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博 士 論 文

Ecological studies of an amphibious plant *Schoenoplectus
lineolatus* with special reference to the mechanism of population
maintenance

両生植物ヒメホタルイ个体群の維持機構に関する生態
学的研究

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神戸大学大学院自然科学研究科

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Doctoral Dissertation

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lineolatus* with special reference to the mechanism of
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SUMMARY

To elucidate the mechanism of maintaining persistent populations of an amphibious plant *Schoenoplectus lineolatus* (Fr. et Sav.) T. Koyama, ecological studies were conducted, including some experiments.

In chapter 1, I cited published works for amphibious plants in various study fields, that is, morphology, physiology and ecology and summarized the mechanism of maintaining persistent populations in other aquatic vascular plants.

In chapter 2, I investigated clonal growth and reproduction in relation to water depth in *S. lineolatus*. The plants were cultivated at three water depths (0, 0.3 and 0.7 m) in an irrigation pond. The number of ramets increased exponentially at three water depths. The clonal growth rate, which was estimated as the increase of \ln transformed number of ramets per day, was the highest under the exposed condition (0 m). Furthermore, the maximum ramet density was also the highest under the exposed condition. Seed production occurred only under the exposed condition, showing that *S. lineolatus* is an amphibious plant without the ability of sexual reproduction under water. Total dry weight of tuber, number of tubers and dry weight per tuber were the highest under the exposed condition.

Maximum ramet density, total dry weight of tuber and number of tubers were higher at 0.7 m site than at 0.3 m site, although there were no significant differences. There was little difference in temperature between

0.3 and 0.7 m sites. The effects of wave exposure and shading of filamentous algae were considered to be the reasons of lower growth at 0.3 m.

In chapter 3, I investigated the mechanism for inhibition of sexual reproduction by submergence and the success of sexual reproduction under fluctuating water level.

Inflorescence formation, flowering (anthesis) and seed setting were observed at three water depths (shallow, middle and deep) in three irrigation ponds. *Schoenoplectus lineolatus* formed inflorescences even under the submerged condition, but the final cumulative percentage of culms which formed inflorescence tended to decrease with increasing water depth in the three ponds. Culms with inflorescence were significantly longer than ones without inflorescence, but culm length tended to decrease with increasing water depth. Plants with the same culm length included the plants with and without producing inflorescence and, furthermore, the minimum length of culm with inflorescence differed among populations, showing that some factors other than plant size could determine the onset of sexual reproduction in *S. lineolatus*.

On the other hand, *S. lineolatus* opened flowers and produced seeds only in case of emergence into the air. In other words, no cleistogamous flowers were found under water. Flowering above the water surface and pollination in air were required for seed production in *S. lineolatus*.

Seed set rates and state of populations in relation to water depth (exposed, emergent and submerged) were examined in ten irrigation ponds

extensively. Overall, seed production was observed in populations where the plants were at least partly exposed or emergent. But the emergence of plants into the air in October and November led to the failure of seed production in some cases probably because of resource limitation.

Although *S. lineolatus* cannot produce seeds under the submerged condition because of the reduced number of inflorescence and the failure of pollination, the plants may produce seeds when they emerge into the air due to temporary drawdown even if they usually grow in deep water. The degree, period and timing of emergence would be important for seed production of the species.

In chapter 4, I compared seed germination and seedling establishment with vegetative propagule (tuber) in relation to water depth (0, 0.3, 0.7 and 1.1 m) in *S. lineolatus*. While all tubers germinated and almost all of the individuals derived from tubers survived at four water depths, seed germination and survival of the individuals derived from seeds were the highest under the exposed condition (0m) and decreased under the submerged conditions (0.3, 0.7 and 1.1 m). Seed germination was observed during drawdown of summer and early autumn at both 0.3 and 0.7 m sites, and the cumulative number of germinating seeds increased by 50% and 114%, respectively. Survival rate of the individuals derived from seeds was the lowest at 0.3 m probably because of the effects of wave exposure or shading of filamentous algae.

All of the individuals derived from tubers flowered when they emerged into the air, and produced tubers. On the other hand, the ones derived from

seeds did not always flower even if they emerged, although all of them produced tubers.

Thus, it was suggested that the establishment from seeds will be the highest around the shoreline and decrease in water, but that it could occur even in usually deeper zones in case of temporary drawdown.

In chapter 5, I investigated the role of water dispersal of seeds in maintaining persistent populations in *S. lineolatus*. Seed release from inflorescence was observed under three conditions (exposed, emergent and submerged). Under the exposed condition, seeds were released only onto the exposed soil. Under the emergent condition, seeds were released both onto water surface or in water. Under the submerged condition, seeds were released in water.

Greenhouse experiments for seed buoyancy showed that seeds released in air stayed floating on the water surface, but that most seeds released in water did not float. Seeds released on the exposed soil could float when the water level increased by pouring water from side as if water flowed into the habitat from upstream after rain, but when exposed directly to rainfall, they did not float when the water level increased.

The duration of seed buoyancy decreased in stirred water in comparison with that in standing water, suggesting that water dispersal of seeds might be limited in wavy environment.

Seeds of *S. lineolatus* can be transported to the shoreline while floating. Thus, the results of this study suggested that the seedlings of *S. lineolatus* had the chance of establishment in exposed soil both after water dispersal

of seeds to shore or after temporary drawdown in the environments with fluctuating water level.

In chapter 6, I investigated the depth distributions of amphibious and other plants. Total coverage of the plants decreased at sites of 2.5 m from the shoreline in three transects probably because of the effects of wave exposure. Overall, the terrestrial and emergent plants were distributed dominantly around the shoreline, and the floating-leaved and submerged plants had very low coverage in the study pond. In contrast, amphibious plants distributed dominantly in water, although the water level was rather constant for three years including the study period. Furthermore, most of amphibious plants were isoetids in the study pond. Accordingly, low nutrients may inhibit propagation of other aquatic plants, and amphibious isoetids may be dominant in water in an oligotrophic pond even if there is little fluctuation of water level.

Schoenoplectus lineolatus tended to distribute dominantly in deeper water than other amphibious plants. Its culm density tended to increase with increasing water depth, although it decreased beyond its peak. Numbers of species tended to become fewer in deeper water. The dominance of *S. lineolatus* in deeper water was considered to be due to less or no competitors in the study pond. In such a habitat, *S. lineolatus* may maintain the persistent population by the establishment from seeds on occasions of drawdown in the long term and clonally reproduce thereafter. The submerged sub-populations of *S. lineolatus* play an important role for the population maintenance in each locality.

Chapter 1 GENERAL INTRODUCTION

Amphibious plants can survive not only under the exposed condition but also under the submerged condition in environments with large water level fluctuations. Some species show typical heterophylly under different conditions, but other species do not change so remarkably in their external morphology. Amphibious plants have been well studied on the morphology (Hostrup and Wiegand, 1991; Pedersen and Sand-Jensen, 1992; Nielsen and Sand-Jensen, 1997; Robe and Griffiths, 1998), physiological response (Braendle and Crawford, 1999), photosynthesis (Ueno et al., 1988; Maberly and Spence, 1989; Nielsen, 1993; Sand-Jensen and Frost-Christensen, 1999) and growth response (Kirkman and Sharitz, 1993; Van der Sman et al., 1993; Nielsen and Sand-Jensen, 1997; Robe and Griffiths, 1998; Sand-Jensen and Frost-Christensen, 1999) under the exposed and submerged conditions. These studies show how they adjust themselves well to drastic environmental changes from exposed to submerged conditions and vice versa. However, little has been known about how amphibious plants having such adaptability in amphibious life maintain persistent populations. In this study, I focused on the mechanism of maintaining persistent populations under fluctuating water level in amphibious plants.

Many vascular aquatic plants can propagate not only sexually but also vegetatively. Rooted vascular aquatic plants with vegetative means of reproduction include the emergent, floating-leaved and submerged plants in addition to the amphibious plants. These plants except amphibious plants

often form zonation, typically arranging in order from shoreline to deeper water along a water-depth gradient (Sculthorpe, 1967; Spence, 1982).

Vascular aquatic plant species with vegetative means have two life cycles, that is, through sexual and vegetative reproductions (hereafter, these life cycles are called the sexual life-cycle and the vegetative life-cycle, respectively) (Fig. 1). Both cycles consist of growth, reproduction, germination and establishment phases. Establishment phase is the process which propagule-derived plants reproduce successfully by sexual or vegetative means after germination.

So far, the mechanisms of maintaining persistent populations have been studied mainly on the emergent, floating-leaved and submerged plants. The mechanism of maintaining persistent populations of each plant species appears to reflect a position within zonation. In case of emergent plants which grow in the shore side within zonation, both growth and reproduction are the highest around the shoreline or in shallower water and decrease with increasing water depth (e.g., Froend and McComb, 1994). Moreover, seed germination and seedling establishment also occur around the shoreline or in shallower water in these plants (e.g., Coops and Van der Velde, 1995). On the other hand, there have been relatively few studies about the mechanisms of maintaining persistent populations in both floating-leaved and submerged plants which grow in the middle and the deeper-water side within zonation, respectively. However, both growth and reproduction are the highest in certain water depth and decrease with increasing or decreasing water depth in these plants (e.g., Cenzato and

Ganf, 2001). Cenzato and Ganf (2001) have conducted the comparative study between the two *Potamogeton* species and have found that optimum depth for growth was shallower in the species with both floating and submerged leaves than in the species with only submerged leaves. This study suggests that growth may be the highest in shallower water in floating-leaved plants than in submerged plants. Seed germination and seedling establishment show two modes in floating-leaved plants. One mode is that seed germination and seedling growth occur at sites exposed to the air, while adult growth favor flooding (Van der Valk and Davis, 1978; Smits et al., 1990). *Nymphoides peltata* is such an example. Another mode is that high seed germination and seedling growth require submerged conditions, not exposed conditions (Van der Valk and Davis, 1978; Smits et al., 1990). However, it is not known what water depth is most favorable to seed germination and seedling establishment in this case. *Nuphar lutea* represents this mode. In submerged plants, seed germination and seedling growth also require submerged conditions, although it is not known what water depth is most favorable to them (Van der Valk and Davis, 1978).

Another characteristic of the mechanisms of maintaining persistent populations of the emergent, floating-leaved and submerged plants is that the vegetative life-cycle is dominant in many species (Sculthorpe, 1967). As mentioned previously, this is the case in emergent plants and in floating-leaved plants such as *N. peltata*. In floating-leaved plants such as *N. lutea* and submerged plants, Barrat-Segretain (1996) and Rogers and Breen (1980) have found that the vegetative life-cycle was dominant in

natural populations.

In contrast with the emergent, floating-leaved and submerged plants, studies on the mechanisms of maintaining persistent populations have been very scarce in amphibious plants. The conspicuous characteristic of amphibious plants is the ability to survive at both sites which are exposed to the air and are submerged, and this ability enable them to dominate the vegetation under fluctuating water level (Casanova and Brock, 2000). Such amphibious plants include the species which can and cannot reproduce sexually under water (Sculthorpe, 1967).

Lobelia dortmanna can reproduce sexually under water. The species has little capacity of propagating vegetatively and maintains persistent populations predominantly through the sexual life-cycle (Moeller, 1978; Szmeja, 1987; Farmer and Spence, 1987a). Both the seedling growth (Farmer and Spence, 1987a) and the proportion of flowering (Moeller, 1978) decrease with increasing water depth. Thus, the mechanism of maintaining persistent populations for *L. dortmanna* is characterized by the high dependency to the sexual life-cycle, as compared to the emergent, floating-leaved and submerged plants.

