (4), C16 : 1; (5) C18 : 0; (6) C18 : 1; (7) C18 : 1.

FA composition of each zooplankton species among distribution layers were examined by a single-classification ANOVA (Sokal & Rohlf, 1981).

Results

On the sampling date, the thermocline was at 15 m, and water temperature in the epilimnion and hypolimnion were 27 °C and 8 °C, respectively (Fig. 1). A maximum of chlorophyll *a* fluorescence 5.1 μg l^{-1} was found slightly below the thermocline. Chlorophyll *a* fluorescence in epi-and hypolimnion were about 1.5 μg l^{-1} and 0.2 μg l^{-1}, respectively. Throughout the water column more than 70% of chlorophyll *a* was found in the edible fraction (< 20 μm).

The three crustacean plankton, *Daphnia galeata*, *Diaphanosoma brachyurum* and *E. japonicus* were the most numerous species. Saturated FA from C14 to C18 and two isomers of monoenoic C16 and C18 FA were identified for each species (Fig. 2). The reactive pyrolysis with TMAH often isomerizes FA (Ishida *et al.*, 1996). The two isomers of monoenoic C16 and C18 FA may be produced by the isomerization, and the authors of the present study were unable to distinguish between isomerized and non-isomerized FA. Thus, these isomers of each monoenoic FA were treated as one FA species for the following analysis. The FA composition of the edible seston showed depth-dependent changes with the C16 : 0 being significantly more abundant above the thermocline (< 15 m deep) while C16 : 0 and C18 : 0 dominated in the seston below the thermocline (Fig. 3). The C16 : 0 was also the most abundant FA in the three zooplankton species except for *Daphnia galeata* collected from the 10–20 m and 20–50 m layers. C18 : 0 did not significantly increase with depth in zooplankton (Fig. 3). The relative abundance of C18 : 1 in zooplankton species was significantly larger than that in the edible seston except for *E. japonicus* collected from the 20–50 m layer. However, the relative abundance of other FA did not significantly differ between zooplankton and edible seston.

To examine the relationship among the relative abundance of FA between the zooplankton and edible seston, the C18 : 1 to C18 : 0 ratio for zooplankton was plotted against C18 : 1 to C16 : 1 ratio for the edible seston (Fig. 4). In all species, both ratios were much higher than in the edible seston (< 20 μm). *Eodiaptomus japonicus* tended to have a smaller C18 : 1 to C18 : 0 ratio than the other species. However, according to the two-way nested ANOVA, the differences in both the C18 : 1 to C16 : 1 and the C18 : 1 to C18 : 0 ratios were not significant among the species (Table 1). This implies that differences within species exceeded differences between species. Within *Daphnia galeata* and *E. japonicus*, individuals collected from the 0–10 m and 10–20 m layers tended to have smaller C18 : 1 to C18 : 0 ratios and larger C18 : 1 to C16 : 1 ratios than those

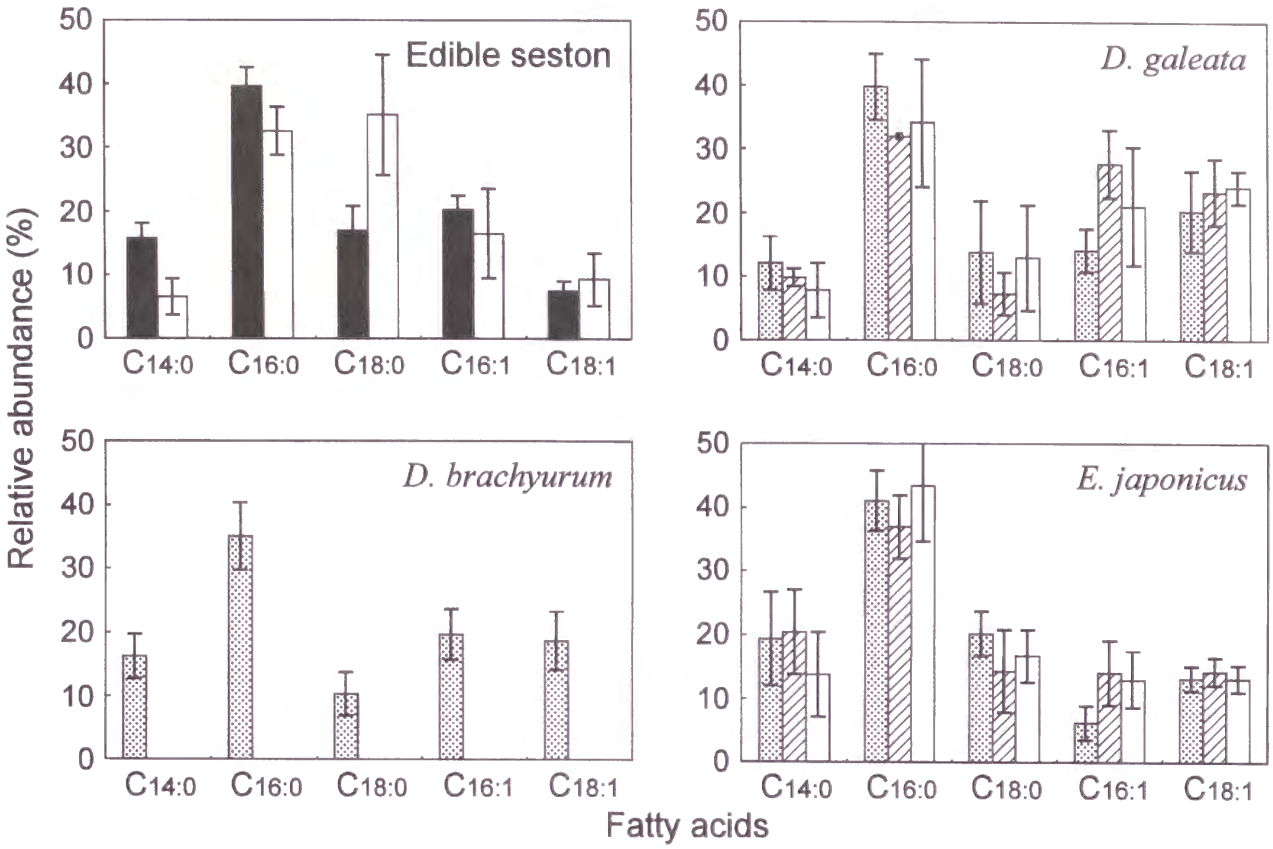

Fig. 3 Relative abundance for each FA in edible seston and zooplankters. Error bars show the 95% confidence intervals. Edible seston: solid bars, seston collected from 0 to 15 m deep ($n = 4$); open bars, seston collected from 20 to 50 m deep ($n = 4$). Zooplankton: dotted bars, individuals collected from the 0–10 m layer (*Daphnia galeata*, $n = 4$; *Diaphanosoma brachyurum*, $n = 7$; *Eodiaptomus japonicus*, $n = 4$); hatched bars, individuals collected from the 10–20 m layer (*D. galeata*, $n = 3$; *D. brachyurum*, no data; *E. japonicus*, $n = 4$); open bars, individuals collected from the 20–30 m layer (*D. galeata*, $n = 3$; *D. brachyurum*, no data; *E. japonicus*, $n = 4$)

from the 20–50 m layers. However, significant differences among depth layers were detected only for the C18 : 1 to C16 : 1 ratio among *E. japonicus* individuals (Table 2). The relative abundance of FA in individuals of *Diaphanosoma brachyurum* was not tested, because not enough individuals were collected from the 10–20 m and 20–50 m layers.

Discussion

The FA composition of each zooplankton species was roughly similar to that of the edible seston (< 20 μm) (Fig. 3). Indeed, C16 : 0 was the most abundant FA in both the zooplankton and the edible seston. This result suggests that the FA composition of zooplankton was basically reflected by the composition of their food. However, the zooplankton's FA composition did not reflect food sources alone. The relative abundance of C18 : 0 did not increase with depth in zooplankton, while the relative abundance of C18 : 0 increased with depth in edible seston. Furthermore, both the C18 : 1 to C16 : 1 and C18 : 1 to C18 : 0 ratios were higher in zooplankton than in the edible seston, suggesting that the relative abundance of C18 : 1 in zooplankton was higher than in the edible seston, and/or the relative abundance of C16 : 1 and C18 : 0 in zooplankton were lower than in the edible seston (Fig. 4). The higher relative abundance of C18 : 1 and the lower relative abundance of C16 : 1 and C18 : 0 in zooplankton could be due to selective feeding or differences in the assimilation, catabolism, synthesis, or modification of FA by the zooplankton. Investigations of FA budgets are required to understand these factors, but these investigations were not carried out in the present study. However, the cladocerans *Daphnia galeata* and *Diaphanosoma brachyurum* do not have the ability to

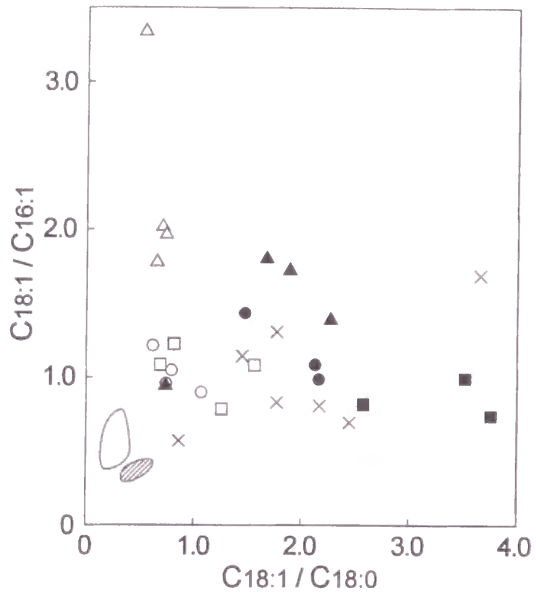

Fig. 4 Ratios of C18 : 1 FA to C18 : 0 and C16 : 1 FA in zooplankters and edible seston. *Daphnia galeata* in 0–10 m (▲), 10–20 m (△), 20–50 m (■); *Eodiaptomus japonicus* in 0–10 m (●), 10–20 m (□), 20–30 m (○) layers; *Diaphanosoma brachyurum* in 0–10 m (×). Open area, edible seston in 0–15 m; hatched area, edible seston in 20–50 m.

select food on the basis of its chemical composition (Butler, Suttle & Neill, 1989; DeMott, 1990). Thus, it is suggested that the differences in the C18 : 1 to C16 : 1 and C18 : 1 to C18 : 0 ratios between these cladocerans and the edible seston might be influenced by processes occurring after ingestion, such as excretion, respiration and the modification of FA. On the other hand, since calanoid copepods have the ability of selective feeding to discriminate between foods on the basis of chemical composition (Butler *et al.*, 1989), we cannot reject the possibility that *E. japonicus* selectively ingested food with high C18 : 1 content and/or food with low C16 : 1 and C18 : 0 contents. Thus, in addition to potential internal physiological differences, selective feeding may influence the differences in the C18 : 1 to C16 : 1 and C18 : 1 to C18 : 0 ratios between *E. japonicus* and edible seston.

A significant difference was detected in the C18 : 1 to C16 : 1 ratio of *E. japonicus* from different depth layers (Table 2). This result implies that depth-related factors have contributed to the differences in physiological status and/or the diet. Water temperature and food abundance expressed as chlorophyll *a* differed in the water column (Fig. 1). These factors are known to influence FA composition of zooplankton (Lee *et al.*, 1971; Farkas, 1979). In the present study, food abundance was highest in the thermocline (10–20 m layer), and lowest in the hypolimnion (20–50 m layer). However, the C18 : 1 to C16 : 1 ratios of *E. japonicus* in the food-rich 10–20 m layer and food-poor 20–50 m layer were similar to each other. Thus, a vertical difference in the C18 : 1 to C16 : 1 ratio of *E. japonicus* cannot be explained by food abundance. However, in accordance with the difference in the C18 : 1 to C16 : 1 ratio, water temperature was higher in the 0–10 m layer than the deeper layer. Thus, differences in the C18 : 1 to C16 : 1 ratio of *E. japonicus* could be explained by changes in temperature with depth. On the other hand, significant differences in the C18 : 1 to C16 : 1 and C18 : 1 to C18 : 0 ratios within *Daphnia galeata* were not detected among depths (Table 2). This result implies that temperature and food abundance changes with depth did not influence the C18 : 1 to C16 : 1 and C18 : 1 to C18 : 0 ratios of *Daphnia galeata*.

It should be noted that the FA composition can differ among developmental stages within a species (Kattner & Krause, 1987). Therefore, intraspecific differences in the C18 : 1 to C16 : 1 and C18 : 1 to C18 : 0 ratios of zooplankton could be related to differences in developmental stage. Furthermore, the vertical distributions of the various developmental stages of *E. japonicus* differ in Lake Biwa (Kawabata, 1987). Hence, the vertical difference in the C18 : 1 to C16 : 1 ratio may have been due to differences in the developmental stage. Unfortunately, since neither the body size nor developmental stage of individuals for FA measurement were recorded, the influence of the developmental stage on the C18 : 1 to C16 : 1 ratios cannot be assessed.

Interspecific differences in the FA composition of zooplankton have been associated with differences in the FA composition of their food (Bourdier & Amblard, 1989). In the present study, the FA composition of each zooplankton species was roughly similar to that of edible seston (Fig. 3). Thus, the results support the hypothesis that the FA composition of zooplankton is basically reflected by the FA composition in their food. However, a significant interspecific difference was not found in the C18 : 1 to C16 : 1 and C18 : 1 to C16 : 1 ratios due to intraspecific differences (Table 1). Assuming that the FA composition of zooplankton is food specific and is not influenced by other factors, lack of interspecific differences in FA composition implies that differences in the FA composition of food ingested

by each zooplankton individual within the same species were greater than the difference among species. However, as noted above, it is suggested that the difference in FA composition does not reflect food source alone, but water temperature and developmental stage also influence FA composition. Thus, in the present study, the lack of interspecific differences in the FA composition of zooplankton implies that the interspecific difference in FA composition in their food is smaller than the intraspecific difference due to the influence of water temperature and developmental stage.