This characteristic is never shared with amphibious plants without the ability of sexual reproduction under water. Robe and Griffiths (1998) have suggested that vegetative reproduction occurred under both exposed and submerged conditions in these plants (Robe and Griffiths, 1998), but the detailed mechanism of maintaining persistent populations has never been elucidated.

This study describes the mechanism of maintaining persistent populations of an amphibious plant without the ability of sexual reproduction under water, *Schoenoplectus lineolatus* (Fr. et Sav.) T. Koyama (Cyperaceae). The survey and experiments were carried out on the following five subjects in the field and greenhouse:

- (1) Clonal growth and reproduction in relation to water depth
- (2) Sexual reproduction under fluctuating water level
- (3) Establishment from seeds under fluctuating water level
- (4) Water dispersal of seeds
- (5) Amphibious plants and water level fluctuations in Shin-ike Pond

Study species

Schoenoplectus lineolatus is a psuedo-annual species with overwintering tubers (vegetative propagules). It is endemic to Formosa and Japan and grows around shores or in shallow water of lakes, rivers and ponds and in paddy fields. Plants have below-ground creeping rhizomes. Figure 2 shows the diagram of growth form of *S. lineolatus*. From each node on rhizomes, a culm (photosynthetic organ) and roots grow out and one branching of new rhizome has often been observed. Thus, they show clonal growth with ramets consisting of a culm, a rhizome and roots as a unit. Columnar culms with 7-25 cm length and a diameter of 0.8-2 mm have one inflorescence with some protogynous flowers (Kadono, 1994). The flowers are borne from July to October in western Japan and

anemophilous. Each flower has one ovule. Tubers are produced at the tip of rhizome and/or at the base of culms in autumn.

Chapter 2 Clonal growth and reproduction in relation to water depth

INTRODUCTION

Aquatic plants usually grow in environments with a water-depth gradient. It has been reported that plant performance such as growth and reproduction are affected by water depth in various aquatic plants. In chapter 2, I investigated growth and reproduction in relation to water depth in *Schoenoplectus lineolatus*.

Plant performance such as growth and reproduction is negatively correlated to water depth in the emergent (Lieffers and Shay, 1981; Rea and Ganf, 1994a; Clevering and Hundscheid, 1998; Blanch et al., 1999), floating-leaved (Cenzato and Ganf, 2001) and submerged plants (Cenzato and Ganf, 2001; Jiang and Kadono, 2001; Strand and Weisner, 2001). Among these plants, however, the optimal water depths for growth and reproduction are different. They reflect their depth distributions in zonation, that is, while the optimal depth is near the shore or shallower water depth in emergent plants, it is deeper water depth in floating-leaved and submerged plants.

Unlike these plants, amphibious plants can survive from terrestrial to totally submerged environments and, therefore, may have potentially the widest range in their depth distributions. Are the optimal water depths for

growth and reproduction above or under water in amphibious plants? This question will be important for understanding the strategy for surviving under fluctuating water level in amphibious plants. For example, amphibious *Lobelia dortmanna* produces cleistogamous flowers (Moeller, 1978) although its optimal water depth for growth and reproduction is not known. The species is considered to be more confined to submerged life (Szmeja, 1987; Pedersen and Sand-Jensen, 1992) and its capacity for producing cleistogamous flowers characterizes the strategy for surviving of the species. In case of *L. dortmanna*, perhaps, the optimal depth for both growth and reproduction might be deeper water depth. From this view, the growth and reproduction along a water-depth gradient and its ecological implications need to be explored in more detail in amphibious plants.

In this study, I investigated the growth and reproduction of an amphibious plant *Schoenoplectus lineolatus* in relation to water depth. Sexual reproduction is not observed under water in this species.

MATERIALS AND METHODS

Transplant experiment in different water depths

In order to investigate the response of growth and reproduction to water depth, the field experiment was conducted in an irrigation pond, Shin-ike Pond in Satani, Kasai City, Hyogo Prefecture, in western Japan. The water of this pond was clear and Secchi-depth was ca. 2.0 m in summer of 2000.

pH was 7.1 ± 0.4 (the mean \pm SD, $n = 30$ from 2000 to 2001) and electrical conductivity was $69.4 \pm 7.6 \mu\text{ S/cm}$ ($n = 30$ from 2000 to 2001), respectively, suggesting that it was a relatively nutrient-poor pond.

The transplant experiment was conducted as follows. The individuals that consisted of two ramets were collected from the study pond on 5 July 2000. Each individual was planted in the plastic tray (area = $22.5 \text{ cm} \times 30 \text{ cm}$, depth = 7 cm) filled with the sediment of neighboring another pond where *S. lineolatus* grew. Eight trays with one individual each were set at three water depths (0, 0.3 and 0.7 m), respectively. The water depths were adjusted within 5 cm of the sediment surface of the trays. When the experiment started, the plants at 0 m were exposed to the air and those at 0.3 and 0.7 m were completely submerged (not emergent). The sites at 0.3 and 0.7 m deep were about 2.5 and 5.0 m away from the shoreline, respectively. Water was added to prevent sediment from drying up when trays were exposed to the air.

The number of new and dying ramets was recorded at least once a week by marking new ramets and the seed production was also checked during the experiment. Ramet density was calculated based on the area of tray. The observation was finished on 8 February 2001 when aboveground parts of all the plants died. The trays were brought back to the laboratory and the tubers produced in the sediment were collected and counted. After drying (80°C for 48 h), their dry weight was measured.

The water level and water temperatures at three water depths were recorded at every observation time during the experimental period. The

water level is described on the basis of 0 m of shoreline at the beginning of the transplant experiment throughout the text.

Data analyses

The effects of water depth on maximum ramet density, clonal growth rate, number of tubers and dry weight per tuber and total dry weight of tubers were tested by one-way analysis of variance (one-way ANOVA) or Kruskal Wallis test.

The numbers of ramets showed exponential increment at three water depths. The clonal growth rate in each depth, therefore, was calculated using the following formula that was given by modifying the one for the relative growth rate (Harper, 1977):

$$\frac{\ln N_2 - \ln N_1}{t_2 - t_1}$$

where N_1 and N_2 are the ramet numbers at time 1 (t_1) and 2 (t_2), respectively.

To meet assumptions of normality and/or homoscedasticity, data were log transformed if necessary. Normality was tested by Shapiro Wilk test and homoscedasticity by Bartlett's test. When data did not satisfy assumptions for ANOVA, Kruskal Wallis test was performed. If significant effects of water depth were detected, unplanned comparisons among the means for ANOVA and for Kruskal Wallis test were performed by Tukey's HSD test and by Games Howell test, respectively. Games Howell test was performed in StatView 5.0 (SAS Institute Inc., USA) and the other tests

were performed in JMP(R) 4.0.5 (SAS Institute Inc., USA).

RESULTS

Environmental conditions

The water level decreased to the minimum level of -0.14 m in early September 2000, but soon recovered and was rather constant throughout the experimental period (Fig. 3). Thus, the plants at 0 m were always exposed to the air and those at deeper water depths always stayed completely submerged. The water temperatures during the experimental period are shown in Fig. 3. They tended to decrease with increasing water depth, but the lowest value was recorded at 0 m in winter. There was little difference in temperature between 0.3 and 0.7 m.

Relationship between clonal growth and water depth

Eight individuals showed a similar trend on seasonal changes of ramet number at each water depth and, therefore, the data are shown as the sums of eight individuals at each water depth (Fig. 4). At three water depths, total number of ramets increased exponentially until September, and the number of ramet deaths was limited during this period (Fig. 4). Total number of ramets reached the peak on 28 September, 11 October and 18 October at 0, 0.3 and 0.7 m, respectively. Maximum ramet density at 0 m was over twice as high as those at 0.3 and 0.7 m (Table 1), but there was a significant

difference only between 0 and 0.3 m (Kruskal Wallis test; $H = 10.478$, d.f. = 2, $P < 0.010$). Maximum ramet density at 0.7 m was 1.8 times as high as that at 0.3 m although no significant difference was detected.

After the date of the peak, the number of ramet deaths increased rapidly at 0 m and all ramets died by 13 December. At 0.3 and 0.7 m, there was little change in ramet number until November and the number of ramet deaths rapidly increased in December. All ramets died by 24 January and 8 February 2001 at 0.3 and 0.7 m, respectively. Thus, the date by which all ramets died was delayed at deeper water depths.

Clonal growth rate was calculated for 28th July to 6th September when ramets were steadily increasing. The increase of \ln transformed number of ramets per day was significantly higher at 0 m than at other water depths, but there was no significant difference between 0.3 and 0.7 m (Fig. 5).

Relationship between reproduction and water depth

All individuals reproduced seeds at 0 m, but none at 0.3 and 0.7 m with constantly and totally submerged states (Table 2). In contrast, tubers were produced at all water depths (Table 2), showing that the species reproduces vegetatively irrespective of these water depths.

Both total dry weight of tubers and the number of tubers at 0 m were significantly higher than under the totally submerged conditions and the number of tubers was 14 and 4 times as large as those at 0.3 and 0.7 m, respectively (Table 3; total dry weight of tubers: Kruskal Wallis test, $H = 15.485$, d.f. = 2, $P < 0.001$; the number of tubers: one-way ANOVA, $F_{2,21} =$

31.727, $P < 0.001$). Total dry weight of tubers and the number of tubers at 0.7 m were 1.2 and 1.6 times as large as those at 0.3 m, respectively, but no significant differences were detected (Table 3). This was a similar tendency with maximum ramet density (Table 1). Tuber size as expressed by dry weight per tuber decreased significantly with increasing water depth (Table 3; Kruskal Wallis test, $H = 1096.934$, d.f. = 2, $P < 0.001$) and it was over 2.4 and 3.3 times as high at 0 m as those at 0.3 and 0.7 m, respectively. So far as tuber size was concerned, it was larger at 0.3 m than at 0.7 m.

DISCUSSION

Relationship between clonal growth and water depth

So far, there have been few studies about the optimal water depth for growth of amphibious plants. Nielsen and Sand-Jensen (1997) have reported growth in relation to water depth in an amphibious *Littorella uniflora*, but they could not detect a significant difference in growth between exposed and totally submerged conditions. They argued that *L. uniflora* does not experience any significant change in inorganic carbon availability between about 40 cm above and about 30 cm below the water surface, which were the water depths in their study, because it can receive CO_2 from the sediment into its extensive lacunar spaces. In Lake Hampen of their study site, it was likely that light availability was enough for the growth of *L. uniflora* because the Secchi-depth was 3.5 m during the study

period.

In this study, the increase of ln transformed number of ramets per day was the highest under the exposed condition (0 m) in *S. lineolatus*, resulting in the highest ramet density. In other words, the growth was the highest under the exposed condition. In *S. lineolatus*, however, it was not negatively correlated with water depth, that is, the maximum ramet density tended to be higher at 0.7 m than at 0.3 m although Farmer and Spence (1987b) found that negative correlation in another amphibious species, *Lobelia dortmanna*. Water temperature is one of the factors affecting the growth of aquatic plants (Barko et al., 1982), but there was little difference between the two water depths in this study (Fig.3). One possibility for the lower growth rate at 0.3 m is the effect of wave exposure near the shore. Wave exposure adversely affects plant growth or distribution both directly through biomass loss (Jupp and Spence, 1977) or indirectly through alteration of substrate particle size, organic content and nutrient concentration (Spence, 1982; Keddy, 1982, 1983; Wilson and Keddy, 1985). The 0.3 m site was nearer to the shore and wave exposure is stronger than at 0.7 m, which may have perhaps caused reduction of clonal growth at 0.3 m although the mechanism is not known in detail. Another possibility might be the effect of shading by filamentous algae (Phillips et al., 1978). Although wave exposure may generally work to remove filamentous algae (Weisner et al., 1997), the growth of filamentous algae was observed in shallower area in summer during the experimental period (Ishii, unpublished observation). Therefore, they may have reduced

photosynthetic production of *S. lineolatus* at 0.3 m during summer when clonal growth was maximal. In this study, the relative contribution of the adverse effect of wave exposure and filamentous algae was not examined.

Relationship between reproduction and water depth

Some amphibious plants have been known to reproduce sexually by cleistogamous flowers underwater, e.g., *Limnophila sessiliflora*, *Rotala hippuris* and *Subularia aquatica* among Japanese plants (Kadono, 1994). However, this study showed that *S. lineolatus* could reproduce sexually only under the exposed condition. On the other hand, vegetative reproduction by tuber production occurred under both exposed and totally submerged conditions.

Total dry weight of tubers, number of tubers and dry weight per tuber were also the highest under the exposed condition. But the number of tubers was smaller at 0.3 m than 0.7 m although dry weight per tuber was larger at 0.3 m. This is most probably due to smaller ramet density at 0.3 m. Tubers of *S. lineolatus* are produced at the tip of rhizomes or at the base of culms and the number of tubers is dependent on the numbers of ramets. On the other hand, dry weight per tuber is determined by storage materials produced by photosynthesis. Improved light conditions after the decay of filamentous algae in autumn, therefore, may have contributed to larger tuber production at 0.3 m.

Ecological implications of clonal growth and reproductive characteristics

for the population maintenance

Schoenoplectus lineolatus showed the highest growth and reproduction under the exposed condition. Its productivity, however, decreased markedly under the totally submerged condition, and in particular *S. lineolatus* never reproduced sexually under that condition. Considering that amphibious plants grow under fluctuating water levels (Casanova and Brock, 2000), it was suggested that *S. lineolatus* might attain the optimal growth and reproduction under the exposed condition due to water drawdown and that its persistent populations might be vegetatively maintained thereafter under the totally submerged condition.

According to our vegetation survey (chapter 6), *S. lineolatus* spread down to 2.5 m deep area as a stable population in Shin-ike Pond. Although the water level fluctuation was relatively small in this pond during our experimental period because the pond was a spare one for other ponds and the water was used only in case of water deficiency in other ponds, the water of the pond had been regularly drawn down once every several years as a traditional way of management. Namely, the water level may be unpredictably lowered down in the years of little rain to supply water for other connected ponds. On these occasions, totally submerged individuals may be exposed to the air and may have a chance of sexual reproduction as well as vigorous vegetative reproduction. On the other hand, with extreme desiccation, the mortality of the individuals of high elevation might become higher, but the individuals at deeper site might have more chance to survive such harsh years. In these situations subpopulations at deeper sites will

contribute to the recovery of the whole population. The individuals at deeper site, therefore, may play an important role for the maintenance of populations even if the productivity is not so large in usual years. Actually the vegetations dominated by *S. lineolatus* often develop in waters with large water level fluctuation where other kinds of vegetation do not develop well. Future study on the comparison of subpopulation dynamics between the shallower and deeper sites in relation to the water level fluctuation will test this hypothesis.

Chapter 3 Sexual reproduction under fluctuating water level

INTRODUCTION

In chapter 2, it was shown that *Schoenoplectus lineolatus* could not reproduce sexually under water. *Schoenoplectus lineolatus* inhabits in the environments with water level fluctuations and, therefore, it often experiences temporary water drawdown. In that case *S. lineolatus* may set seeds successfully above water. In this chapter, I investigated the success of sexual reproduction at the period of temporary water drawdown.

Sexual reproduction of aquatic plants is restricted by growing depth because emergence of the floral organ into the air is required for successful pollination (Uphof, 1938; Sculthorpe, 1967). The species with cleistogamy or hydrophily would not suffer from such pollination-limitation under water, but may be still restricted by water depth through other mechanisms. For example, it has been reported that sexual reproduction is size-dependent in aquatic plants as in terrestrial plants (Farmer and Spence, 1987a; Titus and Hoover, 1991; Olesen, 1999). Water depth affects plant growth, and the success of sexual reproduction may change according to the plant size.

Amphibious plants include the species with and without cleistogamy, but few works have been done on detailed responses of their sexual

reproduction to water depth. For example, although Moeller (1978) has shown that an amphibious plant *Lobelia dortmanna* produced cleistogamous flowers under water and flowering frequency decreased with increasing water depth, it is not known whether its decrease was related to the plant size.

In addition to water depth, water level fluctuation may be another important environmental factor for successful sexual reproduction of amphibious plants. Amphibious plants without cleistogamous flowers cannot reproduce sexually under water and, therefore, increasing water depth strongly inhibits their seed production. Nevertheless, they may produce seeds successfully under some circumstances. The plants growing in deeper zone are sometimes exposed to the air due to temporary water drawdown and may have the chance of pollination in the air (Robe and Griffiths, 1998).

Schoenoplectus lineolatus cannot reproduce sexually under water (chapter 2), but its inhibition mechanism for sexual reproduction under water is not known. In this study, I investigated the sexual reproduction of *S. lineolatus* in relation to water depth and evaluated the importance of temporary water drawdown on the success of seed production.

MATERIALS AND METHODS

Field investigations

Sexual reproduction was observed as a process of the three stages, that is, inflorescence formation, flowering (anthesis) and seed set. The observation was conducted in three irrigation ponds with different water regime, Oku-ike Pond (Satani, Kasai City), Shin-ike Pond (Satani, Kasai City) and an unnamed pond (Umase, Yashiro, Kato-gun; Umase hereafter) in Hyogo Prefecture, western Japan, from June to November in 1997. Three quadrats (25 cm × 25 cm) were set at each of three different water depth sites (shallow, middle and deep) covering distribution zone of the species in each pond. The “shallow”, “middle” and “deep” water depths were 0-15 cm, 15-30 cm and 30-45 cm in Oku-ike Pond, 30-60 cm, 75-105 cm and 120-150 cm in Shin-ike Pond, and 35-45 cm, 50-60 cm and 65-75 cm in Umase, respectively. Water depths are described on the basis of 0 cm water depth at the shoreline on the beginning of the field investigation. Water levels were recorded during the study period. Substratum was sandy only at shallow water site in Oku-ike Pond and silty at other sites in three ponds.

Thirty culms of *S. lineolatus* in each quadrat were tagged and checked for the inflorescence formation, flowering and seed set at intervals of three to seven days. The state of culms was also recorded as submerged, emergent or completely exposed. When flowering started, the height of inflorescences from the water surface was measured for both the male and female stages. At the end of growing period, the culm length was measured for all of the culms.

In addition as an extensive field survey on the relationship between

seed production and fluctuating water level in the population level, seed set rates and water level of populations (exposed, emergent and submerged) were examined at interval of one month in ten irrigation ponds of Hyogo Prefecture from August to November in 1997. The numbers of flowers and mature seeds in inflorescences were counted and seed set rates were calculated as the number of mature seeds / the number of flowers for each inflorescence. Flower number per inflorescence and sample size in each pond are given in Table 4.

Data analyses

To meet assumptions of normality and/or homoscedasticity, data were log, root or arcsine transformed, if necessary. Normality was tested by Shapiro Wilk test and homoscedasticity by Bartlett's test. The effects of water depth on the final cumulative percentage of culms which formed inflorescence was tested by one-way analysis of variance (one-way ANOVA). When the data did not satisfy normality or included only 0 values for any water depth, Kruskal-Wallis test was used. Differences between lengths of culms with and without inflorescence were tested by *t* test. When the data did not satisfy any assumptions for *t* test, Mann Whitney test was used. The effects of water depth on culm length were tested by one-way ANOVA. When the data did not satisfy any assumptions for one-way ANOVA, Kruskal Wallis test was used. The effect of harvest time on seed set rate in each pond was tested by Mann Whitney test and Kruskal Wallis test because of not satisfying assumptions for *t* test and one-

way ANOVA, respectively. In all tests including more than two samples, if significant effects of any factor were detected, unplanned comparisons among the means were performed by Tukey's HSD test. When the data did not satisfy any assumptions for Tukey's HSD test, Scheffe's *F* test was used. Mann Whitney test and Scheffe's *F* test were performed in StatView 5.0 (SAS Institute Inc., USA) and the other tests in JMP(R) 4.0.5 (SAS Institute Inc., USA).

RESULTS

Water regime in three ponds

Water level decreased in August but increased again in September in Oku-ike Pond(Fig. 6). On the other hand, the change was much less in Shin-ike Pond and Umase (Fig. 6). In Oku-ike Pond all of the three water depth sites were exposed to the air during the period of decreased water level in summer (Fig. 7A), but none of them were exposed to the air in Shin-ike Pond (Fig. 7B). In Umase the site of shallow water depth was exposed to the air twice during the short period of repeated water level decrease in September (Fig. 7C).

Relationship between successful seed production and water depth

The formation of inflorescences was observed in August and early September in Oku-ike Pond when water level dropped and the onset of

inflorescence formation was delayed in the plants growing in deeper water (Figs. 6 and 7A). The final cumulative percentage of culms which formed inflorescences was higher at middle water depth than at deep water depth, and the lowest value was observed at shallow water depth. There was a significant difference only between shallow and middle water depths (one-way ANOVA, $F_{2,6} = 5.447$, $P = 0.045$). In Shin-ike Pond, inflorescence formation continued from August to early October under submerged condition and the final cumulative percentage of culms which formed inflorescence was significantly larger at shallow water depth (Figs. 6 and 7B; Kruskal Wallis test, $H = 6.938$, d.f. = 2, $P = 0.031$). Inflorescences were observed in August and September in Umase in shallow and middle water depth sites but not at deep site (Figs. 6 and 7C). The final cumulative percentage of culms which formed inflorescence decreased significantly with increasing water depth (Kruskal Wallis test, $H = 7.448$, d.f. = 2, $P = 0.024$). Thus, the final cumulative percentage of culms which formed inflorescence decreased with increasing water depth in all of the three ponds, except for shallow water depth site in Oku-ike Pond (Fig. 6).

In contrast with inflorescence formation, flowering was observed only in Oku-ike Pond at the time of emergence of the inflorescences into the air (Fig. 8A). Both in Shin-ike Pond where there was no period of emergence and in Umase where period of emergence was limited, flowering was not observed irrespective of inflorescence formation (Figs. 8B and 8C). Except for one flower at the male stage, all flowers opened above the water surface both at male and female stages (Fig. 9). In Oku-ike Pond, *S. lineolatus*

started to open flowers three days after the emergence of inflorescences into the air, and the flowers of most of the culms opened about two weeks later (Fig. 10). Flowered inflorescences could produce seeds, but unflowered inflorescences did not produce seeds (Fig. 11). In case that inflorescences became submerged again after flowering (Fig. 7A), seed set was normally observed (Fig. 11).

Size-dependence of the formation of inflorescences

Figure 12 shows the frequency distribution of length of the culms with and without inflorescences. Culms with inflorescence were significantly longer than the ones without inflorescence in three ponds investigated (Oku-ike Pond: t test for log transformed data, $t = 3.012$, $P = 0.003$; Shin-ike Pond: Mann Whitney test, $U = 2729$, $P < 0.001$; Umase: $U = 1579$, $P < 0.001$). The minimum length of culms with inflorescence was 4.2 cm in Oku-ike Pond, 9.4 cm in Shin-ike Pond and 15.1 cm in Umase, respectively. Thus the critical threshold size for sexual reproduction was different among populations.