Several studies in this special issue have emphasized the importance of long-chain highly unsaturated fatty acids (HUFA) in zooplankton nutrition. In the present study, FA longer than C20 and polyunsaturated FA could not be detected because of the pyrolysis conditions in the analysis (Ishida *et al.*, 1996). However, the present study suggests that intraspecific differences in the FA composition can exceed the interspecific differences among crustacean plankton. These results suggest that large intraspecific differences in HUFA composition could also occur. Since traditional GC methods require a large number of individual zooplankton to measure FA composition, differences among individuals cannot be detected. Due to this technical problem, previous studies have analysed the FA composition of zooplankton species without considering intraspecific difference. However, to examine whether changes in FA composition are species-specific, the intraspecific difference should now be considered.

Acknowledgments

We are grateful to Mr T. Koitabashi and Mr T. Ueda for their kind assistance with sampling. We also thank Drs J. Urabe and J.J. Frenette for their comments on this manuscript. Financial support by the Showa Shell Sekiyu Foundation for Promotion of Environmental Research, the Ishida Foundation and the Research Foundation for the Electrotechnology of Chubu are gratefully acknowledged. The present study was also supported in part by a grant-in-aid from the Ministry of Education, Science and Culture, Japan (No. 06304048) and a grant-in-aid from the Institute for Hydrospheric-Atomospheric Sciences, Nagoya University (1994–95).

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DIEL VERTICAL MIGRATION OF ZOOPLANKTON: OPTIMUM MIGRATING SCHEDULE BASED ON ENERGY ACCUMULATION

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Key Words: DVM, nutritional status, reproduction cycle, predation, trade-off, Daphnia

Running Head: diel vertical migration of zooplankton

ABSTRACT

Zooplankton perform diel vertical migration (DVM) to avoid predators at the upper layer, while often stay at the upper layer throughout a day in spite of presence of the predators. This difference in migrating behavior has been explained by difference in environmental condition or genetic difference. Here, we theoretically examined how internal conditions of zooplankton individuals relate to determining different migrating behavior based on their reproductive success. A simple optimization model demonstrates that zooplankton individuals change their migrating behavior depending on energy accumulation for reproductive investment. Such energy accumulation and its investment for reproduction are repeated every reproduction cycle. Therefore, if the reproduction cycle is not synchronized among individuals, different migrating behaviors will be observed within a population regardless of neither difference in environmental condition nor genetic difference. Our model demonstrates that such coexistent of the two migrating behaviors is possible in natural Daphnia populations, and suggests that internal conditions of zooplankton individuals can not be neglected as a factor for determining migrating behavior of zooplankton.

Diel vertical migration (DVM) is general phenomenon in marine and fresh water zooplankton. Usually, zooplankton ascend at dusk after staying at the deeper layer during daytime, and then descend at dawn after staying at the upper layer during nighttime (Cushing 1951; Hutchinson 1967). Several hypotheses have been proposed for the reason of DVM (Kerfoot 1985; Lampert 1989, 1993). Among these, 'predator avoidance hypothesis' has been most supported by many researchers (Zaret and Suffern 1976; Stich and Lampert 1981; Wright et al. 1980). This hypothesis states that zooplankton stay at the deeper layer with dark and low food condition to avoid visually searching predators such as fish during daytime, but stay at the food-rich upper layer to gain food during nighttime.

Huntley and Brooks (1982) reported that when food supply was limited, zooplankton stayed at the upper layer throughout a day in spite of presence of predators. This fact suggests that whether zooplankton perform DVM or stay at the upper layer throughout a day is a result of a trade-off between avoidance from visually searching predators and food gain (Johnsen and Jakobsen 1987; Lampert 1989). Therefore, it has been explained that seasonal changes in balance of the trade-off between predation pressure and food abundance cause seasonal changes between the two migrating behaviors. Apart from these seasonal changes, two inter- or intraspecific groups with the different migrating behaviors are observed within lakes (Stich and Lampert 1981; Weider 1984; Guisande et al. 1991). Since prey body size influences on visibility for predator (Zaret and Kerfoot 1975; Brooks and Dodson 1965; Write et al. 1980), it is supposed that genetic difference in body size causes difference in vulnerability of predation, resulting differentiation of the migrating behavior (De Meester et al. 1995). However, Sekino and Yoshioka (1995) reported that the different migrating behaviors, which cannot be explained by the body size, coexist within a population. They suggested that difference in nutritional status is important to explain migrating behavior of zooplankton.

Zooplankton invest most of accumulated energy through feeding for reproduction after maturation (Tessier and Goulden 1982). Thus, zooplankton with more accumulated energy can produce more offsprings and have higher potential of reproductive fitness. In this respect, zooplankton which stay at the upper layer throughout a day have more advantages than those which perform DVM. Ingested zooplankton by predators, however, lose whole accumulated energy otherwise used for the reproduction. If the expected loss of accumulated energy due to predation is larger than the net energy gain, zooplankton may move to the deeper layer to avoid predator at the expense of energy gain. Assuming that environmental conditions are constant, the net energy gain is proportional to time period when they stay at the upper layer. Whereas the expected loss of accumulated energy due to predation increases with increasing amount of accumulated energy. Therefore, it is expected that zooplankton begins to perform DVM when the amount of accumulated energy exceeds a certain threshold. Since energy accumulation and reproductive investment are repeated (Tessier and Goulden 1982), zooplankton may alter their migrating behavior according to reproduction cycle. If the reproduction cycle is not synchronized in a population, different migrating behavior will be observed within the population because amount of accumulated energy exceeds the threshold in some individuals but not in the others.

Several theoretical models about DVM of zooplankton have been proposed in previous studies (demographic simulation model, Wright et al. 1980; game model, Iwasa 1982; ESS model, Gabriel and Thomas 1988a, b; life history model, Fiksen 1997). However, there were no studies which consider a fact that the amount of accumulated energy changed according to reproduction cycle. In the present study, we investigate an optimum migrating schedule within a reproduction cycle to maximize reproductive fitness using a simple mathematical model. We further examine how such a schedule change according to food abundance and predation

pressure. Here, we define the ratio and order of the two migrating behaviors within a certain period as a migrating schedule. The present study is carried out using Daphnia (Cladocera: Crustacea) as a model animal, because they are dominant zooplankton in most lakes.

MODEL

Since Daphnia produce eggs at each molt after maturation, a reproduction cycle corresponds to molt cycle (fig. 1). Within each molt, Daphnia accumulate energy and invest them to egg production at the end of inter molt. The produced eggs are released into brood porch where they develop until next maternal molt when the juveniles leave from the maternal individuals (Tessier and Goulden 1982). Since these eggs in the brood porch obtain no nutrition from maternal individual, duration of egg development depends only on water temperature. Since egg development time roughly equal to inter molt duration (Tessier and Goulden 1982), our model assumes that a reproduction cycle corresponds to egg development time.

The reproductive fitness over a given reproduction cycle (W) can be expressed as

$$W = S(\alpha + \beta \cdot pR) \ , \tag{1}$$

where S and R are the survival probability ($cycle^{-1}$) and the amount of accumulated energy ($J \cdot cycle^{-1}$) during the reproduction cycle, and p is the number of eggs produced by unit energy ($eggs \cdot J^{-1}$). Here, the expected clutch size in next reproduction cycle is expressed as pR. α is the reproductive value of maternal individual including eggs in its brood porch, and it is expressed as

$$\alpha = 1 + NM \ , \tag{2}$$

where 1 means reproductive value of a given maternal individual; N is the number of eggs in brood porch; These eggs are produced in previous reproduction cycle and develop in the

brood porch; M is the survival probability of a juvenile from leaving the brood porch to maturation. β is the reproductive value of a newly produced egg and is expressed as

$$\beta = S' \cdot M \ , \tag{3}$$

where S' is the expected value of survival probability of an egg during development in the next reproduction cycle until leaving the brood porch.

When an individual stays at the upper layer for the former i days ($0 \le i \le T$) and performs DVM for the latter T - i days in T days of a reproduction cycle, the survival probability (S) and the amount of accumulated energy during the reproduction cycle (R) are expressed as

$$S = {s_s}^{i} \cdot {s_m}^{(T-i)} \tag{4}$$

$$R = r_s i + r_m (T - i) \ , \tag{5}$$

where s_s and r_s are the daily survival rates (day^{-1}) and the daily amounts of accumulated energy ($J \cdot day^{-1}$), when the individual stays at the upper layer throughout a day; s_m and r_m are the daily survival rate and the daily amount of accumulated energy, when the individual performs DVM. Here, we assume $s_s < s_m$, because the death rate due to predation is lower for the migrating individuals than for the staying individuals. On the other hand, we assume $r_s > r_m$, because the migrating individuals stay at the food-poor deeper layer during daytime. It should be noted that there is a trade-off between S and R because S decreases with increasing i while R increases, and *vice versa*. Applying equation (4) and (5) for equation (1), we can represent the fitness when an individual stays at the upper layer for i days (W_i) as

$$W_i = {s_s}^{i} \cdot {s_m}^{(T-i)} \{\alpha + \beta \cdot p(r_s i + r_m (T - i))\} \quad (i = 0, 1, 2, \cdots, T) \ . \tag{6}$$

We consider that migrating schedule evolves so that the fitness (W_i) is maximized. The optimal solution can be obtained by the following procedure.

We define w_i as the ratio of W_{i+1} to W_i:

$$w_i = \frac{W_{i+1}}{W_i} = \frac{s_s}{s_m} \cdot \frac{\alpha + \beta \cdot p((r_s - r_m)(i+1) + r_m T)}{\alpha + \beta \cdot p((r_s - r_m) i + r_m T)} \qquad (i = 0, 1, 2, \cdots, T-1) \tag{7}$$

Since $r_s > r_m$, w_i decrease monotonously with increasing i. Neglecting special cases with $w_i = 1$, this means that W_i has a unique maximum value at a certain i under any condition.

If $w_0 < 1$, W_0 is maximum because $w_i < 1$ for any i so that

$$W_o > W_1 > W_2 > \cdots > W_T \ .$$

If $w_{i-1} > 1$ and $w_i < 1$, W_i is maximum $(i = 1,2,\cdots,T-1)$ because

$$W_0 < \cdots < W_{i-1} < W_i \quad \text{and } W_i > W_{i+1} > \cdots > W_T \ .$$

Finally, if $w_{T-1} > 1$, W_T is maximum because $w_i > 1$ for any i so that

$$W_o < W_1 < W_2 < \cdots < W_T \ .$$

Applying Equation (7), the above inequalities for w_i can be rewritten as follows:

W_0 is maximum when

$$\frac{s_m}{s_s} > \frac{\frac{\alpha}{\beta} \cdot \frac{1}{pr_s} + 1 + \frac{r_m}{r_s}(T-1)}{\frac{\alpha}{\beta} \cdot \frac{1}{pr_s} + \frac{r_m}{r_s} T} \ ; \tag{8}$$

W_i is maximum for $i = 1,2,\cdots,T-1$ when

$$\frac{\frac{\alpha}{\beta} \cdot \frac{1}{pr_s} + i + \frac{r_m}{r_s}(T-i)}{\frac{\alpha}{\beta} \cdot \frac{1}{pr_s} + i - 1 + \frac{r_m}{r_s}(T-i+1)} < \frac{s_m}{s_s} < \frac{\frac{\alpha}{\beta} \cdot \frac{1}{pr_s} + i + 1 + \frac{r_m}{r_s}(T-i-1)}{\frac{\alpha}{\beta} \cdot \frac{1}{pr_s} + i + \frac{r_m}{r_s}(T-i)} \ ; \tag{9}$$

W_T is maximum when

$$\frac{s_m}{s_s} < \frac{\frac{\alpha}{\beta}\cdot\frac{1}{pr_s}}{\frac{\alpha}{\beta}\cdot\frac{1}{pr_s}+T-1+\frac{r_m}{r_s}} . \qquad (10)$$

Inequality (10) is the condition where staying at the upper layer during a whole reproduction cycle (non-DVM) is optimum migrating schedule. Inequality (8) is the condition where DVM everyday during a whole reproduction cycle (continuous-DVM) is optimum migrating schedule. Inequality (9) is the condition where it is optimum migrating schedule that the individual stays at the upper layer for i days, and then performs DVM for T-i days (occasional-DVM). When parameters T, pr_s and α / β are given, conditions for these optimum migrating schedules can be identified as regions in the space of the ratios of s_m / s_s and r_m / r_s according to inequalities (8), (9) and (10).

RESULTS

To examine relationship between optimum migrating schedule and environmental conditions, we used literature values on Daphnia species for standard values of parameters T, pr_s and α / β. We also examined effect of variation from these standard values. Since duration of egg development for Daphnia species is about 5 days at 15 °C (Bottrell et al. 1976), we set T at 5 days. If individuals continue to stay at the upper layer during a whole T days of reproduction cycle, the number of produced eggs within the reproduction cycle (i.e. the clutch size) is expressed as pr_sT. Assuming this egg number as clutch size for the next reproduction cycle (pr_sT), it was set at 15 which fall within usual clutch size for Daphnia species (Hall 1964). Therefore, pr_s was set at 3. The number of eggs in brood porch in this reproduction cycle (N) was set at 15 from usual clutch size for Daphnia species. Survival probabilities of M and S' were tentatively assumed as 0.2 and 0.8, though they may vary in the natural population.

Therefore, α / β was set at 25 from assumed N, M through S' and equations (2) and (3)

Using values mentioned above, we illustrated the number of days for the staying at the upper layer (i) to maximize the fitness (W_i) (fig. 2). When the value of s_m / s_s is low, the fitness is maximum at $i = T$. This means that the optimum migrating schedule is staying at the upper layer during the whole reproduction cycle (non-DVM). As s_m / s_s increases, the optimum value of i, the number of days for the staying at the upper layer, is decreased. This implies that the increase in s_m / s_s enhances DVM. When optimum value of i is 0, the individual performs DVM everyday during the whole reproduction cycle (continuous-DVM). Whereas, when optimum value of i is between 1 and 4 (i.e. T-1), the individual continues to stay at the upper layer for i days, and then performs DVM for T-i days (occasional-DVM). The result indicates that it is advantageous for Daphnia to change their migrating behavior depending on the ratio of survival rates of the two migrating behaviors within a reproduction cycle. As r_m / r_s increase at a low value of s_m / s_s, the optimum value of i gradually decreases. This means that with decreasing the daily amount of energy accumulation at the upper layer, the optimum migrating schedule change from non-DVM to continuous-DVM through occasional-DVM. However, when s_m / s_s takes a higher value, non-DVM never becomes the optimum migrating schedule for any r_m / r_s. As s_m / s_s increases further, optimum migrating behavior is only continuous-DVM for any r_m / r_s. In this sense, we can say that s_m / s_s affects the optimal behavior more strongly than r_m / r_s.