Culm length tended to decrease with increasing water depth in three ponds, except for shallow water depth in Oku-ike Pond (Fig. 13). The final cumulative percentage of culms which formed inflorescence showed the same tendency (Fig. 6). In Oku-ike Pond, culm length was the longest at middle water depth, but there were no significant difference between shallow and deep water depths (Kruskal Wallis test for log transformed data, $H = 38.002$, d.f. =2, $P < 0.001$). In Shin-ike Pond, culm length was

significantly longer at shallow water depth than at the other water depths (one-way ANOVA for root transformed data, $F_{2,267} = 318.974$, $P < 0.001$). In Umase, culm length decreased significantly with increasing water depth (one-way ANOVA for root transformed data, $F_{2,267} = 238.121$, $P < 0.001$).

Field survey on seed production in ten irrigation ponds

Inflorescence formation was observed in eight out of ten ponds (Table 4). Number of flowers per inflorescence were 9.9 ± 3.5 (mean \pm SD) and ranged from 1 to 23 ($n = 471$) in eight ponds.

Table 5 shows seed set rates and the water level of populations. Seed productions were observed when the populations were at least partly exposed or emergent in Oku-ike Pond, Saka-ike Pond, an unnamed pond, Naka-ike Pond, Shin-ike Pond, Oh-ike Pond, Nobuka-ike Pond and Kamino-ike Pond. On the other hand, seed production was not observed even though the population was exposed on 2nd October and 5th November in Sedaiko-ike Pond. Seeds were not produced in Sanda-ike Pond where the population was always submerged,.

Seed set rates were not significantly different among harvest times in Oku-ike Pond (Kruskal Wallis test, $H = 0.025$, d.f. = 2, $P = 0.988$), Saka-ike Pond (Kruskal Wallis test, $H = 6.650$, d.f. = 3, $P = 0.084$), an unnamed pond (Mann Whitney test, $U = 199.000$, $P = 0.978$) and Nobuka-ike Pond (Kruskal Wallis test, $H = 3.068$, d.f. = 2, $P = 0.216$), although water level increased on 2nd October, on 31th August, on 2nd October and 5th November and on 2nd October, respectively. In Shin-ike Pond, the seed set

rate significantly increased when water level decreased on 31st August (Kruskal Wallis test for arcsine transformed data, $H = 17.905$, d.f. = 3, $P < 0.001$). Seed set rates decreased significantly on 2nd October and 5th November in Naka-ike Pond (Kruskal Wallis test, $H = 12.713$, $P = 0.002$) and on 5th November in Kamino-ike Pond (Kruskal Wallis test, $H = 16.247$, d.f. = 2, $P < 0.001$).

DISCUSSION

Successful sexual reproduction and water depth

The present observations showed that inflorescence formation does not necessarily require the emergence of culms into the air in *S. lineolatus*. However, anthesis was observed only under exposed condition when water level temporarily decreased. Some amphibious plants have cleistogamous or pseudocleistogamous flowers which set seeds under submerged condition, e. g., *Limnophila sessiliflora* and *Rotala hippuris* (Kadono, 1994). In this study *S. lineolatus* was proved to lack such cleistogamic habit. However, seed set is possible underwater if pollination has already occurred in the air.

Sexual reproduction is size-dependent in various freshwater aquatic plants, e.g. *Lobelia dortmanna* (Farmer and Spence, 1987a), *Vallisneria americana* (Titus and Hoover, 1991) and a seagrass, *Zostera marina* (Olesen, 1999). In *S. lineolatus*, the culms with inflorescence were

significantly longer than the ones without inflorescence. In our other study I found that culm length was correlated with the dry weight of the culm (Ishii, unpublished data). The difference in length between the culms with and without inflorescence, therefore, suggested that inflorescence formation depends on plant size.

Culms became short with increasing water depth. Considering the size-dependent inflorescence formation, fewer inflorescence formation in deeper water will be ascribed to smaller size of the culms. Poor growth and fewer inflorescence formation at shallow site in Oku-ike Pond may be caused by sandy substratum there because *S. lineolatus* prefers to silty substratum (Ishii, unpublished observation).

In most of cases including this study, only plant size cannot determine the onset of sexual reproduction. Plants with the same size include the plants with and without inflorescence or flowering (Farmer and Spence, 1987a; Titus and Hoover, 1991; Olesen, 1999). Moreover, the minimum plant size for sexual reproduction was found to differ among populations in Farmer and Spence (1987a) and also in this study. Difference in onset of sexual reproduction among plants may be due to the genetic and/or the environmental factors such as day length, temperature (Titus and Hoover, 1991) and resource availability for flowering (Farmer and Spence, 1987a). Integrated regulation of ramets may also be involved to form inflorescence in plants with clonal growth such as *S. lineolatus*.

Cost and benefit of inflorescence formation under water

This study showed that submergence inhibited the flowering of *S. lineolatus*, but inflorescence formation was not restricted. Then, why does the species form inflorescence under water?

If the plants could not have a chance of flowering above the water surface, the cost of formation of inflorescence would be in vain. One reason might be the possibility that the plants do not have physiological mechanisms to distinguish between exposed and submerged conditions and inflorescences are formed when culms attain to some degree of size. In this case, if the inflorescence stays under water, inflorescence formation should be maladaptive.

Another possibility is related to the characteristics of habitats of *S. lineolatus*. Amphibious plants usually grow under fluctuating water level in their habitats (Casanova and Brock, 2000). Inflorescences formed under water will lead to quick flowering under the exposed condition when water level decreases. From this viewpoint, it might be referred to as a “waiting strategy” for the decrease of water level. The case of flowering in Oku-ike Pond seems to support this hypothesis. The plants growing in deeper zone could flower and set seeds when they became exposed or emergent due to temporary drawdown. However, when the period of emergence at deeper site was limited, seed set would not occur. It took about two weeks for most of culms to open flowers after emergence. Therefore, the degree and period of water level decrease are important for successful sexual reproduction in *S. lineolatus*.

Seed production in ten irrigation ponds

The results of the field survey on seed production in ten irrigation ponds clearly described the success or failure of sexual reproduction under fluctuating water level in *S. lineolatus*. While *S. lineolatus* produced seeds under the exposed or emergent conditions in most of ten ponds investigated, even inflorescence formation was not observed in Sanda-ike Pond where the plants stayed under the submerged condition. Populations became submerged due to the increase of water level after seeds were produced under the exposed or emergent conditions on 31st August, 4th August, 2nd October, 4th and 31st August and 31st August, respectively, in Oku-ike Pond, Saka-ike Pond, an unnamed pond, Shin-ike Pond and Nobuka-ike Pond. However, seed set rates were not significantly different among harvest times in their ponds. These results indicate that new inflorescences may have been not formed under the submerged conditions after seeds were produced in their ponds. In contrast, seed set rates decreased significantly on 2nd October and 5th November, respectively, in Naka-ike Pond and Kamino-ike Pond when water level increased. The increase of water level may have affected pollination as well as formation of new inflorescences in both ponds. When water level decreased, seed set rates significantly increased on 31st August in Shin-ike Pond, but significantly decreased on 5th November in Naka-ike Pond. In Shin-ike Pond, number of flowering culms was low on 4th August, but increased on 31st August when water level decreased (Ishii, unpublished observation). There may have been more pollen grains for seed production on 31st August than on

4th August and, then, seed set rates was higher on 31st August. On the other hand, the decrease of seed set rates on 5th November in Naka-ike Pond may be due to seasonal reason, that is, too late for sexual reproduction in *S. lineolatus*. In fact, inflorescences were not formed despite exposure on 2nd October and 5th November in Sendaiko-ike Pond, although seeds were produced on 2nd October in Oh-ike Pond. The significant decrease of seed set rates on 5 November in Kamino-ike Pond may also be due to too late season rather than the increase of water level. These results suggest that the timing of decreasing water level is one of the important factors for successful sexual reproduction in *S. lineolatus*.

In conclusion, the importance of the degree, period and timing of decreasing water level was suggested for successful seed production of *S. lineolatus*. Adequate water drawdown would make the species possible to reproduce sexually. Water level fluctuations are often unpredictable, as seen in ten irrigation ponds of Hyogo Prefecture. In such circumstances, a “waiting strategy” may enhance the productivity of seeds in *S. lineolatus*.

Chapter 4 Establishment from seeds under fluctuating water level

INTRODUCTION

The results of chapters 1 and 2 showed that *Schoenoplectus lineolatus* had chances to produce seeds in the whole range of its depth distributions. In this chapter, the phases of seed germination and seedling establishment were focused on.

Williams (1975) has stated that seeds have higher rates of mortality than vegetative propagules in growth into adult plants. This implicates that establishment from seeds may be restricted to limited sites as compared with vegetative propagules. In aquatic plant species, some researchers have found such establishment patterns in relation to water depth in field studies (Van der Valk and Davis, 1978; Rea and Ganf, 1994b; Clevering, 1995). Establishment from seeds was limited in shallower water depth as compared with establishment from vegetative propagules in their species. On the other hand, little is known about the establishment phase of amphibious species in relation to water depth, much less in relation to water level fluctuation.

Schoenoplectus lineolatus produces seeds and tubers as sexual and vegetative overwintering propagules, respectively. In the previous chapter, I found that *S. lineolatus* did not have cleistogamous flowers and could not

produce any seeds underwater. However, the plants that grow in deeper zone and are totally submerged still have chances of seed production. This is because *Schoenoplectus lineolatus* can produce seeds when it emerges into the air due to temporary water drawdown even for relatively short periods. Thus, unlike other aquatic plants, *S. lineolatus* has chances of seed production in the whole range of its depth-distribution from terrestrial to submerged conditions.

In this study, I investigated the success of seed germination and seedling establishment in relation to water depth and analyzed the effects of water level fluctuations on them in comparison with the reproductive success of the tubers in *S. lineolatus*. This leads to understanding the whole life cycle through sexual means under fluctuating water level in *S. lineolatus*.

MATERIALS AND METHODS

Field experiment

Field experiment was conducted in an irrigation pond, Shin-ike Pond (Satani, Kasai City, Hyogo Prefecture), which was the experimental pond of chapter 2, in 2001.

Schoenoplectus lineolatus distributed up to about 2.5 m water depth when the pond was nearly filled with water (chapter 6). The seeds were collected from sixteen plants of *S. lineolatus* cultivated under the terrestrial

condition in containers set in the field. Mature seeds fell on the sediment in the containers and left until next spring in the field. Tubers were also collected from the pond on 4th April 2001 and were kept outdoors under the dark and submerged condition to prevent germination.

Field experiments were planned to examine the responses of germination and establishment of both seeds and tubers in relation to water depth. For the germination experiment, the well-mixed sediment with seeds was divided into 72 plastic petri dishes (9 cm in diameter, 1.5 cm in height) and the sediment surface was adjusted to 0.7 cm in height. Eleven tubers were covered with 0.7 cm sediment layer in each of other twelve plastic petri dishes. Seventy two and twelve petri dishes for seeds and tubers were randomly set into twelve plastic trays (area = 22.5 cm × 30 cm, height = 7 cm) for each tray to include six petri dishes for seeds and one for tubers. The seven petri dishes were randomly placed within each tray. Three trays were placed at each of four water depths (0, 0.3, 0.7 and 1.1 m). Plastic covers with some small holes were used for each petri dish to prevent outflow of the seeds or tubers and for the sediment to keep contact with outside conditions.

For the establishment experiment, one-week-old seedlings were collected from the containers and were transplanted in each of 144 vinyl pots (9 cm in diameter, 7 cm in height) filled with sediment to avoid density effect. One hundred forty four vinyl pots were divided into twelve plastic trays. One-week-old individuals derived from tubers, which had been cultivated in the container, were also planted in each of 120 vinyl pots

filled with sediment. These pots were divided into twelve trays as well. Three trays for seeds and tubers, respectively, were placed by turns in each of same four water depths as the germination experiment. Because germination dates were different between seeds and tubers, the establishment experiment started on 30th April for seeds and on 20th April for tubers.

In these experiments, water depths were those of 6th April when the germination experiment started, and were adjusted within 5 cm at the sediment surface. All sediments used for experiments were collected from the other pond where *S. lineolatus* grew. Water was added to prevent sediment from drying up when trays were exposed to the air.

The numbers of germinating seeds and tubers were recorded, and whether individuals derived from seeds and tubers survived were checked at least once in every two-week. Flowering and tuber production were also checked. This allows us to evaluate their reproductive success. The experiments were continued to 8th November until all individuals at 0 m disappeared. Water level and water temperatures at four water depths were recorded during the experimental period.

Data analyses

After testing for normality and homoscedasticity, the effects of water depth on germination and establishment were analyzed by one-way analysis of variance (one-way ANOVA). When data did not satisfy the assumptions for one-way ANOVA, Kruskal Wallis test was performed.

Normality was tested by Shapiro Wilk test and homoscedasticity by Bartlett's test. If significant effects of water depth were detected, unplanned comparisons among the means were performed using Tukey's HSD test. When data did not satisfy the assumptions for Tukey's HSD test, Scheffe's *F* test was performed. Scheffe's *F* test was performed in StatView 5.0 (SAS Institute Inc., USA) and the other tests were performed in JMP(R) 4.0.5 (SAS Institute Inc., USA).

RESULTS

Environmental conditions

Water level decreased temporarily from August to early September and the minimum level, -0.818 m, was recorded on 7th August (Fig. 14). Thus, 0 m site was always exposed to the air, and 0.3 and 0.7 m sites were exposed to the air during some period. In contrast, 1.1 m site was always submerged. On August, water temperatures reached the peak at four water depths (Fig. 14). They were 37.5°C, 38.5°C, 35.5°C, and 29.0°C at 0, 0.3, 0.7 and 1.1 m. Water temperatures tended to decrease with increasing water depth throughout the experiments.

Germination at four water depths

Many seeds germinated from April to early May within about four weeks after the experiment started at four water depths (Fig. 15A). At 0.3

and 0.7 m, seeds germinated newly in response to exposure to the air due to water drawdown in August and early September. The cumulative number of germinating seeds increased by 50% at 0.3 m and by 114% at 0.7 m. The final cumulative number of germinating seeds were over 3.2 times higher at 0 m than at 0.3, 0.7 and 1.1 m, and significant differences were detected (Fig. 15A; Kruskal Wallis test, $H = 8.197$, d.f. = 3, $P < 0.050$). The final cumulative number of germinating seeds at 0.3 and 0.7 m showed similar values and there was no significant difference. It was over 3.4 times higher than at 1.1-m, but there were no significant differences.

On the other hand, most of tubers germinated in two weeks within April, and the final cumulative percentage of geminating tubers was 100% at four water depths (Fig. 15B).

Establishment and reproductive success at four water depths

The survival rate of individuals derived from seeds decreased gradually with the lapse of days, and no one died after September at four water depths (Fig. 16A). At the period of water drawdown, it was rather constant at 0.3 and 0.7 m. The final survival rate of individuals derived from seeds was the highest at 0 m (58.3%) and the lowest at 0.3 m (11.1%). It decreased with increasing water depth, except at 0.3 m, but there were significant differences only between 0 and 0.7 m and 0.3 m (Fig. 16A; one-way ANOVA; $F_{3,8} = 7.322$, $P < 0.050$).

Individuals derived from tubers died only in July and early August at 0 and 1.1 m (Fig. 16B), and no one died at 0.3 and 0.7 m at the period of

drawdown. The final survival rate of individuals derived from tubers was very high at four water depths, ranging from 90 to 100%, and there was no significant difference (Fig. 16B; Kruskal Wallis test; $H = 2.212$, d.f. = 3, $P = 0.530$).

Schoenoplectus lineolatus grow to 7-25 cm in height (Kadono, 1994). In the establishment experiment, the individuals at 0, 0.3 and 0.7 m were exposed to the air from August to early September, but not at 1.1 m (Fig. 14). While all individuals derived from seeds flowered at 0 m, the percentage of flowering individuals decreased at 0.3 and 0.7 m (Table 6). In contrast, all individuals derived from tubers flowered at 0, 0.3 and 0.7 m (Table 6). Both individuals derived from seeds and tubers produced tubers at four water depths (Table 6).

DISCUSSION

Seed germination and seedling establishment in relation to water depth

So far, little work has been done on favorable water depths for establishment from seeds in amphibious plants. For example, Szmeja (1987) found that amphibious *Lobelia dortmanna* established frequently from seeds under water. But its optimal water depth for establishment is not known.

In *S. lineolatus*, seed germination and seedling establishment occurred most frequently under the terrestrial condition (0m). Under the submerged

conditions (0.3, 0.7 and 1.1 m), the number of germinating seeds highly decreased. In contrast, all tubers germinated and almost all of the individuals derived from tubers survived at four water depths. These results suggest that establishment from seeds is restricted to terrestrial or shallower sites, and establishment from tubers dominates under water in natural populations.

However, with the drawdown, seeds could germinate newly even at usually submerged sites. In our study pond, the number of germinating seeds increased by 50% and 114% at 0.3 and 0.7 m sites, respectively, with the drawdown from August to early September. A similar pattern of seed germination has been found in flood-tolerant *Rumex* species, *R. crispus* and *R. palustris* (Voeselek and Blom, 1992). These species inhabit in the habitats with erratic and catastrophic summer floods in river flood-plains, and are characterized by late spring germination and multiple post-flood germination. Both *S. lineolatus* and *Rumex* species experience unpredictable fluctuations of water level in their habitats. Germination in response to drawdown may be an adaptation to establishment from seeds in such environments.

On the other hand, the final survival rates of individuals derived from seeds tended to decrease with increasing water depth in *S. lineolatus*. This suggests that the probability of establishment from seeds still decreases with increasing water depth after water level increases and the plants become submerged again. Nielsen and Sand-Jensen (1997) have found negative effects of water level change from submerged to terrestrial

conditions on plant growth in an amphibious *Littorella uniflora*. In this study, the survival of individuals derived from seeds and tubers was not affected by water level fluctuations.

Germination in response to drawdown may be stimulated by high temperature in summer. Baskin et al. (1993) have examined annual dormancy cycles of seeds at different temperature regimes in summer annual mudflat species of Cyperaceae which grow in habitats with unpredictable fluctuations of water level. The results suggested that high temperature stimulated seed germination at exposed sediments in summer. In this study, water temperatures were over 30°C at 0.3 and 0.7 m sites at the time of emergence due to drawdown in summer.

In this study, survival rate of the individuals derived from seeds was the lowest at 0.3 m site. Such a decrease at 0.3 m had been also found in clonal growth and vegetative reproduction of this species in our another experiment which was carried out in the same pond in 2000 (chapter 2). These findings strongly suggest that there is any factor to reduce plant performance in shallower water in our study pond. Spence (1982) and Keddy (1982, 1983) demonstrated that wave exposure affects littoral vegetation around the shoreline. The site at 0.3m was nearer to the shoreline than those at 0.7 and 1.1 m, and then wave exposure may have reduced the survival of the individuals derived from seeds of *S. lineolatus*. On the other hand, filamentous algae were observed around the site of 0.3 m in this study (Ishii, unpublished observation), as also seen in chapter 2. Therefore, it may have been alternative factor for the reduction in the

survival rate of the individuals derived from seeds.

Sexual and vegetative reproductions of individuals derived from seeds

All of the individuals derived from tubers flowered when they were exposed to the air, and also produced tubers irrespective of their water depths. On the other hand, individuals from seeds did not always flower when they were exposed to the air, although all of them produced tubers. Because seeds are smaller than tubers in initial size, seedlings might usually grow slower than juveniles derived from tubers. As a result, it is probable that the plants derived from seedlings are of smaller size and flowering is limited due to the size-dependence of sexual reproduction (chapter 3), as compared with tuber-derived plants.

Thus, individuals derived from seeds placed a higher priority on tuber production against seed production in *S. lineolatus*. Such a priority on production of vegetative propagules is found in a submerged plant, *Vallisneria americana* (Titus and Hoover, 1991), but is not found by amphibious *Lobelia dortmanna* which cannot propagate vegetatively without first flowering (Farmer and Spence, 1987a). Vegetative propagules have the greater capacity for establishment than seeds (this study; Silander, 1985) and, therefore, the higher priority on production of vegetative propagules may suggest that it is important for individuals derived from seeds to establish and propagate locally (Silander, 1985). On the other hand, *L. dortmanna* populations are exclusively maintained through sexual reproduction under water and vegetative propagation is of minor

importance (Farmer and Spence, 1987a; Szmeja, 1987). The higher priority on seed production of *L. dortmanna* appears to fit such mechanisms of maintaining persistent populations.

Life cycle through sexual means under fluctuating water level

Results of this study show that establishment from seeds of *S. lineolatus* is restricted to the shore, but that it could occur even in usually deeper zones in case of temporary drawdown. Although sexual reproduction is usually inhibited under water, *S. lineolatus* can also produce seeds in response to temporary exposure to the air in deeper zones (chapter 3). Therefore, I conclude that *S. lineolatus* have chances of life cycle through sexual means in the whole range of its depth distributions under fluctuating water level. When the populations stay submerged, they are maintained through vegetative means. Water level fluctuations are often large and unpredictable in the habitats of amphibious plants such as *S. lineolatus*. They appears to maintain persistent populations successfully through sexual and vegetative means (see Brock, 1991; Robe and Griffiths, 1998). This is apparently different from that of amphibious plants with cleistogamous flowers, such as *Lobelia dortmanna*, in which establishment from sexual means dominates under water (Farmer and Spence, 1987a; Szmeja, 1987).

Chapter 5 Water dispersal of seeds

INTRODUCTION

With the emergence into the air due to temporary drawdown, *Schoenoplectus lineolatus* could produce seeds at sites of deep water depth (chapter 3). On the other hand, the results of chapter 4 suggested that *S. lineolatus* may frequently establish from seeds along the shore. When it produces seeds at sites of deep water depth in response to temporary drawdown, such seeds may have chances to establish around the shoreline if they can be dispersed there by water. Or, *S. lineolatus* may wait until next drawdown because it may establish from seeds in response to temporary drawdown (chapter 4). In this case, water dispersal of seeds is not required. In this chapter, I investigated the role of water dispersal of seeds in maintaining persistent populations.

Vascular aquatic plants grow in water and, therefore, seeds will frequently fall in water by gravity unless they are adapted for the dispersal by animals or wind. In such vascular aquatic plants, water is the most significant seed dispersal agent. Potentials for water dispersal of seeds (hydrochory) are determined by degrees of buoyancy (Skoglund, 1990; Nilsson et al., 1991). The degrees of buoyancy are different among species (Parker and Leck, 1985; Smits et al., 1989; Coops and Van der Velde, 1995) and may characterize life-history strategies of the species.