Since the period of a reproduction cycle (T) is affected by water temperature (Hall 1964; Bottrell et al. 1976), we examined the effect of water temperature on optimum migrating schedule. The optimum migrating schedules in the space of s_m / s_s and r_m / r_s were calculated at 20 and 10 °C, where reproduction cycle of Daphnia (T) is 3 and 8 days (fig. 3). In the calculations, the other parameters were fixed to those when $T = 5$ in fig. 2. The regions of

continuous-DVM for $T = 3$ and $T = 8$ is scarcely different from that for $T = 5$, implying that water temperature does not greatly influence on the condition where continuous-DVM is optimum. On the other hand, the region of occasional-DVM is larger for larger T values, while the region of non-DVM is smaller for larger T values, implying that when T increases due to low water temperature, the condition where occasional-DVM is an optimum is expanded, while the condition where non-DVM is optimum is restricted.

To examine the effect of changes in parameter α / β, the optimum migrating schedules when α / β is 10 and 50 were calculated (fig. 4). In the calculations, the other parameters were fixed to those when $\alpha / \beta = 25$ in fig. 2. The region of continuous-DVM is larger for larger α / β values, whereas the regions of non-DVM and occasional-DVM are smaller for larger α / β values. These results imply that increase in α / β enhances DVM. According to equation (2) and (3), α / β increases when M and / or S' decrease and when N increases. Thus, the condition for each migrating schedule to be advantageous is connected with juvenile survival probability (M), egg survival probability in the next reproduction cycle (S') and the number of eggs in the brood porch (N).

The optimum migrating schedules when pr_s is 1 and 5 were calculated (fig. 5). In the calculation, the other parameters were fixed to those when $pr_s = 3$ in fig. 2. Since T is set at 5, the expected clutch size in next reproduction cycle for an individual of non-DVM (pr_sT) is 5 eggs for $pr_s = 1$ and 25 eggs for $pr_s = 5$. The region of continuous-DVM is smaller for larger pr_s values, whereas those of occasional-DVM and non-DVM are larger for larger pr_s values. It should be noted that p is inversely proportional to the egg mass, and r_s is proportional to food abundance at the upper layer. Since fluctuation of egg mass is smaller than that of food abundance (Trubetskova and Lampert 1995), pr_s depends mainly on food abundance at the upper layer in actual cases. Thus, the effect of changes in parameter pr_s on the optimum

migrating schedule indicates that the increase in food abundance at the upper layer under a constant ratio of r_m / r_s enhances staying at the upper layer throughout a day.

DISCUSSION

The present study suggests that Daphnia may change its migrating behavior during a reproduction cycle depending on the amount of accumulated energy. Since the reproduction cycle of Daphnia is not generally synchronized among individuals (Hall 1964), we may observe two individual groups with different migrating behaviors within a population: one individual group stays at the upper layer throughout a day and the other individual group performs DVM. In the previous studies, coexistence of different migrating behaviors has been explained by genetic differences (Guisande et al. 1991; DeMeester et al. 1995). In support with this explanation, some genotypes are known to coexist simultaneously in a lake (Weider 1984; Müller and Seitz 1993; Spaak and Hoekstra 1993). However, our result suggests that difference in migrating behavior can be also explained simply by difference in the amount of the accumulated energy among individuals within the same population. Some previous studies showed that changes in migrating behavior of zooplankton can be explained by phenotypical polymorphism because they were induced by increasing predators (Ringelberg 1991; DeMeester 1993), suggesting that phenotypical changes in migrating behavior may also be induced by changes in the amount of the accumulated energy.

The survival rate for each migrating behavior (s_m and s_s) depends on predation pressure at different depths. When predators do not exist at both layers, s_m / s_s is 1. As predation pressure at the upper layer increases, s_m / s_s increases due to decreasing s_s. As less than 0.5 of survival rate per day for Daphnia is not usually observed in a natural population (Hall, 1964; Byron et al., 1986; Lueck et al. , 1990; Walters et al., 1990; Lampert, 1991), the maximum s_m / s_s is not

larger than 2. Thus, most of s_m / s_s value for natural population is likely in the range between 1 and 2. In addition, Lampert (1987) reported that daily death rates of migrating individuals and of staying individuals at the upper layer were 0.019 and 0.100 in a natural population of Daphnia, respectively, in Lake Constance. In this case, since $s_m = 0.981$ and $s_s = 0.900$, s_m / s_s is 1.090. Therefore, the range of s_m / s_s examined in the present study is realistic in natural populations.

Daily amount of energy accumulation (r_m and r_s) depends on food abundance at the upper and the deeper layers. The vertical difference in food abundance changes between seasons (Huntley and Brooks 1982). When the vertical difference in food abundance is small, r_m / r_s approaches 1. On the other hand, when food abundance at the deeper layer is smaller than that at the upper layer, r_m / r_s is less than 1. If vertical difference in food abundance is great, r_m / r_s is roughly equal to ratio of nighttime length per day because nighttime length determines the period that migrating individuals can gain food at the food-rich upper layer. Thus, r_m / r_s can be much less than 0.5 in summer except for low latitude areas. In addition, since migrating individuals require time for migration between the deeper and the upper layer, the period that migrating individuals gain food at the upper layer is shorter than nighttime length. Therefore, r_m / r_s may often approach 0. Consequently, it is supposed that the value of r_m / r_s in natural population may take various values between 0 and 1 in which we examined the optimal migrating behavior.

As shown in figure 2, occasional-DVM appears generally in region where $s_m / s_s \cong 1.1$ and $r_m / r_s < 0.5$ when other parameters take standard values for Daphnia species. The above discussion implies that these values are not unrealistic in natural populations, and therefore, the occasional-DVM may be realistic as an optimum migrating schedule.

Occasional-DVM was defined as the migrating schedule that zooplankton perform DVM

after staying at the upper layer within a reproduction cycle. In our model, however, the fitness does not change between individuals performing DVM before and after staying at the upper layer, if number of days when they stay at the upper layer is the same (see equation (6)). Nevertheless, in natural populations, it is supposed that Daphnia perform DVM after staying at the upper layer in occasional-DVM, by the following reason which was not included in the model assumption. Daphnia accumulate lipid as energy for reproductive investment (Tessier and Goulden 1982). These lipids are combined with carotenoids, and therefore are colored (Green 1957). In addition, colored eyes appear in eggs in the brood porch with egg development (Threlkeld 1979). Therefore, individuals with colored lipid and developed eggs during the latter period of a reproduction cycle are more visible than individuals during the earlier period of the reproduction cycle. Generally, transparency and color of zooplankton body affect on visibility for predator (Zaret and Kerfoot 1975; Kerfoot 1985). Thus, predation pressure for individuals are larger during latter period than earlier period of the reproduction cycle. This difference in predation pressure between the periods implies that individuals should stay at the upper layer during earlier period of the reproduction cycle in occasional-DVM. This is consistent with an observation by Sekino and Yoshioka (1995) that Daphnia with early stage eggs stayed at the upper layer throughout a day, while Daphnia with late stage eggs performed DVM. It is also easily expected that if the model is modified so that the survival probability decreases more strongly in s_s than s_m as reproduction cycle proceeds, the above order of migrating behavior becomes optimal.

In our model, the period of a reproduction cycle (T) is assumed to be constant, and does not change depending on migrating behaviors. Decrease in water temperature actually causes an increase in the period of a reproduction cycle. Therefore, migrating zooplankton which stay at the cool deep layer during daytime have longer period of a reproduction cycle than

zooplankton which stay at the warm upper layer throughout a day (Orcutt and Porter 1983; Ringelberg et al. 1991). How does the result of our mode change if this factor is incorporated? Suppose that the reproduction cycle of migrating Daphnia is 8 days and that of staying Daphnia at the upper layer is 3 days. In this case, the boundary between the region where continuous-DVM and occasional-DVM are optimal will be almost the same as the boundary in fig. 3b, because Daphnia with occasional-DVM, near the boundary, have also a reproduction period near $T = 8$. By the same reason, the boundary between the regions where non-DVM and occasional-DVM are optimal will be almost the same as the boundary in fig. 3a where $T = 3$. Since, as we have already noted, the former boundary scarcely changes between fig. 3a and fig. 3b, the pattern of optimal migrating behavior in the $(r_m / r_s, s_m / s_s)$ space in this case is almost the same as that in fig. 3a. Thus, we can approximately apply the result of our model with constant reproduction cycle for the case where migrating Daphnia require a longer reproduction period.

Ratio of reproductive values of the given maternal individual to a newly produced egg (α / β) strongly affected the range of condition where each migrating schedule is optimum: as seen in equation (2) and (3), decrease in the survival probability of a juvenile during maturation (M) and the survival probability of an egg in the brood porch (S') enhances DVM (fig. 4). Zaret and Suffern (1976)suggested that zooplankton perform DVM when the survival rate decreases with increasing predation pressure. This suggestion referred to the direct predation pressure on individuals which are represented by s_m and s_s in our model. Since M and S' are the expected survival probabilities after this reproduction cycle, the optimal migrating behavior is also affected by future predation pressure. Thus, zooplankton may behave more adaptively if they can estimate future predation pressure.

Effect of α / β on migrating schedule suggests that increase in the number of eggs in the

brood porch (N) also enhances DVM (see equation (2) and (3)). It is known that Daphnia with more eggs in brood porch is more vulnerable to predation by fish (Tucker and Woolpy 1984; O'Brien 1987). Therefore, it is considered that increasing egg number in brood porch enhance DVM according to increasing predation pressure. The present model, however, showed that changes in N affects on the migrating behavior without affecting directly on survival rates of Daphnia. They can obtain more fitness through DVM when they carry more eggs because zooplankton protect their existent eggs from predators.

In the previous studies, migrating behavior of zooplankton has been explained by the trade-off between avoidance from visually searching predators and food gain (Johnsen and Jakobsen 1987; Lampert 1989). Our model explains this mechanism by changes in s_m / s_s and r_m / r_s which relate to vertical difference in predation pressure and food abundance, respectively. In addition, our model demonstrates that not only relative food abundance at the upper layer but also absolute food abundance under constant r_m / r_s enhances staying at the upper layer throughout a day (fig. 5). However, some previous studies demonstrate contrarily that decreasing in absolute food abundance reduces DVM (Huntley and Brooks 1982; Johnsen and Jakobsen 1987). According to these studies, zooplankton cease DVM at low food abundance to avoid starvation even under a high predation pressure. However, our model assumed that Daphnia are not starved, and invest net energy gain for reproductive investment. Therefore, at high food abundance, energy gain by staying at the upper layer exceeds the mortality loss. The difference in the present and previous studies suggests that the influence of absolute food abundance on migrating behavior differs between starved and unstarved conditions.

In conclusion, difference in migrating behavior of Daphnia can be explained by behavioral changes depending on the amount of accumulated energy. This conclusion implies that

nutritional status of zooplankton individuals are important to determine their migrating behavior. Nutritional status has scarcely been studied as the factor of migrating behavior, though nutritional status as a result of the migrating behavior has been studied (Guisande et al. 1991; Duncan et al. 1993). Our model considers such internal condition, and therefore, can provides a theoretical base which explains various types of migrating behavior with neither genetic difference nor fluctuation of environmental conditions.

ACKNOWLEDGMENTS

We wish to thank Drs. J. Urabe and M. Nakanishi for their helpful comments.

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Table 1. Glossary of symbols

Symbol	Interpretation	Unit
(Variables)		
i	number of days for staying at the upper layer	days
W	reproductive fitness over a given reproduction cycle	
W_i	reproductive fitness for i days staying at the upper layer during T days of reproduction cycle	
S	survival rate over a given reproduction cycle	$cycle^{-1}$
R	amount of accumulated energy over a given reproduction cycle	$J \cdot cycle^{-1}$
(Parameters)		
s_s	daily survival rate for staying at the upper layer	day^{-1}
s_m	daily survival rate for DVM	day^{-1}
r_s	daily amount of accumulated energy for staying at the upper layer	$J \cdot day^{-1}$
r_m	daily amount of accumulated energy for DVM	$J \cdot day^{-1}$
T	number of days for a reproduction cycle	days
α	reproductive value of a given maternal individual	
β	reproductive value of a newly produced egg	
N	number of eggs in brood porch	eggs
M	survival probability of a juvenile during maturation	
S'	survival probability of an egg in brood porch during development	
p	number of eggs produced by unit energy	$eggs \cdot J^{-1}$

FIGURE LEGENDS

Fig. 1. - A reproduction cycle of Daphnia. 1. A maternal Daphnia produces eggs from accumulated energy, and moves the newly produced eggs into the brood porch in its back. 2. The Daphnia develops the eggs in the brood porch, and accumulates energy for the next reproductive investment. 3. The Daphnia releases eggs at the brood porch.

Fig. 2. - The number of days for the staying at upper layer (i) to maximize the fitness (W_i) illustrated as regions in the space of the ratio of survival rates (s_m / s_s) and the ratio of daily amounts of energy accumulation (r_m / r_s). T, pr_s and α / β are set at 5, 3 and 25, respectively.

Fig. 3a, b - Optimum migrating schedule in the space of the ratios of s_m / s_s and r_m / r_s . Left panel (a), $T = 3$; Right panel (b), $T = 8$. pr_s and α / β are set at 3 and 25, respectively.

Fig. 4a, b - Optimum migrating schedule in the space of the ratios of s_m / s_s and r_m / r_s . Left panel (a), $\alpha / \beta = 10$; Right panel (b), $\alpha / \beta = 50$. T and pr_s are set at 5 and 3, respectively.

Fig. 5a, b - Optimum migrating schedule in the space of the ratios of s_m / s_s and r_m / r_s . Left panel (a) , $pr_s = 1$; Right panel (b) , $pr_s = 5$. T and α / β are set at 5 and 25, respectively.

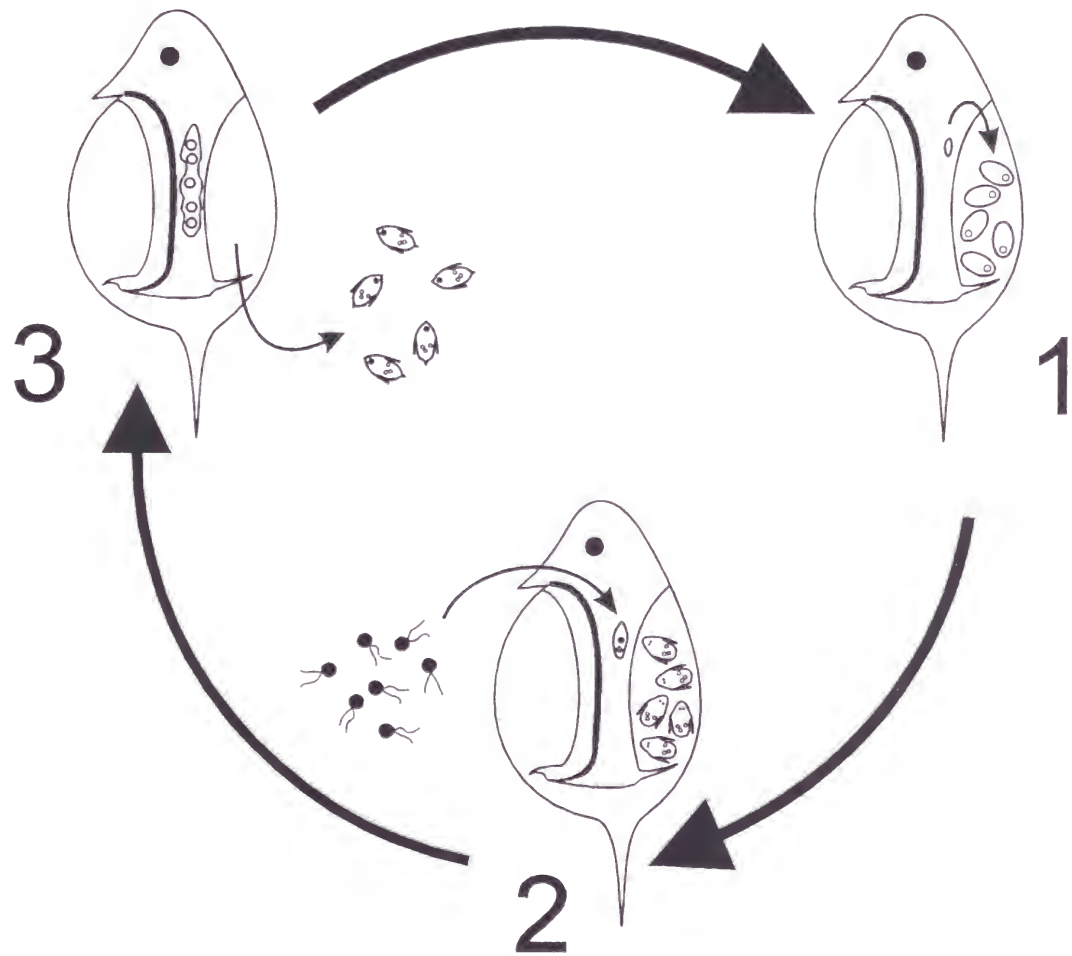

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Fig. 1

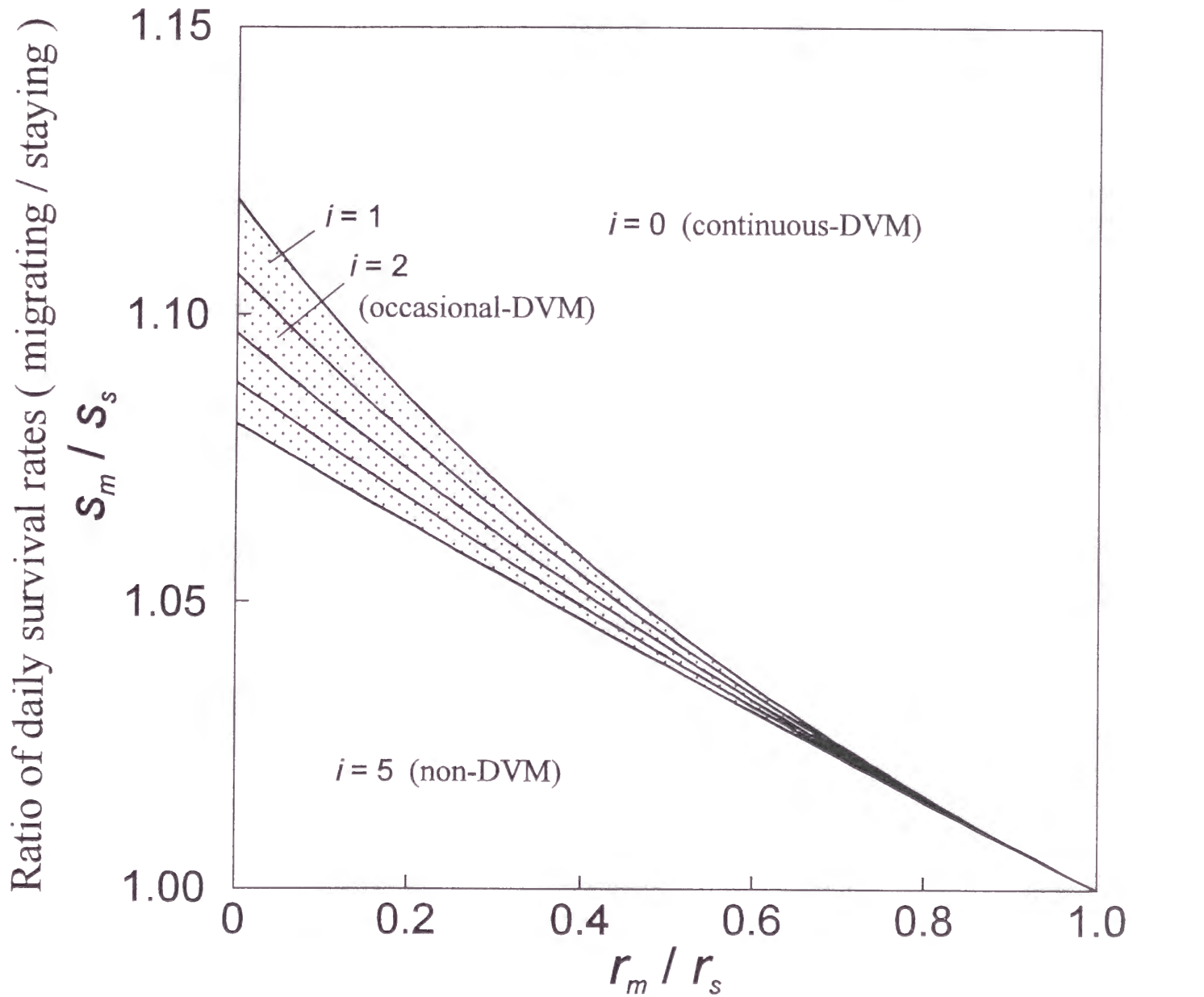

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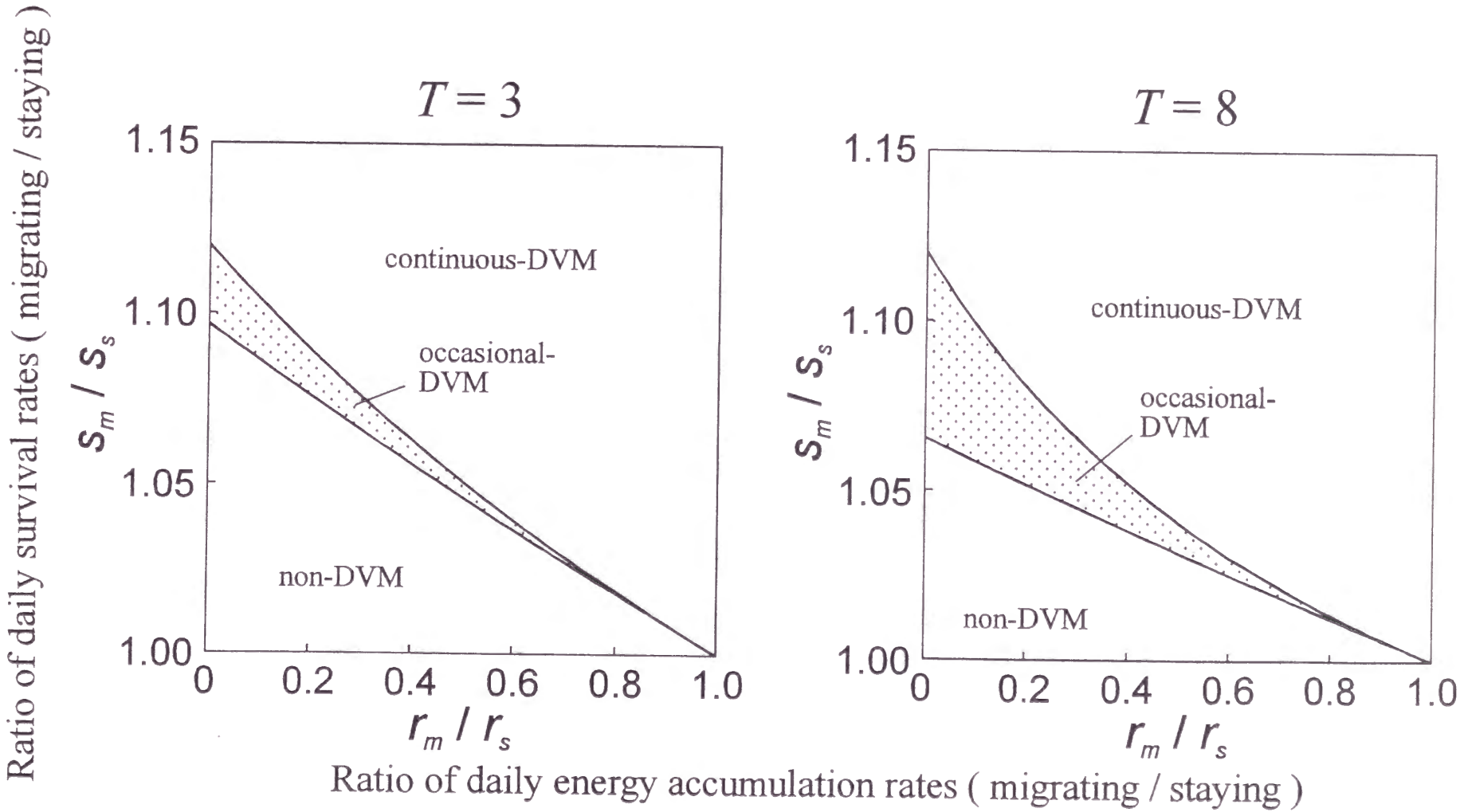
fig. 2



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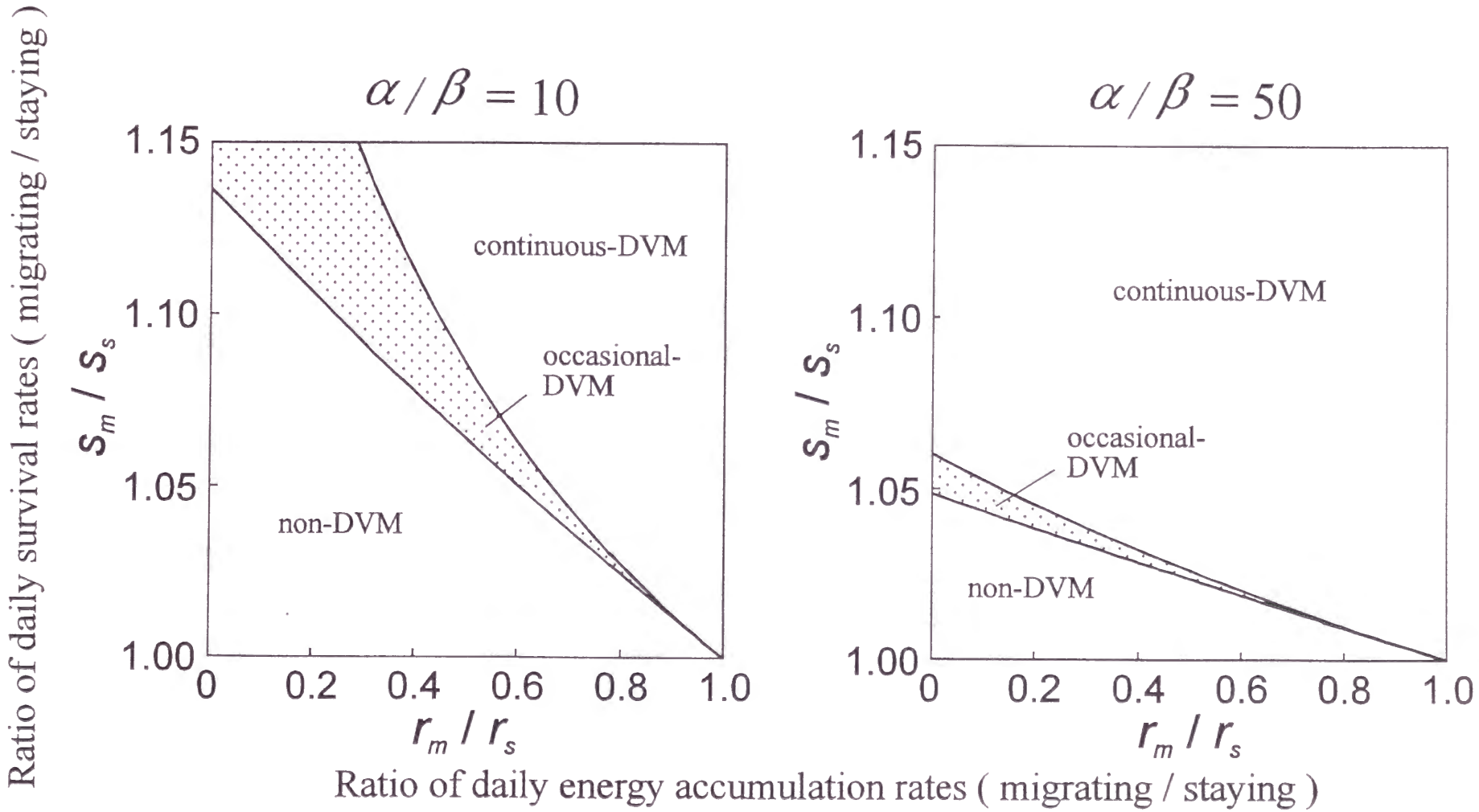