Water dispersal of seeds may influence population dynamics and/or metapopulation dynamics. Seeds with low buoyancy are locally dispersed and may contribute to maintaining populations (Grelsson and Nilsson, 1991; Titus and Hoover, 1991). On the other hand, seeds with high buoyancy can travel long distances among populations within metapopulation in a water system (Skoglund, 1990; Titus and Hoover, 1991; Kudoh and Whigham, 2001). When other dispersal agents for seed such as animals and wind play an important role, the degrees of buoyancy may not necessarily reflect the capacity of dispersal (Nilsson et al., 1991) and water dispersal will have only minor effects.

Several researchers have found that the establishment from seeds is limited in shallower water depth within adult stands in vascular aquatic plants, such as emergent plants (Rea and Ganf, 1994b; Clevering, 1995), floating-leaved plants (Smits et al., 1990) and amphibious plants (chapter 4, Moeller, 1978). If regeneration from seeds play a role for maintaining populations in those plants, seed dispersal to shallower water depth must be required for establishment (Titus and Hoover, 1991). Coops and Van der Velde (1995) have suggested such dispersal patterns in some emergent plant species. However, whether this trait is common in other aquatic plants remains to be investigated.

Schoenoplectus lineolatus produces seeds with a length of 1-2 mm which cannot be dispersed by wind and fall by gravity. It is not known whether they can be dispersed by animals or not. Although *S. lineolatus* can survive under water, it is suggested that both seed production and

establishment from seeds may frequently occur along the shore in natural populations (chapters 3 and 4). Nonetheless, they also occur even at deeper water sites when there is the emergence into the air due to temporary water drawdown. In such a case, are seeds produced transported to the shore for the establishment when water level increases again? Or, do those seeds, instead of being transported, wait for next emergence because *S. lineolatus* grows in habitats with water level fluctuations? One directional dispersal to the shore is likely to be attained by water, but not by animals that may behave unpredictably. Thus, water dispersal should be discussed as an important subject.

In this study, I observed seed release from inflorescence and conducted seed-buoyancy experiments in order to elucidate the role of water dispersal in the establishment from seeds in *S. lineolatus*. Because wave and rainfall are common in aquatic environments, I estimated their effects on seed buoyancy.

MATERIALS AND METHODS

Observation and experiments in greenhouse

The release of seeds from inflorescence was observed and seed-buoyancy experiments were conducted for *S. lineolatus* in greenhouse of Kobe University in 2000. Plants with mature seeds were collected from an irrigation pond, Saka-ike Pond (Tamano, Kasai City, Hyogo Prefecture) on

11th September. The plants for seed-release observations were gently sampled as growing state together with substrate. On the other hand, for seed-buoyancy experiments only the plants were collected and seeds were taken out from inflorescences in the laboratory. Seeds were kept in room temperature until the experiments started.

For observation of seed release from inflorescence, plants with mature seeds were planted together with substrate of 2.5 cm depth in each of three plastic pots (18 cm in diameter, 3.5 cm in height) and with substrate of 7 cm depth in each of six plastic pots (18 cm in diameter, 15 cm in height). Plants were set in three water-depth treatments (exposed, emergent and submerged). For exposed treatments, three pots with 15 cm in height were used. For emergent treatments, another three pots with 15 cm in height were placed in larger pots (22 cm in diameter, 29 cm in height) filled with tap water and emergent state (7 cm in water depth) was set by placing pedestals under pots. For submerged treatments, three pots with 3.5 cm in height were also placed in the larger pots filled with tap water. It was observed where the seeds were released, that is, on the exposed soil, on the water surface or in water.

Two seed-buoyancy experiments were conducted. First, possibility of water dispersal of seeds, that is, whether seeds float or not on the water surface, was tested in four treatments: releasing of seeds above 1 cm from water surface, releasing of seeds below 1 cm from water surface, putting of seeds on exposed soil surface and increasing water level by pouring water from side, putting of seeds on exposed soil surface and increasing water

level by setting under rainfall. In former two treatments, the relationships between buoyancy and the position of releasing seeds were investigated. In the latter two treatments, the relationships between buoyancy and increasing water level under two different conditions by direct exposure to rainfall or not were investigated. Pouring water from side simulated the inflow of water into the habitat after raining upstream. In each treatment, three plastic pots (16 cm in diameter, 19 cm in height) and 100 seeds per pot were prepared. Former two treatments were performed using pots filled with tap water. Latter two treatments were performed using pots with 100 seeds sown on soil. Soil surface was adjusted at 1.5 cm height. In a set of pots, water was poured from side. Other set of pots were exposed to rainfall directly (second rainfall on 30th September for Table 7). In each treatment number of floating seeds was counted.

Secondly, effects of wave and rainfall on buoyancy were investigated in three treatments: standing, stirred on occasion of every measurement and exposed to rainfall. In each treatment, three plastic pots with 100 floating seeds and filled with tap water were prepared. Wave was simulated by stirring water in the pots by glass rod at 50 times per 30 seconds on occasion of every measurement. In rainfall treatment, pots were exposed to rainfall outside at three times (Table 7: on 26th and 30th September and on 6th August) and each exposure was performed for 24 hours from noon. Pots were covered except for the measurement times. In each treatment, number of floating seeds was counted at least once six days for 103 days.

All of the observation and experiments were performed or started on

23rd September. Precipitation data of Kobe and Rokko-san meteorological stations (http://www.jma.go.jp/JMP_HP/jma/indexe.html) were used for Kobe University that is located at the midpoint of both sites (Table 7).

Data analyses

Effects of the position to release of seeds and of increasing water level to initial buoyancy were tested by Kruskal Wallis test, after testing assumptions of normality and homoscedasticity. When significant effects of treatments were detected, unplanned comparisons among the treatments were performed using Scheffe's *F* test. Effects of rainfall (for first trial on 26th September) to buoyancy were tested by a Mann Whitney test because the data did not satisfy assumptions for *t* test. Normality and homoscedasticity were tested by Shapiro Wilk test and by Bartlett's test, respectively. Shapiro Wilk test and Bartlett's test were performed in JMP(R) 4.0.5 (SAS Institute Inc., USA) and the other tests in StatView 5.0 (SAS Institute Inc., USA).

RESULTS

Seed release from inflorescence

The release of seeds from inflorescence was observed in three water-depth treatments (exposed, emergent and submerged). The results are summarized in Table 8. Under the exposed condition, seeds were released

only onto the exposed soil. Under the emergent condition, seeds were released onto the water surface or in water when culms fell down. Under the submerged condition, seeds were released in water. Thus, the position of seed release shifted from the exposed soil to the water surface and finally in water with increasing water depth.

Seed buoyancy

There was no seed germination during the experiments. Seed buoyancy varied significantly among four treatments (Table 9; Kruskal Wallis test, $H = 9.962$, d.f. = 3, $P < 0.050$). Seeds released in air stayed floating on the water surface, but most seeds released under water did not float. On the other hand, seeds released on the exposed soil could float when water level increased due to pouring water from side, but when exposed to rainfall, they did not float even if water level increased.

The percentage of floating seeds in standing water decreased gradually with the lapse of days up to the 41st day, but slightly decreased beyond that day (Fig. 17). The percentage of floating seeds in standing water was 32.3% at 103rd day. In contrast, percentage of floating seeds in stirred water rapidly decreased, and the maximum duration of seed buoyancy was 20 days. Furthermore, the seeds sank much sooner in exposure to rainfall than in standing water (Fig. 17), and almost all of the seeds sank in first rainfall (Fig. 18; t test, $t = 118.149$, $P < 0.001$), despite a little precipitation of only 1.0 mm within 24 hours which was the mean value in Kobe and Rokko-san (Table 7). After exposures to rainfall three times, there were no

floating seeds.

DISCUSSION

Seed buoyancy of S. lineolatus

In this study, seeds released in air floated up to 100%, but only 3% when released in water in *S. lineolatus*. This result indicates that once seeds sink, they have little chance to float up on the water surface. Accordingly, seeds released on the water surface can float and there are chances of water dispersal, but much less chances when they are released in water although strong currents may disperse even such seeds. Seeds of *S. lineolatus* are small (with a length of 1-2 mm), and appear to float by using surface tension (Howe and Smallwood, 1982).

Seeds floating on the water surface may be exposed by wave and/or rainfall. In the experiment to simulate wave action, the result showed that wave highly reduced the duration of seed buoyancy in *S. lineolatus*. This suggests that water dispersal of seeds may be limited in wavy environment. Such effects of wave on seed buoyancy have been found in other aquatic plants, *Scirpus lacustris*, *Typha angustifolia* and *Typha latifolia* (Ridley, 1930; Coops and Van der Velde, 1995). Considering the common wave action in aquatic environments, it may be not negligible in water dispersal. On the other hand, in the experiment for exposure to natural rainfall, a little rainfall, e.g. 1 mm within 24 hours, made almost all of the seeds sunken in

S. lineolatus. Smits et al. (1989) have also found that the duration of seed buoyancy strongly reduced by pouring water to simulate rain in three floating-leaved species, *Nymphoides peltata*, *Nymphaea alba* and *Nuphar lutea*. These findings strongly suggest that rainfall as well as wave action may be not negligible in water dispersal of seeds for aquatic plants.

Despite limitations by wave and/or rain, several researchers have suggested that water dispersal may play an important role in the establishment from seeds (Parker and Leck, 1985; Coops and Van der Velde, 1995). Hart and Cox (1995) released fruits of *Nuphar luteum* in lakes and observed water dispersal. The result was that many fruits moved about 20 m to 134 m within a few hours. Although this does not apply directly to the seeds, seed buoyancy even with short periods might be enough to function for water dispersal. Assuming that the wave simulation in this study was natural, seeds of *S. lineolatus* are expected to float for 20 days at maximum in field exposed to wave. Perhaps, *S. lineolatus* could disperse seeds by water not only for a short distance but also for a long distance.

The role of water dispersal in establishment from seeds

In *S. lineolatus* both seed production and establishment from seeds are inhibited under submerged conditions (chapters 3 and 4). When the water level is constant, they will occur along the shore, but not in deeper water zone. Along the shore, plants become exposed or emergent. The observation of seed release from inflorescence in this study showed that the

positions of seed release of *S. lineolatus* were on the exposed soil for plants under the exposed condition, and both on the water surface and in water when culms fell down for plants under the emergent condition. Due to seed buoyancy, even the seeds released on the water surface under the emergent condition have chances to be transported to the exposed soil. Accordingly, water dispersal will lead to greater establishment from seeds along the shore in *S. lineolatus*.

On the other hand, both seed production and establishment from seeds occur in deeper water zone if there is the emergence into the air due to temporary drawdown in *S. lineolatus* (chapters 3 and 4). Such water level fluctuations are likely to be common in habitats of amphibious plants (Casanova and Brock, 2000). In that case, the seeds that are produced and are released on the exposed soil may also experience increasing water level. Results of our experiment of increasing water level showed that pouring water from side kept most of seeds floating on the water surface, but in direct rainfall most of seeds did not float. Pouring water from side simulated the inflow of water into the habitat after raining upstream. Therefore, when there is such an increase of water level, the seeds produced in deeper water zone may be transported to the shore, resulting in the establishment. In direct rainfall, however, the seeds will never be dispersed by water and will wait for next emergence for establishment.

In conclusion, both water dispersal and no water dispersal of seeds to the shore were suggested in *S. lineolatus*. In other amphibious plant, *Lobelia dortmanna*, which has cleistogamous flowers, the seeds are with

little buoyancy (Farmer and Spence, 1987a; Szmeja, 1987). *Lobelia dortmanna* can not only produce seeds but also establish from seeds under water. The lack of seed buoyancy, therefore, is adaptive for the establishment. Unlike this species, *S. lineolatus* with few chances of establishment from seeds under water may have more chances of establishment on the exposed soil both after water dispersal to the shore and after temporary water drawdown.

Chapter 6 Amphibious plants and water level fluctuations in Shin-ike Pond

INTRODUCTION

I have showed the mechanism of maintaining persistent populations of *Schoenoplectus lineolatus* in previous chapters. The aim of this chapter is to elucidate how this mechanism functions in the natural population. I investigated the distributions of plants including *S. lineolatus* in an irrigation pond and some factors determining their distribution, and I inferred how to maintain the persistent population of *S. lineolatus*.

Amphibious plants can survive both under the terrestrial condition and under the submerged condition, and have physiological adaptations to life under water level fluctuations (Braendle and Crawford, 1999). Casanova and Brock (2000) have demonstrated, in the greenhouse experiment, that water level fluctuations in establishment of plant communities resulted in dominance of amphibious plants.

Nonetheless, there are few studies on the distributions of amphibious plants in natural communities and on the factors determining their distributions. In particular, various factors such as water depth (Spence, 1982; Casanova and Brock, 2000), water chemistry (Riis et al., 2000; Vestergaard and Sand-Jensen, 2000), wave exposure (Keddy, 1983) and competitive interaction (Grace and Wetzel, 1981) as well as water level

fluctuations may affect the distributions of aquatic vascular plants. Hence, amphibious plants may be dominant due to additional environmental and/or biological factors, despite less fluctuations of water level.

In this study, in order to estimate the importance of water level fluctuations in the dominance of amphibious plants in a natural plant community, I investigated plant distribution and water level fluctuations, water depth and some chemical variables as environmental factors determining its distribution in an irrigation pond, Shin-ike Pond. In Japan amphibious plants often grow in irrigation ponds where water level fluctuates due to use of water for irrigation purpose, and the dominance of amphibious plants has been observed in Shin-ike Pond. I also investigated the relationships in distribution between an amphibious plant, *Schoenoplectus lineolatus*, and other aquatic plant species, and discussed its mechanism for maintaining a population in the study pond.

MATERIALS AND METHODS

Investigation of vegetation

The vegetation was surveyed on 18-24th November 2000 in an irrigation pond, Shin-ike Pond (Satani-cho, Kasai City, Hyogo Prefecture; secchi-depth = 2.01 m on 16th August 2000) when the pond was nearly filled with water. Three transects with 40 m length were placed in parallel five meters away from each other, according to pond size. Eleven quadrates

(50×50 cm) on each transect were set at the exposed (Ex) and flooded side (Fl) on the shoreline and 2.5, 5, 7.5, 12.5, 17.5, 22.5, 27.5, 32.5 and 37.5 m from the shoreline. Species composition, coverage (%) and water depth were examined for all quadrates, by diving survey. Water depths are described on the basis of 0 m of the shoreline on 18th November and shown as the means of the depths of four corners of each quadrate. Culm density was measured for *Schoenoplectus lineolatus* using quadrates with smaller size (25×25 cm) in each quadrate.

Environmental measurements

The water level fluctuations, pH and conductivity were examined as environmental factors that might affect the species composition. Both pH and conductivity were used to estimate trophic state of the study pond. Because the water level may change within and/or between years, it was recorded for about three years, from 30th July 1999 to 2nd August 2002. Both pH and conductivity also may change within a year and, therefore, they were measured for about one year, from 22nd November 1999 to 21st November 2000, using a twin pH Model B212 compact pH meter (Horiba Co.) and a personal SC meter Model SC82 conductivity meter, Yokogawa (Electric Co.), respectively.

RESULTS

Environmental conditions

The water level was nearly constant and decreased up to -0.811 m only from August to early September 2001 in Shin-ike Pond for three years (Fig. 19). On the other hand, both pH and conductivity did not show any seasonal changes remarkably, and the means \pm SD were 6.95 ± 0.48 ($n = 45$) and 68.75 ± 6.66 μ S/cm ($n = 46$), respectively for one year (Fig. 20).

Plant distribution in Shin-ike Pond

Figure 21 and Table 10 show water depth, species composition and coverage along three transects. Ten terrestrial and emergent species, one floating-leaved species, one submerged species and six amphibious species were observed in Shin-ike Pond. Water depth increased gradually with increasing distance from the shoreline in three transects in a similar way, and was 2.7, 3.1 and 3.3 m deep at 37.5 m distance from the shoreline in transects 1, 2 and 3, respectively. Total coverage of plants decreased once at the sites of 2.5 m from the shoreline in three transects. Water depth at these sites ranged from 0.35 to 0.45 m deep. Overall, terrestrial and emergent plants distributed dominantly along the shoreline. They had low or no coverage at sites of 2.5 m from the shoreline, and did not distributed beyond 2.5 m from the shoreline. On the other hand, amphibious plants including *Schoenoplectus lineolatus* distributed dominantly in water where emergent plants had low or no coverage. In transect 1, amphibious plants had no coverage along the shoreline, and they distributed dominantly from 2.5 to 7.5 m from the shoreline. Water depth at these sites ranged from 0.45

to 0.99 m deep. From 12.5 to 37.5 m from the shoreline, amphibious plants had low or no coverage, and only one submerged species, *Potamogeton getonoctandrus*, occurred with low coverage. In transect 2, amphibious plants also had low coverage along the shoreline, and they distributed dominantly from 2.5 to 17.5 m from the shoreline. Water depth at these sites ranged from 0.40 to 2.02 m deep. From 22.5 to 37.5 m from the shoreline, only *P. getonoctandrus* occurred with low or no coverage. In transect 3, amphibious plants were absent in the exposed side of the shoreline, and they distributed dominantly from the flooded side of the shoreline to 22.5 m from the shoreline. Water depth at these sites ranged from 0 to 2.47 m deep. From 27.5 to 37.5 m from the shoreline, only *P. getonoctandrus* occurred with low or no coverage. One floating-leaved plant, *Potamogeton distinctus*, occurred with low coverage only in transect 2. Thus both floating-leaved and submerged plants had very low coverage as a whole.

Schoenoplectus lineolatus tended to distribute dominantly in deeper water than other amphibious plants (Fig. 21, Table 10). Namely, this species distributed from 0.35 to 2.47 m water depth, while other amphibious plants distributed from 0 to 1 m water depth. Culm density tended to increase with increasing water depth although it decreased beyond its peak in *S. lineolatus* (Fig. 22). In contrast, number of species except *S. lineolatus* tended to decrease with increasing distance from the shoreline in three transects in Shin-ike Pond (Fig. 23).

DISCUSSION

Distribution of amphibious plants

In Shin-ike Pond, terrestrial and emergent plants distributed only along the shoreline, and both floating-leaved and submerged plants had very low coverage. In contrast, amphibious plants distributed in gaps of stands of terrestrial and emergent plants, if any, along the shoreline and were rather dominant in water. Although Casanova and Brock (2000) have showed that water level fluctuations in establishment resulted in the dominance of amphibious plants, the water level was rather constant during three years investigated in Shin-ike Pond. This does not suggest that the dominance of amphibious plants in water may have been caused by frequent fluctuations of water level in Shin-ike Pond. The dominance of terrestrial and emergent plants, instead of amphibious plants, along the shoreline also suggests little effect of water level fluctuations on the plant community in Shin-ike Pond.

On the other hand, pH and conductivity indicates that Shin-ike Pond is an oligotrophic pond. Moreover, most of amphibious plants dominating in Shin-ike Pond are isoetids (sense Den Hartog and Segal (1964); e.g. *Eriocaulon nakkasimanum*, *Eleocharis acicularis*, *Isoetes japonica* and *Schoenoplectus lineolatus* in this study) that typically have rosettes with a short stem and well-developed roots. Isoetids are the aquatic plants that show adaptations in growth for nutrient- and carbon- poor environments (Sand-Jensen and Søndergaard, 1979; Boston, 1986; Boston and Adams,

1987). It is, therefore, likely that low nutrients may have inhibited propagation of other aquatic plants in water and that amphibious isoetids may have been dominant mainly due to low nutrient condition in Shin-ike Pond. Sand-Jensen and Søndergaard (1979) reported a similar distribution pattern of aquatic plants in oligotrophic Lake Kalgaard in Denmark where the emergent and floating-leaved plants poorly developed and an amphibious isoetid, *Littorella uniflora*, was dominant due to low nutrient condition. Although Shin-ike Pond showed less fluctuations of water level in the study period, such fluctuations may be still observed in the long term. In this context, long-term studies of vegetation dynamics will be needed enough to know the effects of water level fluctuations. In Shin-ike Pond, however, the dominance of amphibious plants did not necessarily require water level fluctuations at least in the short term.

Total coverage for all plants decreased at sites of 2.5 m from the shoreline in three transects in Shin-ike Pond. Spence (1982) and Keddy (1982, 1983) have provided evidence that wave exposure affected shoreline vegetation. Spence (1982) found that in case of Loch Maberry the soil was sandy in the shore that was exposed to wave and possessed a reduced plant cover. In Shin-ike Pond, the sites of 2.5 m were covered by sandy soil instead of silty and/or muddy soils (Ishii, unpublished observation) and, therefore, wave may have decreased total coverage at the sites of 2.5 m.

Distribution of Schoenoplectus lineolatus

In chapter 2, I have found that *S. lineolatus* showed optimal growth

under the terrestrial condition, but not under the submerged condition. Nonetheless, it was dominant in deeper water as compared with other amphibious plants in Shin-ike Pond. Number of species tended to be small in deeper water, and this may suggest that *S. lineolatus* was excluded by interspecific competition in shallower water with more competitors and was dominant in deeper water with less or no competitors.

In *S. lineolatus* both seed production and establishment from seeds are inhibited under the submerged condition, but they occur even in deeper water zone when there is emergence into the air due to temporary drawdown (chapters 3 and 4). Accordingly, *S. lineolatus* has limited chances of the establishment from seeds in growing depth in Shin-ike Pond because of less water drawdown (Fig. 24). On the other hand, tuber production and establishment from tubers occur under the submerged condition as well as under the terrestrial condition in *S. lineolatus* (chapters 2 and 4) and, therefore, they will vegetatively maintain the persistent population at the regular years of high water level in Shin-ike Pond (Fig. 24). Brock (1991) has suggested a similar mechanism for maintaining persistent populations in *Myriophyllum varriifolium* growing in a shallow Australian lake. The capacity for the reproduction and establishment at the period of temporary water drawdown is common between both species.

In conclusion, it was suggested that the dominance of amphibious plants might not necessarily require frequent fluctuations of water level. In Shin-ike Pond, low nutrients may have inhibited propagation of other aquatic plants and, therefore, amphibious isoetids were dominant in water.

In such a habitat, *S. lineolatus* appears to maintain the persistent population in deeper water due to the establishment from seeds at the period of drawdown with limited chances and vegetative reproduction thereafter, despite stressful environment.

CONCLUSION

The goal of this paper was to clarify how to maintain persistent populations in an amphibious plant without the ability of sexual reproduction under water. In this study, *Schoenoplectus lineolatus* showed the highest growth and reproduction under the exposed condition. It also established most frequently from seeds and tubers under that condition. These results show that the terrestrial condition is most favorable in both life cycles through sexual and vegetative reproductions in *S. lineolatus*. However, remarkable difference between both life cycles is revealed in responses to a water-depth gradient. In the sexual life-cycle, seed production always fails under water, and therefore there is no sexual life-cycle beyond the water depth where culms are totally submerged. In contrast, the vegetative life-cycle always occurs under both the exposed and submerged conditions, although tuber production tends to decrease with increasing water depth.