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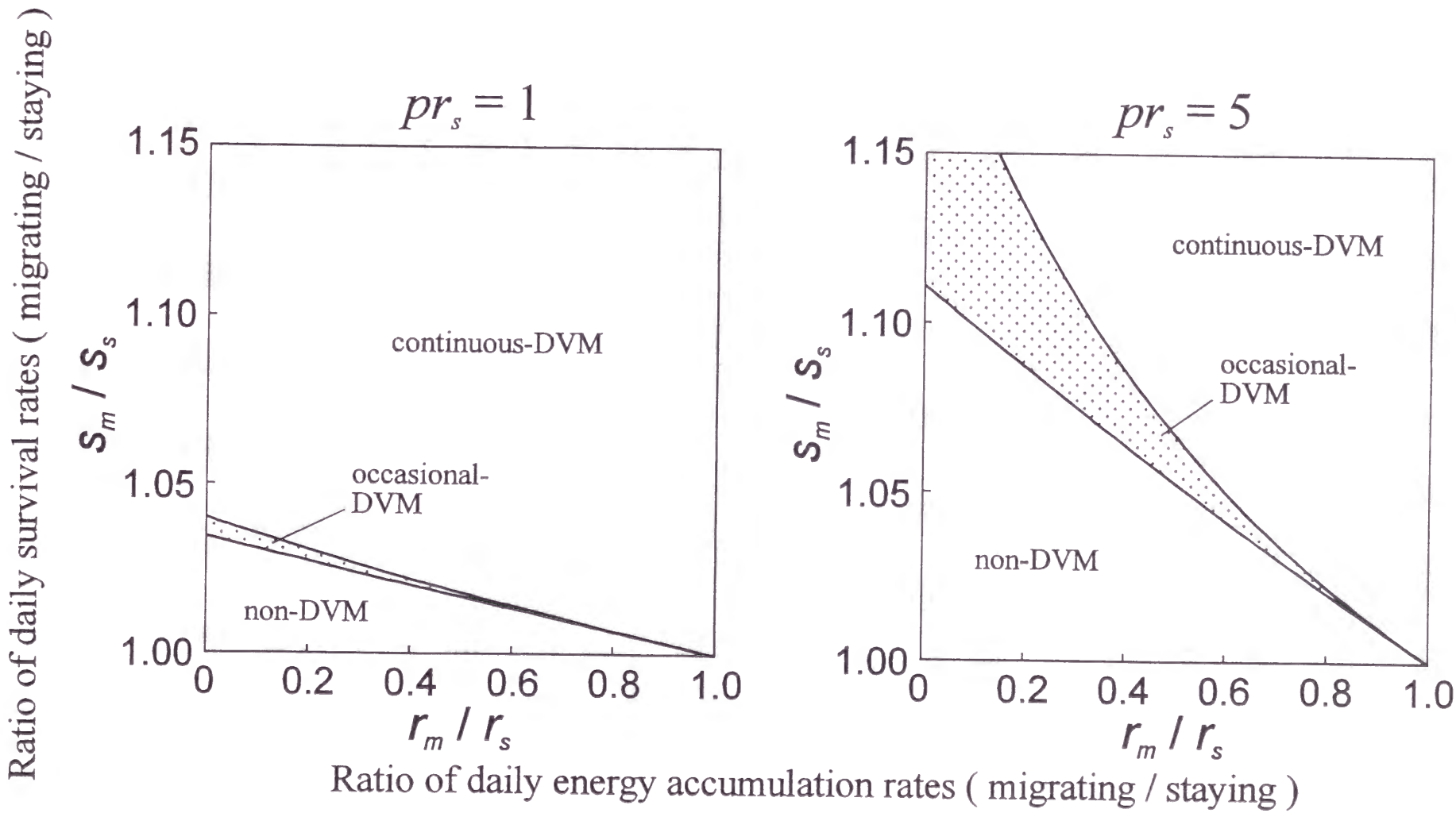

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fig 5

Seasonal changes in vertical distribution of Daphnia galeata: Effects of migration and food conditions

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Key Words: population dynamics, Daphnia galeata,

Running Head: Vertical distribution

Abstract

Daphnia galeata population in Lake Biwa was examined to clarify factors inducing seasonal changes in vertical distribution. Birth and death rate of D. galeata were estimated at each depth and compared with vertical changes in individual density. The analyses revealed that vertical distribution of D. galeata was changed by vertical difference in birth rate when their nutritional status was high, while that was changed by migration when their nutritional status was low. Thus, seasonal change in vertical distribution of zooplankton is caused by migration and vertical difference in birth or death rate. Daphnia increase their density at depths where food is abundant, but they migrate and dispersed to search for food vertically, when food is depleted.

Zooplankton change their vertical distribution diurnally and seasonally. Diurnal changes in vertical distribution of zooplankton, which is called diel vertical migration, are explained by avoidance from visually searching predators at daytime in the deeper layer and migration to the upper layer to obtain food at nighttime (Zaret and Suffern 1976, Stich and Lampert 1981 and Wright et al. 1980). Compared to diurnal changes, seasonal changes in vertical distribution of zooplankton have been less focused. A limited number of studies, however, suggested that these are relate with various environmental factors such as food abundance (Leibold 1990, Cummigel 1983, George 1983, Sameoto 1984 and Paffenhöfer 1983), predation pressure (Leibold 1990 and Mclvilleal and Maly 1981), water temperature (Sameoto 1985 and Castro et al. 1991) and dissolved oxygen (Horppila 1997, Sameoto 1986 and Weikert 1982). These studies were made by comparing simply patterns of vertical distribution of zooplankton with environmental factors.

However, it should be noted that vertical distribution of zooplankton is determined by two immediate causes. It is considered that 1) immigration to and emigration from certain depths; 2) vertical differences in birth and death rates .Seasonal changes in vertical distribution induced by immigration and emigration are a response to on present environmental condition, while those induced by vertical difference in birth and death rates are a result of past environmental condition. Thus, the two causes should be examined separately to clarify environmental factors inducing seasonal changes in vertical distribution of zooplankton . However, previous studies have not separated these two causes, and therefore, not been sufficient to examine the relationship between the vertical distribution and environmental factors.

In the present study, I estimated depth-specific birth and death rates of Daphnia population and their vertical migration in Lake Biwa. The estimated rates were used to clarify why they seasonally change their vertical distribution.

Methods

Lake Biwa is located in the center of Honshu Island in Japan at an altitude of 85 m, and is 674.4 km^2 in surface area and 104.0 m in maximum depth (Horie 1984). Samplings were carried out biweekly except for winter during the period from April 1994 to March 1995 at the station in the north basin (35°10'30"N, 135°56'30"E, 43m deep). In winter time (form Jan. to Mar.), samplings were made monthly.

At sampling, zooplankton were collected in the morning (from 10:00 to 12:00) from 0m to 42m deep at 3 m intervals with a Schindler zooplankton sampler (10L, 100μm mesh). The samples were fixed with 5% sugar formalin (Haney and Hall 1973), and were counted under a microscope. At the same time of zooplankton sampling, lake water for measurement of chlorophyll a concentration were collected from 0, 5, 10, 15, 20, 30 and 40 m depth with a modified 10 L Van-Dorn water sampler. Since crustacean zooplankton in Lake Biwa ingest food particles smaller than 20μm (Okamoto 1984 and Urabe et al. 1996), water samples were passed through a 20-μm plankton net, and aliquots of its filtrate were filtered onto Whatman GF/F filters for determining chlorophyll a concentration in the < 20 μm size fraction. Chlorophyll a concentration were determined fluorometrically by the method of Lorenzen (1967). Vertical profile of water temperature was measured with a thermistor thermometer (Tohodentan, RB-2). A part from these sampling, Daphnia individuals werer collected by vertical tows from bottom to surface with a plankton-net (200 μm mesh; 30 cm diameter) These individuals were used to measure lipid index (Goulden and Hornig 1980). When more than 50 individuals of Daphnia could be collected for this measurement, an average of lipid index was used for the following analysis.

To analyze dynamics of the Daphnia population, average population density in the water column was calculated from population density at each depth by the following formula.

$$X = \frac{1}{42}\sum_{i=0}^{39}\frac{(x_i + x_{i+3}) \times 3}{2} \qquad (i = 0, 3, \cdots, 39), \qquad (1)$$

where X is the average population density in the water column, and x_i is the population density at i m deep. Using this value, instantaneous change rate of population density in the water column (r, day^{-1}) between two sampling days (t_1 and t_2) was calculated according to Edomondson 1960:

$$r = \frac{\ln(X_{t_2}) - \ln(X_{t_1})}{t_2 - t_1} \qquad (2)$$

where X_{t_1} and X_{t_2} are average population density in the water column at time t_1 and t_2. Instantaneous birth rate in the water column (b, day^{-1}) was calculated from instantaneous birth rate at each layer 3 m intervals. Egg development time of the layer from i to i+3 m depth (D_{i-i+3}, days) was estimated from average water temperature between i and i+3 m depth (T_{i-i+3}) by

$$\ln(D_{i-i+3}) = 3.3956 + 0.2193\ln(T_{i-i+3}) - 0.3414(\ln(T_{i-i+3}))^2$$

$$(i = 0, 3, \cdots, 39) \qquad (3)$$

(Bottrell et al. 1976). Instantaneous birth rate of the layer from i to i+3 m deep (b_{i-i+3}, day^{-1}) was calculated from average of egg ratio between i and i+3 m depth (E_{i-i+3}, eggs · individual^{-1}) and the estimated egg development time of the layer (D_{i-i+3}) according to the egg ratio method:

$$b_{i-i+3} = \frac{\ln(E_{i-i+3} + 1)}{D_{i-i+3}} \qquad (i = 0, 3, \cdots, 39) \qquad (4)$$

(Edmondson 1968, 1971 and Paloheimo 1974). If no mortality is assumed, population density of the layer between i and i+3 m at t_2 ($x_{t_2, i-i+3}$) is estimated as

$$x_{t_2,i-i+3} = x_{t_1,i-i+3} \times \exp(b_{t_1,i-i+3} \cdot (t_2 - t_1)) \qquad (i = 0, 3, \cdots, 39), \qquad (5)$$

where $x_{t_1,i-i+3}$ and $b_{t_1,i-i+3}$ are population density and instantaneous birth rate at time t_1 estimated by formula (4). Therefore, average population density in the water column at t_2 ($\hat{X}_{t_2}$) is expected as

$$\hat{X}_{t_2} = \frac{1}{14}\sum_{i=0}^{39} x_{t_2,i-i+3} \qquad (i = 0, 3, \cdots, 39), \qquad (6)$$

Using this value, instantaneous birth in the water column (b, day^{-1}) between two sampling days (t_1 and t_2) was estimated as

$$b = \frac{\ln(\hat{X}_{t_2}) - \ln(X_{t_1})}{t_2 - t_1}, \qquad (7)$$

where X_{t_1} is average population density in the water column at t_1 calculated by formula (1). Average death rate in the water column (d, day^{-1}) between two sampling days is estimated by:

$$d = b - r \qquad (8)$$

(Edmondson 1968, 1971).

Instantaneous birth and death rates of Daphnia at each depth were also calculated for analyzing vertical difference in demographic parameters. Moving average of population density and egg ratio were used for the calculation to reduce influence of random error. Instantaneous birth rate at each depth is calculated from egg ratio and egg development time estimated from water temperature at each depth (Bottrell et al. 1976) according to egg ratio method (Edmondson 1968, 1971 and Paloheimo 1974). Instantaneous growth rate at each depth was calculated from population density at each depth according to Edomondson 1960. Using calculated instantaneous birth and growth rates at each depth, instantaneous death rate at each depth was estimated (Edmondson 1968, 1971). This analysis was carried out for data series when population density was (more than 0.2 in the average in the water column).

The immigration ans emigration within water column weredetermined from the death rate at each depth. When the estimated death rate is less than 0, the growth rate of the population is larger than estimated the birth rate. The negative death rate is caused by immigration from the different depth or recruitment from resting eggs (DeBernardi 1974 and Lampert 1978). If recruitment from resting eggs can be neglected, the negative death ratecan be used as an index of immigration from the different depth.

Influence of water temperature on vertical distribution of Daphnia was tested statistically. If Daphnia is distributed according to water temperature, Daphnia should be distributed at the depths with the same water temperature between two sampling days, even though distribution depth is changed. In the present study, I also estimated Daphnia density in the layers which are divided vertically according to water temperature of distributed depth at 2 °C intervals. Distributions of Daphnia in each layer with the same water temperature were compared for successive sampling date by Kolmogorv-Smirnov Test (Sokal and Rohlf 1995).

Results

Environmental condition

During the study period, thermocline was formed around 10-15 m depth from June to September (Fig. 1). Thereafter, the thermocline gradually shifted toward the deeper layer, and then, disappeared in December. Chlorophyll a concentration in the < 20 μm size fraction showed three peaks during the investigation period (Fig. 1). Chlorophyll a increased in early July, and reached to peak with density of 5.0 $\mu g \cdot L^{-1}$ at 15 m depth in late June. The next peak was at the thermocline in August with density of 5.3 $\mu g \cdot L^{-1}$. This second chlorophyll a peak gradually declined toward the deeper layer, and disappeared in October. The third peak of chlorophyll a formed above the thermocline from September to December, and extended to deeper layer along the thermocline. Comparing the first and the second chlorophyll a peak, the

third chlorophyll a peak was small, and was less than 2.5 $\mu g \cdot L^{-1}$.

Population dynamics of Daphnia population

Daphnia population density showed three peaks during the study period (Fig. 2). Daphnia population density increased from mid June but decreased immediately after the population density reached to a peak with density of 6.2 individuals $\cdot L^{-1}$ in early August. In mid September, Daphnia population density increased and formed second peak with 28.2 individuals $\cdot L^{-1}$ in late October. Thereafter, the population density once decreased. However, it recovered again fin early November, and reached to 11.8 individuals $\cdot L^{-1}$ in early December. The Daphnia population was low in winter (form December to March).

Seasonal changes in average instantaneous birth and death rates with through water column are shown in figure 3. The birth rate increased rapidly from early June, and reached to the maximum, being 0.22. After rapid decrease, the birth rate increased again from early July and reached to 0.09 in late August. After September, the birth rate decreased gradually, and was less than 0.01 in December. Similar seasonal trends were found in lipid index (Fig. 4). Indeed, birth rate of Daphnia population significantly correlated with the lipid index (Kendall's rank correlation, $p < 0.01$). However, the birth rate did not correlate the average water temperature which was weighted according to density of Daphnia at each depth (Kendall's rank correlation, $p > 0.05$).

Average of instantaneous death rate of Daphnia population through water column showed three peaks during the study period (Fig. 3). The death rate was higher than 0.10 in mid June, and late July, and decreased from August to September. In November and December, the death rate was also high but less than 0.10. The high death rate in June was apparently due to food limitation, because lipid index showed high values. However, second (June) and third (November) peaks in death rate were appeared when lipid index was low.

Seasonal change in vertical distribution

Corresponding to the seasonal changes in population density (Fig. 2), Daphnia formed several assemblages with distinct vertical distribution (Fig. 5). From June to August, Daphnia assemblage was found in layer from 10 m to 20 m depth (Fig. 2). The maximum density of this assemblage was 30.6 individuals · L^{-1} at 15 m deep in late July. At this time, other assemblages of Daphnia were found at 25 m deep and at near the surface in early August. In late August, Daphnia seemed to assemble around 20 m depth. This assemblage moved toward the deeper layer during September, and then disappeared in late September. Corresponding to the increase in population density in October (Fig. 2), Daphnia formed two different assemblages above and below 15 m depth. The maximum densities were 176.0 individuals · L^{-1} in the upper assemblage and 24.4 individuals · L^{-1} in the lower assemblages. In November, an other assemblage was appeared at 10 m depth, moved toward deeper depth in late November and. in late December .

Water temperature did not correlate with seasonal change in vertical distribution of Daphnia after late June when the thermocline was developed (Kolmpogorv-Smirnov test, $p < 0.05$). However, vertical changes in Daphnia density correlated with chlorophyll a concentration in summer (Table 1).

Causes for seasonal changes in vertical distribution

Instantaneous birth and death rates at each depth were estimated during the period from mid June to December when average population density of Daphnia in water column was more than 0.2 individuals · L^{-1} (Fig. 6). The birth rate was high at the depth from 10 m to 25 m in June, and correlated with individual density at each depth in next sampling date (Table 2). Similarly, the death rate was also high at depths where Daphnia was abundant. Thus, relatively

high density of Daphnia around 15 m was caused by high birth rate. In mid July, the death rate was smaller than 0 at depth from 10 m to 30 m. This negative value means that Daphnia immigrated to these depths during the period from mid to late July. In the same way, the negative death rate at depths from 3 m and 10 m deep in late July means that Daphnia immigrated to these depths during the period from late July to early August. During the period from mid July to early August, most Daphnia individual moved from 10 m to 15 m, but some individuals moved to near the surface. These results indicate that changes in vertical distribution were due to immigration of Daphnia.

In early August, the birth rate was high around 15 m, and correlated with individual density in next sampling date (Table 2). However, the death rate had small vertical change, and was not smaller than 0 during August. This result indicates that high density of Daphnia individuals around 15 m deep in mid August was caused by vertical changes birth rate caused, but was not related with vertical changes in death rate and immigration. In the term from early to mid September, most Daphnia individuals moved to about 20m when the negative death rate was found around 20 m deep. This result indicates that vertical distribution of Daphnia was changed by immigration to 20 m deep.

In early October, the birth rate was not high in whole of the water column, and did not correlate with Daphnia density on next sampling date (Table 2). However, the negative death rate was found above 15 m deep in late September and above 30m deep in early October. These results indicate that the the high density of Daphnia individuals above and below 15 m in early October was not formed by vertical difference in the birth rate, but was caused by vertical migration.

During the term from late October to early November, the birth rate was high above 20 m, and correlated with vertical distribution of Daphnia on next sampling date (Table 2). Nevertheless, the death rate was not smaller than 0 in whole of water column in late October.

These results indicate that high density of Daphnia individuals around 10 m in November was caused by vertical difference in birth rate, but was not caused by vertical difference in the death rate and immigration. However, In mid November, the negative death rate was found from 20 m and 30 m. This result indicates that extending of Daphnia toward to the deeper layer in late November was caused by immigration.

Discussion

In the present study, cause of seasonal changes in vertical distribution was separated into spatial heterogeneity in demographic parameters and seasonal vertical migration. Assuming that Daphnia emigrated always from depth with the maximum population density of that, seasonal changes in vertical distribution of Daphnia can be illustrated in Fig. 7. The figure shows that vertical difference in birth rate and seasonal vertical migration alternately influence on vertical distribution of Daphnia.

Although migrations of Daphnia between depths were estimated based on immigration, individuals may immigrate form not only different depth but also by horizontally different site. However, most of the average death rate of Daphnia in the water column was more than 0 between June to December (Fig. 3). This result implies that population dynamics of Daphnia can be explained without immigration from horizontally different site. In the present analysis also assumed that recruitment originated from resting eggs was negligible. Since no Daphnia with resting eggs was found during the study period, this assumption seems to be appropriate.

Zooplankton can vertically migrate either by swimming or by water movement. If migration of Daphnia is due to water movement, changes in vertical distribution of Daphnia should correlate with vertical profile of water temperature, because water movement relates closely with vertical profile of water temperature (Wetzel 1983 and Goldman and Horne 1983). However, change in vertical distribution of Daphnia was not explained by vertical profile of

water temperature during the period from June to December. Therefore, this implies that seasonal vertical migrations found in the present study were due to swimming of Daphnia themselves.

Several factors can be considered to induce seasonal vertical migration of Daphnia. Many researchers examined influence of harmful UV light on vertical distribution of zooplankton (Bollons and Frost 1990, Williamson et al. 1994 and Hessen 1994). Unfortunately, measurements on UV light did not be made out in the present study. Nevertheless, although harmful UV light influence on zooplankton distributed shallower than at most 20 cm deep in natural lake (Zagarese 1994), Daphnia do not distribute such a shallow layer in Lake Biwa. Thus, effect of harmful UV light did not a factor to the seasonal vertical migration of Daphnia.

As diel vertical migration is caused by avoidance from predators (Zaret and Suffen 1976), the seasonal vertical migration of Daphnia may be also caused by the avoidance from predators. Although several species of fish in Lake Biwa prey on zooplankton (Nagoshi 1982), most of the species are not abundant except for sweetfish (Plecoglossus altivelis altivelis) (Nakanishi and Sekino 1996). Sweetfishes ingest zooplankton at the surface layer in pelagic area from late spring to summer (Azuma 1964). Therefore, predation by the sweetfishes may have influenced on population dynamics of Daphnia in the summer, when the death rate was high (Fig. 4) even though lipid index was high from June to July (Fig. 5). However, Daphnia distributed in the surface layer above 10 m deep in June, and migrated vertically to the surface layer in August (Fig. 7). Thus, it is difficult that avoidance from predators is a factor of the seasonal vertical migration of Daphnia. In addition, it is known that most of crustacean zooplankton in Lake Biwa did not show diel vertical migration (Nagoshi 1982, Okamoto 1984 and Kawabata 1987). This tendency implies that influence of predation pressure on zooplankton is little in Lake Biwa.

Vertical changes in individual density of Daphnia correlated significantly with chlorophyll a concentration in most date during the period from June to September (Table 1). In addition,

distribution of Daphnia extended toward the deeper layer when chlorophyll a was high even in the deeper layer in December. Such relationships between of Daphnia and chlorophyll a were found when vertical migrations were occurred (Fig. 8). Furthermore, lipid index of Daphnia was low when vertical distribution of Daphnia was changed by vertical migration (Fig. 3 and 7). Thus, poor food condition for Daphnia may stimulate changes in vertical migration. Swimming or migrating behavior of zooplankton are known to be changed according to food condition (Cudding and McCauley 1994, VanDuren and Videler 1995 Johnsen and Jacobsen 1987). It is supposed that when food condition deteriorated, Daphnia migrate vertically and dispersed in the water column to search food.

It is known that abundance of zooplankton influence on their own food condition, and often cause food limitation (Tessier et al. 1992 and Motchell and Wiliams 1982). The maximum population density was found when the lipid index was low (Fig. 2 and 4). Thus, it is likely that increase in Daphnia caused food limitation for themselves.

In summerize process of seasonal changes in vertical distribution of the Daphnia population is considered as follows. Birth rate of Daphnia increased at the depth where food patch was formed, so that Daphnia was assembled that depth (spatial heterogeneity in demographic parameters). However, as population density increase, food is depleted. Under such situation, Daphnia migrate vertically and dispersed in the water column to search for food. Among the dispersed Daphnia, a part of the individuals distributed at the depth where next food patch was formed could obtain enough food to colonize there. Birth rate of Daphnia increased again, so that a new assemblage of Daphnia was formed at that depth (spatial heterogeneity in demographic parameters).

Increase in population density after dispersion implied that a part of population is selected. Thus, this selection may induce changes in not only vertical distribution but also genetic structure of Daphnia population. However, such a process can not be noted at all by the

previous studies which examine correlation between environmental factors and vertical distribution of zooplankton. To understand more deeply the cause and effect of genetic and population structure of zooplankton, it should be examine seasonal changes in vertical distribution. Seasonal changes in vertical distribution of zooplankton should be examined, separating the two causes which are vertical differences in birth and death rates and vertical migration.

Acknowledgments

I wish to thank Drs. J. Urabe and M. Nakanishi for their helpful comments. I also grateful to Mr. T. Koitabashi and Mr. A. Kawabata for their kind assistance with sampling

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Figure legends

Fig. 1 Seasonal changes in vertical profiles of water temperature and chlorophyll a concentration in the < 20 μm size fraction.

Fig. 2 Seasonal changes in population density of D. galeata within water column.

Fig. 3 Seasonal changes in instantaneous birth and death rate of D. galeata population. The rates are average over water column (se text).

Fig. 4 Seasonal changes in mean lipid index of D. galeata population.

Fig. 5 Seasonal changes in vertical distribution of D. galeata.

Fig. 6 Vertical changes in instantaneous birth and death rate of D. galeata.

Fig. 7 Schematic diagram showing changes in vertical distribution of D. galeata in Lake Biwa. Red arrows, multiplication; Blue arrows, migration. Vertical distribution was changed by vertical difference in birth rate when the red arrows were shown, and was changed by migration when the blue arrows were shown.

Table 1

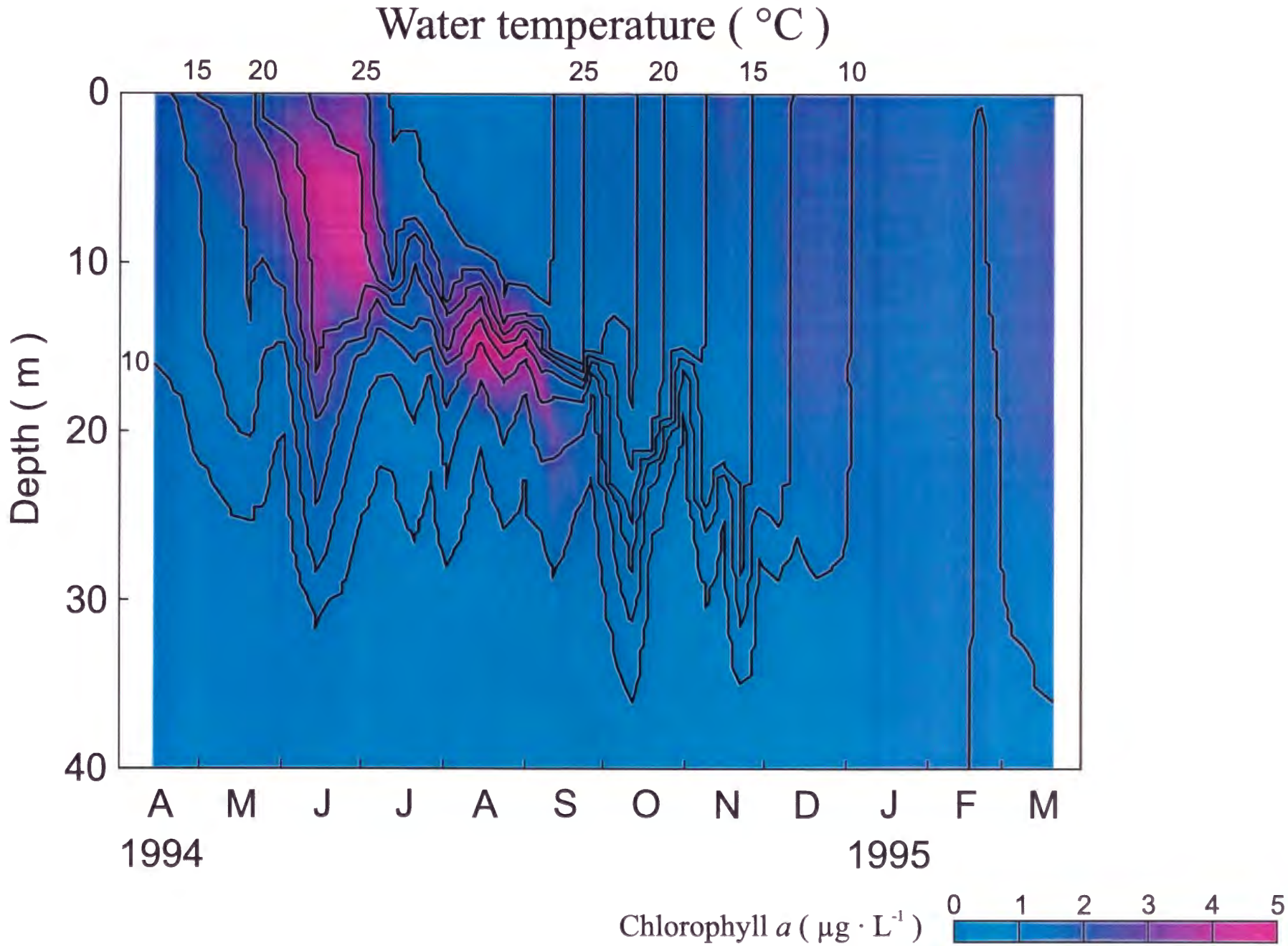
Correlation coefficient between daphnia density and Chlorophyll a concentration in < 20 μm size fraction at different depths.

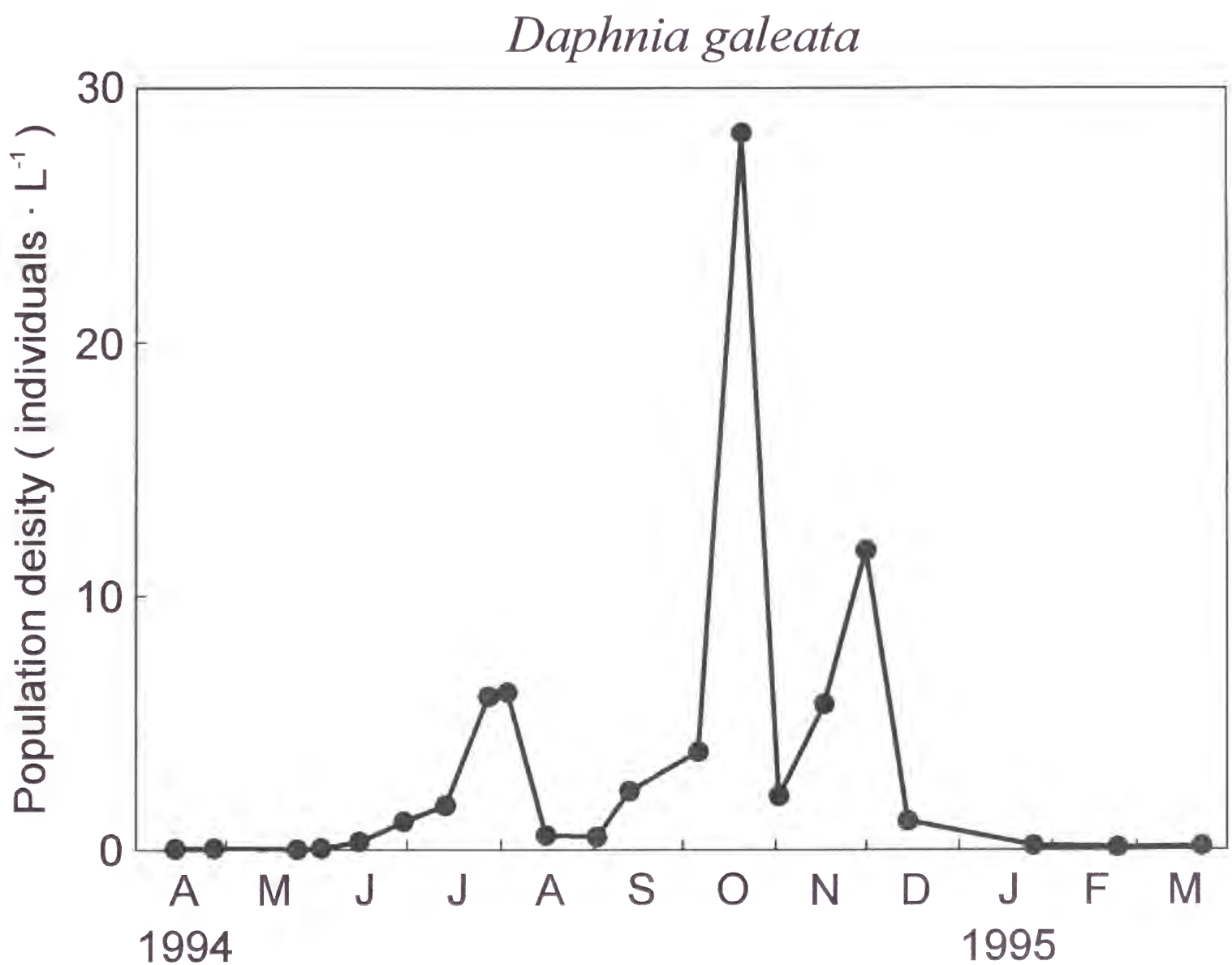
Date	Jun 15	Jun 30	Jul 14	Jul 28	Aug 3	Aug 16	Sep 2	Sep 13	Oct 6	Oct 20	Nov 2	Nov 17	Dec 1	Dec 15
r	0.488	0.787*	0.776*	0.699	0.892*	0.911**	0.968**	0.826*	0.265	0.402	0.616	0.212	0.429	0.403

Table 2

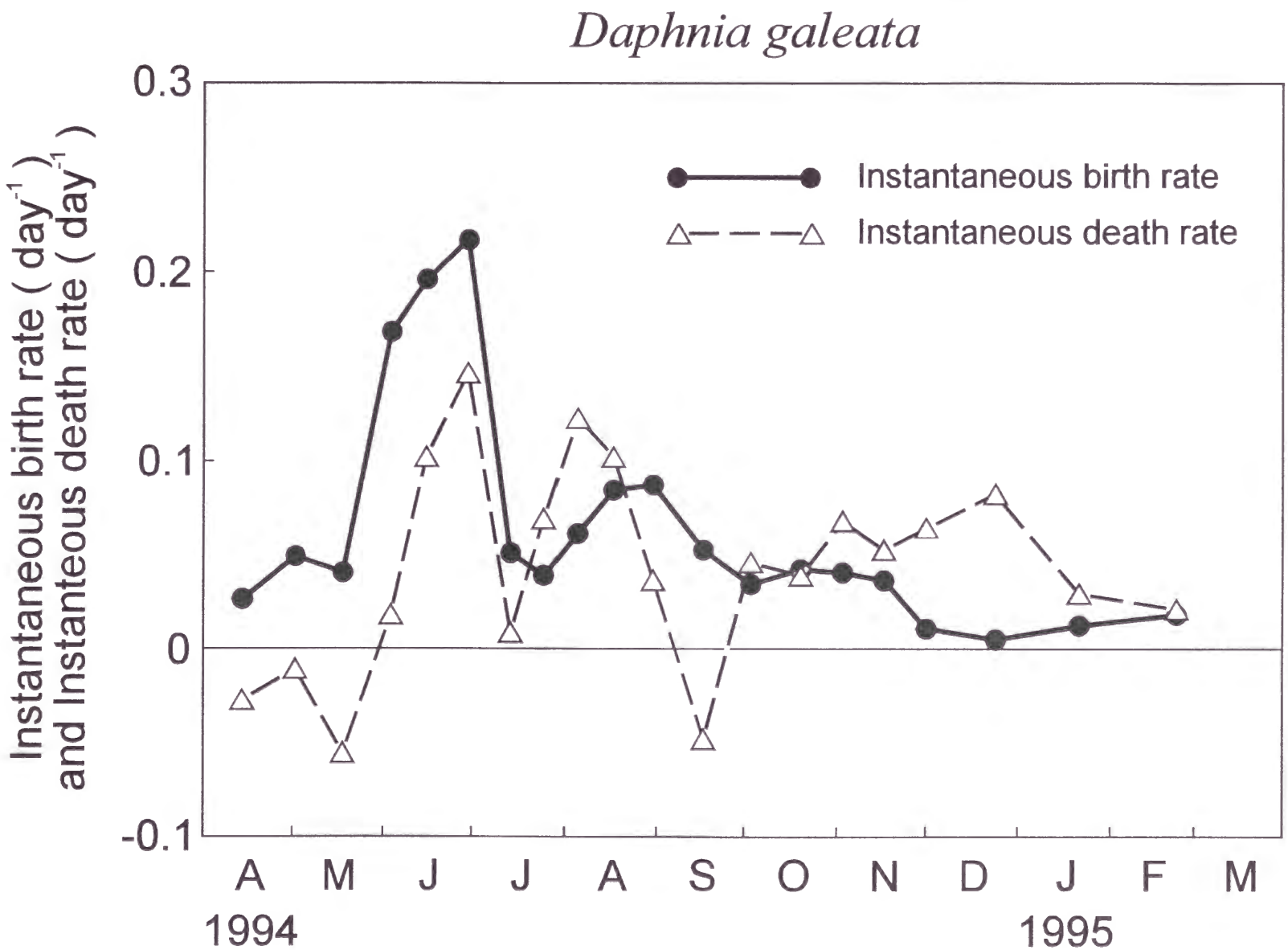
Correlation coefficient between instantaneous birth rate at each depth and individual density at next sampling date.

Date	Jun 15	Jun 30	Jul 14	Jul 28	Aug 3	Aug 16	Sep 2	Sep 13	Oct 6	Oct 20	Nov 2	Nov 17	Dec 1	Dec 15
τ	0.495*	0.419*	0.086	0.105	0.429*	0.238	0.219	0.000	-0.010	0.457*	0.410*	0.114	0.495*	0.086

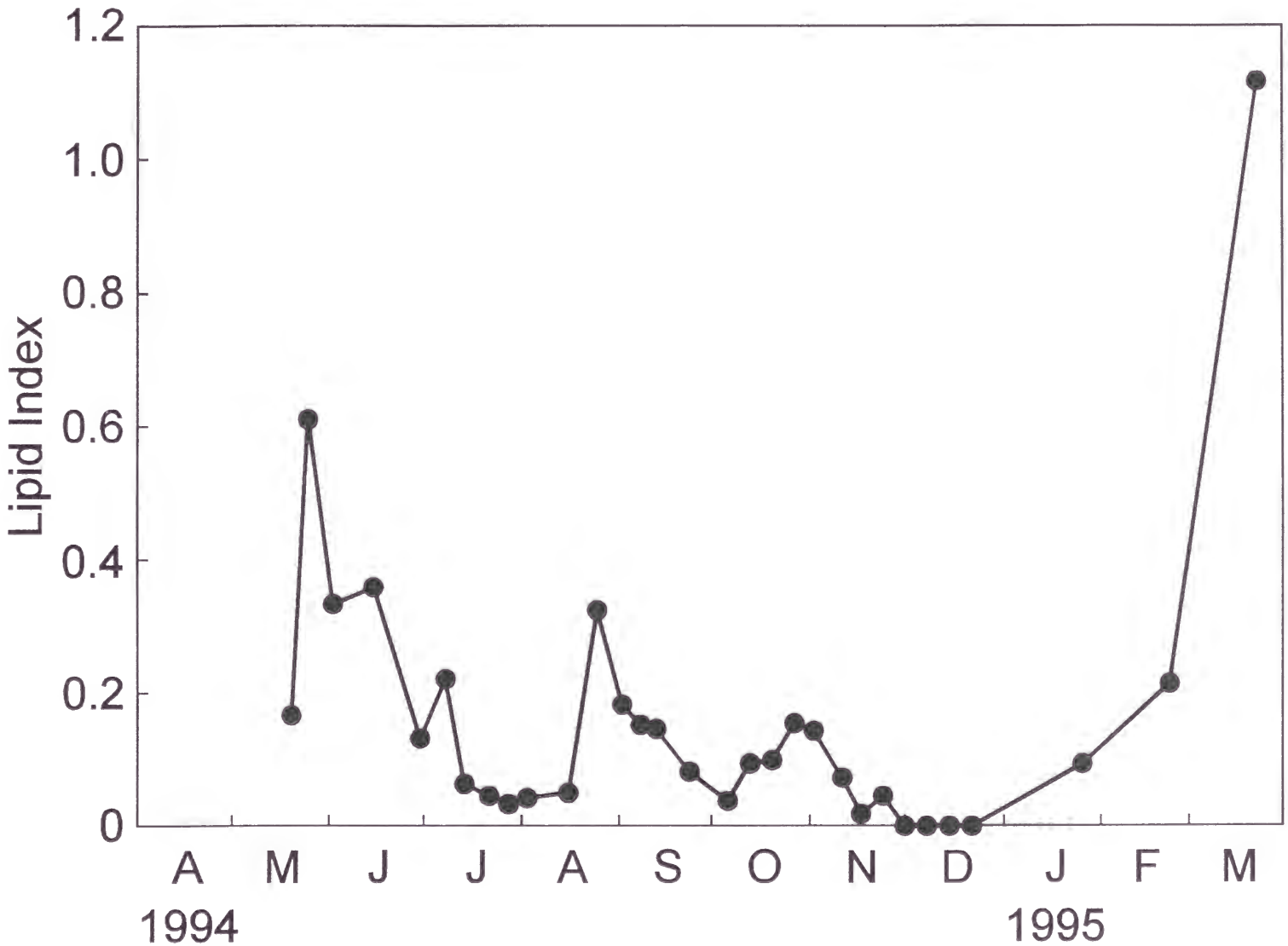

Water temperature (°C)
15
20
25
25
20
15
10
0
10
20
30
40
Depth (m)
10
A
M
J
J
A
S
O
N
D
J
F
M
1994
1995
Chlorophyll a (µg · L-1)
0
1
2
3
4
5


Daphnia galeata
Population deisity (individuals · L-1)
30
20
10
0
A
M
J
J
A
S
O
N
D
J
F
M
1994
1995

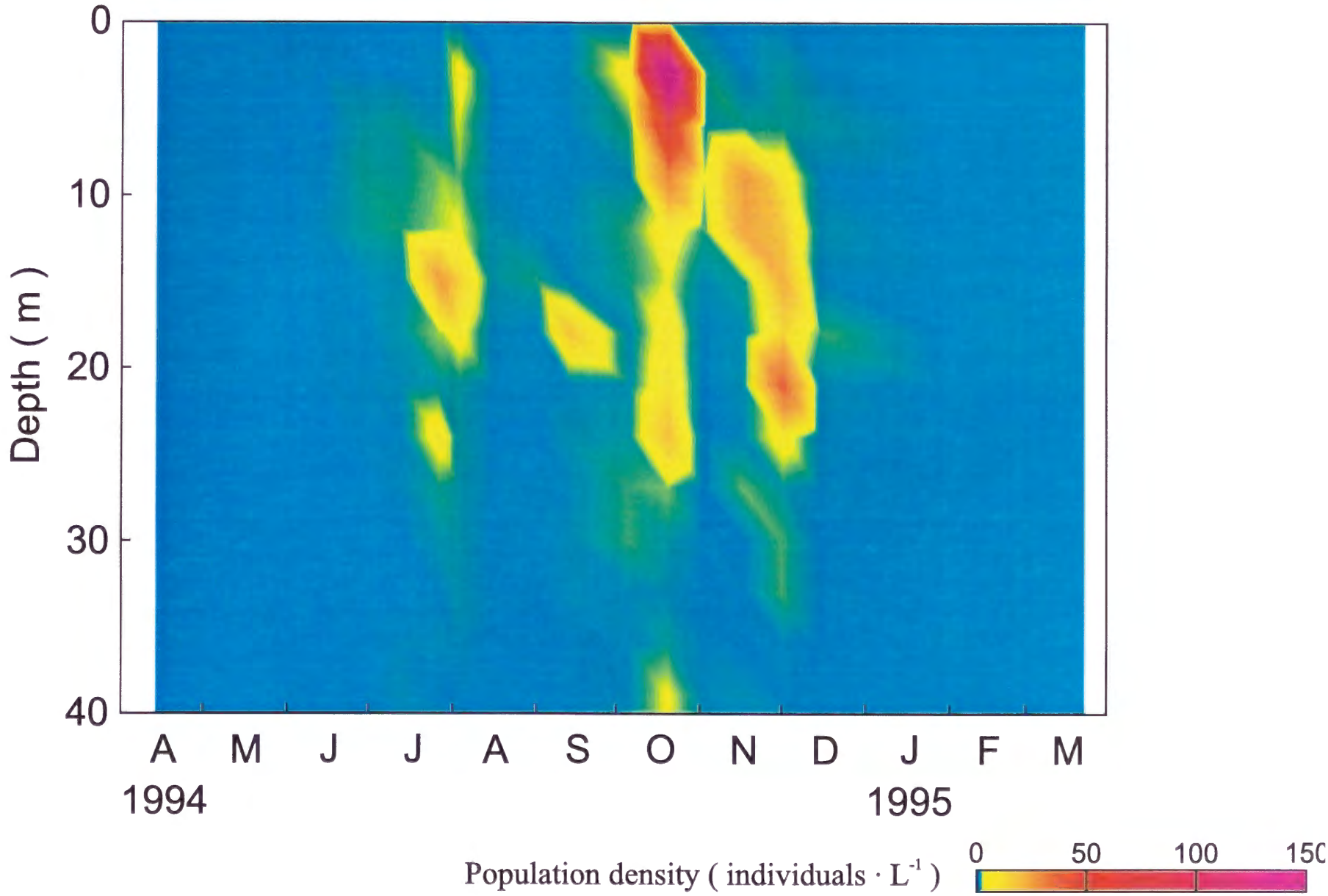
Tekino Fig 2

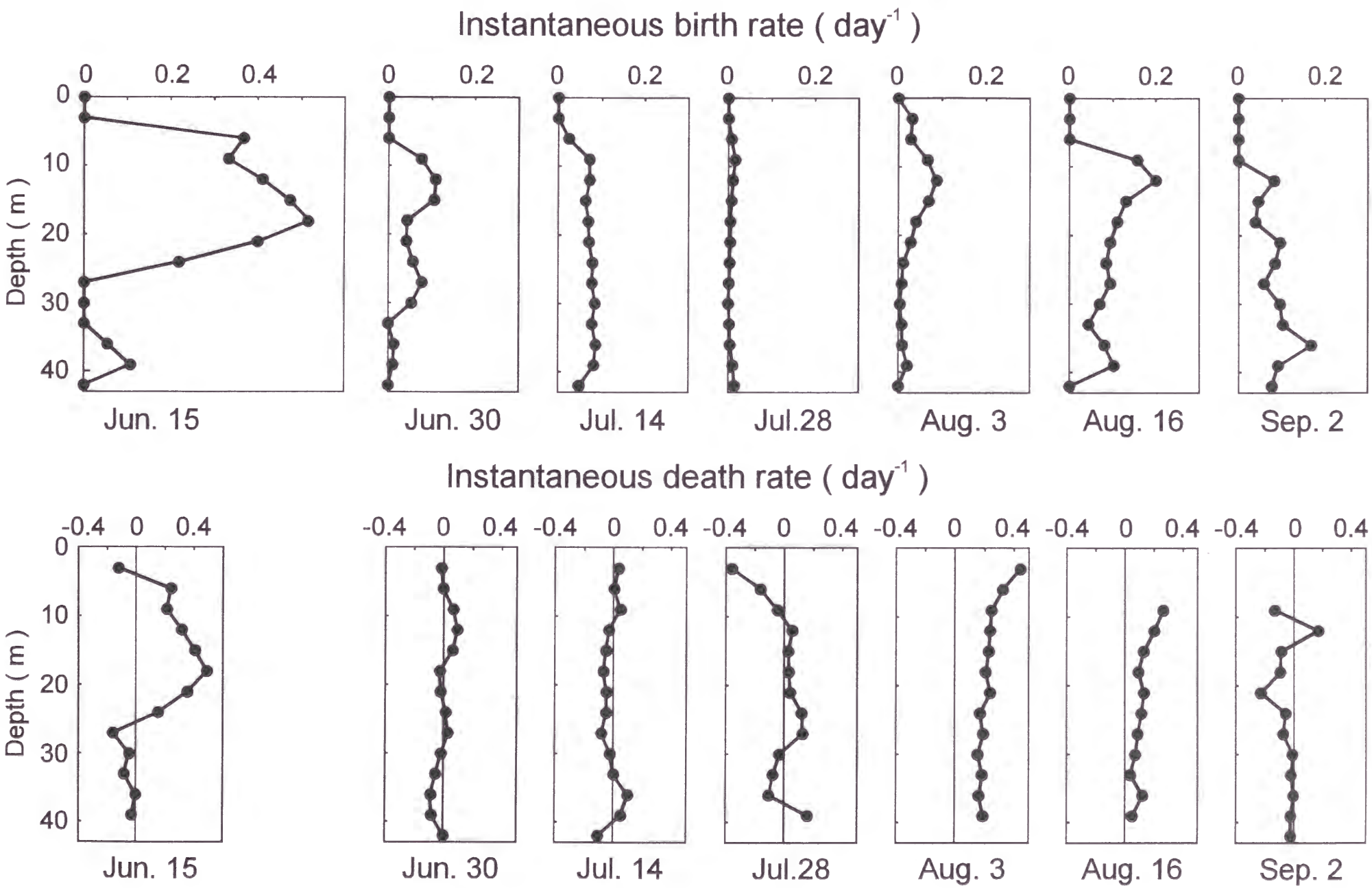

Daphnia galeata
Instantaneous birth rate (day-1) and Instanteous death rate (day-1)
0.3
0.2
0.1
0
-0.1
Instantaneous birth rate
Instantaneous death rate
A
M
J
J
A
S
O
N
D
J
F
M
1994
1995

Sekiko Fig 3

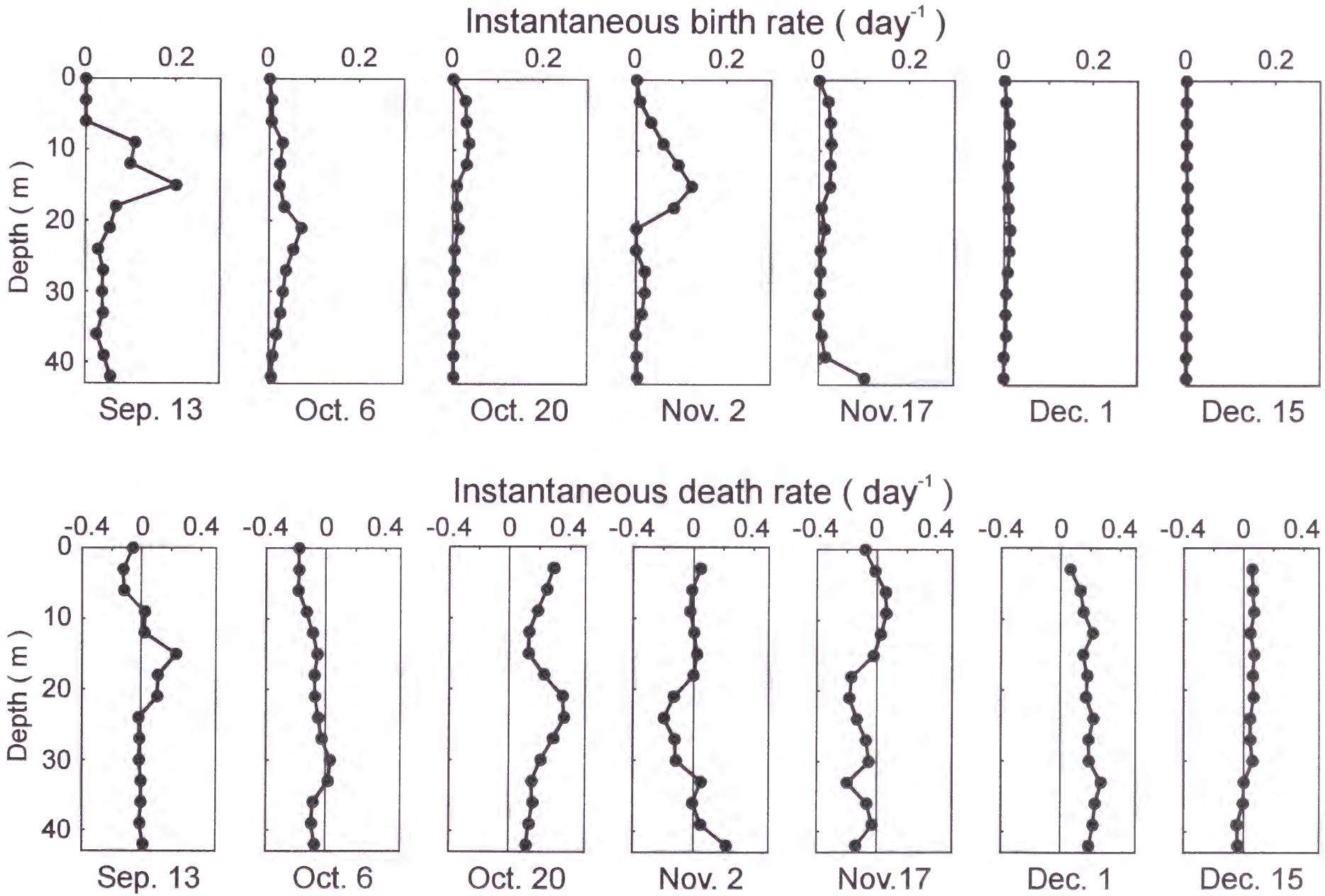


Sekino Fig. 4

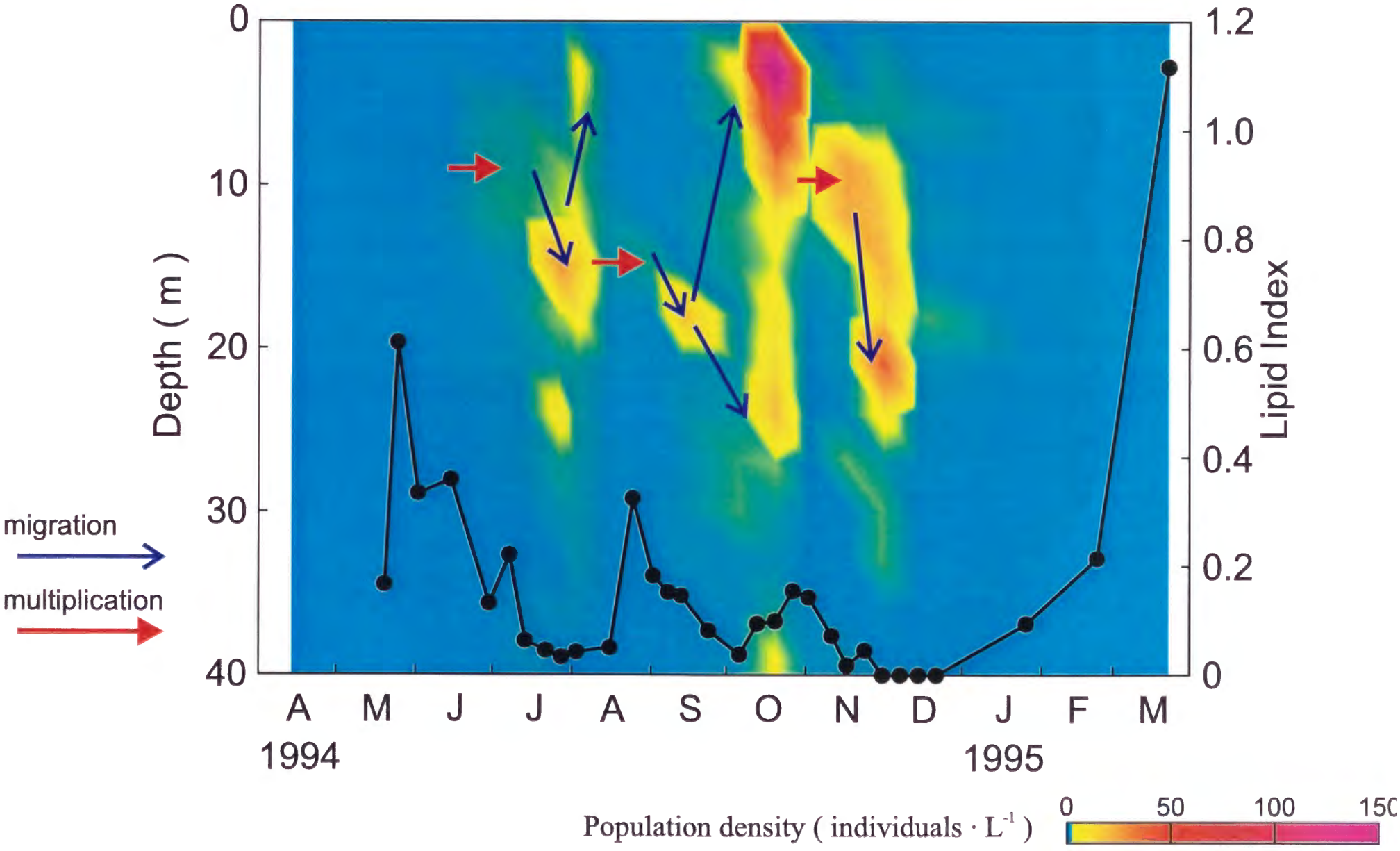

0
10
20
30
40
Depth (m)
A
M
J
J
A
S
O
N
D
J
F
M
1994
1995
0
50
100
150
Population density (individuals · L-1)


Instantaneous birth rate (day-1)
0
0.2
0.4
Depth (m)
0
10
20
30
40
Jun. 15
Jun. 30
Jul. 14
Jul.28
Aug. 3
Aug. 16
Sep. 2
Instantaneous death rate (day-1)
-0.4
0
0.4
Depth (m)
Jun. 15
Jun. 30
Jul. 14
Jul.28
Aug. 3
Aug. 16
Sep. 2

Sekiw Fig 6-1



Sekino Fig.6-2


0
10
20
30
40
Depth (m)
1.2
1.0
0.8
0.6
0.4
0.2
0
Lipid Index
A M J J A S O N D J F M
1994
1995
migration
multiplication
Population density (individuals · L-1)
0
50
100
150