These findings suggest that persistent populations should be maintained through both sexual and vegetative life-cycles around shoreline, and only through the vegetative life-cycle in deeper water in *S. lineolatus*. On the other hand, the results of this study also showed that both seed production and establishment may occur in response to exposure to the air due to temporary drawdown in *S. lineolatus*. Such temporary drawdown will induce the sexual life-cycle even at sites which are usually in deep water.

Seeds of *S. lineolatus* may be dispersed to shore by water when seeds

are released onto the water surface or when seeds are released on the exposed soil and water level increases due to the inflow of water after raining upstream. However, seeds will remain in positions of release when seeds are released in water or are exposed to rainfall. Such seeds will wait for next exposure into the air due to temporary drawdown for establishment. This seed dispersal pattern may enhance establishment from seeds in environments with the unpredictable fluctuation of water level in *S. lineolatus*.

This mechanism of maintaining persistent populations of *S. lineolatus* has a similarity to that of emergent plants in two ways. First, both growth and reproduction are the highest around the shoreline. Second, the sexual life-cycle is limited around the shoreline when the water level do not fluctuate. Consequently, when there is no fluctuation of water level, both plants should differ only in the ability to survive and to maintain persistent populations through the vegetative life-cycle under water. When water level fluctuates, the areas where seed production and establishment occur will expand within a population according to the exposure to the air resulting from temporary drawdown in *S. lineolatus*. In conclusion, this paper suggested that *S. lineolatus* has both the mechanism of maintaining persistent populations through the vegetative life-cycle when completely submerged and the one through the sexual life-cycle when exposed to the air due to temporary drawdown, in addition to the mechanism of maintaining populations of emergent plants.

Habitats of amphibious plants including *S. lineolatus* are not

necessarily the environments where water level frequently fluctuates. For amphibious plants may dominate in oligotrophic habitats if they are isoetids, which are considered to be adaptive in growth for nutrient- and carbon- poor environments, at the same time. In such a case, whether the water level fluctuations have effects on the dominance of amphibious plants or not remains to be investigated. Only monitoring population dynamics in the long term will provide its answer.

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Table 1. Maximum ramet density of *Schoenoplectus lineolatus* ($n = 8$) at three water depths. Values are shown as means and the lower and upper 95% confidence limits (in parentheses). All values are back transformed. Different letters show significant differences between water depths due to Games Howell test ($P < 0.05$)

Water depth	Maximum ramet density (m^{-2})
0 m	4876 (3025 - 6726) a
0.3 m	1380 (791 - 1968) b
0.7 m	2452 (1649 - 3255) ab

Table 2. Percentages of individuals producing propagules (seeds and tubers) at three water depths in *Schoenoplectus lineolatus*.

Water depth	Individuals producing propagules (%)	
	Seed	Tuber
0 m	100	100
0.3 m	0	100
0.7 m	0	100

Table 3. Tuber production of *Schoenoplectus lineolatus* at three water depths. Total dry weight of tubers of each individual is shown as the means \pm SE. For number of tubers and dry weight per tuber, values are the means, and lower and upper 95% confidence limits are shown in parentheses. For number of tubers and dry weight per tuber, all values are back transformed. Different letters show significant differences between water depths due to Games Howell test for total dry weight of tubers and dry weight per tuber and due to Tukey's HSD test for number of tubers ($P < 0.05$).

Water depth	Total dry weight of tubers (mg)	Number of tubers	Dry weight per tuber (mg)
0 m	8173 \pm 1845 a (n = 8)	372 (201 - 543) a (n = 8)	21.0 (20.4 - 21.6) a (n = 2978)
0.3 m	493 \pm 46 b (n = 8)	57 (33 - 81) b (n = 8)	8.6 (7.9 - 9.3) b (n = 456)
0.7 m	580 \pm 69 b (n = 8)	90 (63 - 117) b (n = 8)	6.3 (6.0 - 6.7) c (n = 720)

Table 4. Flower number per inflorescence of *Schoenoplectus lineolatus* and sample size (*n*). Means \pm SD and ranges (in parentheses) are shown in ten ponds of Kasai and Kakogawa Cities, Hyogo Prefecture. Note that same pond names are often used for different ponds. (0): No production of inflorescence, (–): data unavailable.

Pond	Harvest time			
	4th August	31th August	2nd October	5th November
Kasai City				
Oku-ike	0	12.4 \pm 2.2	9.2 \pm 3.1	9.4 \pm 3.0
Hata		(9 - 17) <i>n</i> = 20	(5 - 18) <i>n</i> = 20	(6 - 18) <i>n</i> = 20
Saka-ike	10.2 \pm 3.3	11.7 \pm 2.9	10.7 \pm 4.1	9.2 \pm 3.0
Tamano	(6 - 18) <i>n</i> = 20	(8 - 19) <i>n</i> = 20	(5 - 18) <i>n</i> = 20	(5 - 16) <i>n</i> = 20
<i>unnamed</i>	–	0	9.1 \pm 2.4	8.7 \pm 2.3
Toyokura			(4 - 13) <i>n</i> = 20	(6 - 13) <i>n</i> = 20
Naka-ike	–	13.3 \pm 2.9	7.2 \pm 2.6	8.1 \pm 4.2
Toyokura		(6 - 17) <i>n</i> = 20	(4 - 12) <i>n</i> = 20	(4 - 18) <i>n</i> = 11
Sanda-ike	–	0	0	0
Toyokura				
Sendaiko-ike	0	0	0	0
Nojo				
Shin-ike	10.5 \pm 3.3	10.1 \pm 2.6	12.0 \pm 2.6	9.2 \pm 2.9
Uzurano	(6 - 7) <i>n</i> = 20	(6 - 15) <i>n</i> = 20	(9 - 19) <i>n</i> = 20	(5 - 18) <i>n</i> = 20

Table 4. Continued.

Pond	Harvest time			
	4th August	31th August	2nd October	5th November
Kakogawa City				
Oh-ike Shikata-cho, Hara	0	0	13.3 ± 2.7 (8 - 19) <i>n</i> = 20	—
Nobuka-ike Shikata-cho, Hata	0	14.5 ± 3.6 (9 - 23) <i>n</i> = 20	8.8 ± 1.9 (4 - 13) <i>n</i> = 20	10.5 ± 2.8 (4 - 14) <i>n</i> = 20
Kamino-ike Shikata-cho, Hiro	0	7.0 ± 1.5 (5 - 9) <i>n</i> = 20	8.9 ± 3.5 (3 - 15) <i>n</i> = 20	5.5 ± 2.2 (1 - 10) <i>n</i> = 20

Table 5. Seed set rates (% , means) and states of populations (Ex: exposed, Em: emergent, S: submerged) in *Schoenoplectus lineolatus* of ten ponds of Kasai and Kakogawa Cities, Hyogo Prefecture. Samples for Table 4 were used for measurements. Values are back transformed for Shin-ike Pond, Uzurano and are untransformed for the others. Different letters show significant differences among harvest times in each pond due to Scheffe's *F* test ($P < 0.05$). See the caption of Table 4 for other explanations.

Pond	Harvest time			
	4th August	31th August	2nd October	5th November
Kasai City				
Oku-ike	0	70.0	68.5	72.5
Hata	S	Ex	S	S
Saka-ike	65.3	70.0	47.7	46.8
Tamano	Ex, Em, S	S	S	S
<i>unnamed</i>	—	0	29.5	30.8
Toyokura		Em	Em, S	S
Naka-ike	—	76.4 a	50.9 b	14.1 c
Toyokura		Em	S	Ex, Em, S
Sanda-ike	—	0	0	0
Toyokura		S	S	S
Sendaiko-ike	0	0	0	0
Nojo	S	S	Ex	Ex
Shin-ike	51.7 a	80.5 b	88.4 b	71.2 ab
Uzurano	Ex, Em, S	Ex	S	S
Kakogawa City				
Oh-ike	0	0	48.6	—
Shikata-cho, Hara	S	S	Ex	
Nobuka-ike	0	75.9	54.8	66.9
Shikata-cho, Hata	Em, S	Em, S	S	S
Kamino-ike	0	46.2 a	55.8 a	23.7 b
Shikata-cho, Hiro	Ex, Em, S	Ex, Em, S	Ex	Em, S

Table 6. Sexual and vegetative reproductive success of individuals derived from seeds and tubers of *Schoenoplectus lineolatus* at four water depths. Flowering and tuber production show percentages of individuals which flowered and produced tubers, respectively. *n*: sample size.

Water depth	Reproductive success	Individuals derived from seeds		Individuals derived from tubers	
0 m	Flowering	100.0%	<i>n</i> = 21	100.0%	<i>n</i> = 27
	Tuber production	100.0%		100.0%	
0.3 m	Flowering	75.0%	<i>n</i> = 4	100.0%	<i>n</i> = 30
	Tuber production	100.0%		100.0%	
0.7 m	Flowering	88.2%	<i>n</i> = 17	100.0%	<i>n</i> = 30
	Tuber production	100.0%		100.0%	
1.1 m	Flowering	0.0%	<i>n</i> = 11	0.0%	<i>n</i> = 29
	Tuber production	100.0%		100.0%	

Table 7. Precipitation (mm) for 24 hours from noon in each date in Kobe and Rokko-san in Hyogo Prefecture.

	Total	Precipitation per hour		
		average	min	max
Kobe (N34° 41.6'; E135° 12.9')				
First rainfall (26th Sept.)	0.5	-	-	-
Second rainfall (30th Sept.)	14.0	2.0	1.5	3.0
Third rainfall (6th Oct.)	36.0	4.5	1.0	10.0
Rokko-san (N34° 45.6'; E135°, 16.5')				
First rainfall (26th Sept.)	1.5	-	-	-
Second rainfall (30th Sept.)	15.5	2.2	1.0	3.5
Third rainfall (6th Oct.)	62.0	6.2	1.0	18.0

Table 8. The position of seed release from inflorescence under exposed, emergent and submerged conditions in *Schoenoplectus lineolatus*.

Treatment	Seed release
Exposed	on the exposed soil
Emergent	on the water surface or in water when culm fell down
Submerged	in water

Table 9. Seed buoyancy of *Schoenoplectus lineolatus* in different release treatments. Values are means \pm SD. Different letters show significant differences among treatments due to Scheffe's *F* test ($P < 0.05$). $n = 3$ for each of all treatments.

Treatment	Seed buoyancy (%)
Releasing 1 cm above water surface	100.0 \pm 0.0 a
Releasing 1 cm below water surface	3.0 \pm 0.0 b
Putting on exposed soil surface and increasing water level due to pouring water from side	97.7 \pm 2.1 a
Putting on exposed soil surface and increasing water level due to rainfall	1.0 \pm 1.7 b

Table 10. Species composition and depth distribution in Shin-ike Pond in 2000. Data of three transects are pooled. Asterisks show the presence of species.

Species	Distance of quadrat from the shoreline											
	Ex	Fl	2.5m	5.0m	7.5m	12.5m	17.5m	22.5m	27.5m	32.5m	37.5m	
Terrestrial and emergent plants												
<i>Bidens frondosa</i>	*	*										
<i>Carex thunbergii</i>	*	*										
<i>Cyperus brevifolius</i> var. <i>brevifolius</i>	*	*										
<i>Equisetum arvense</i>		*										
<i>Eleocharis congesta</i>	*	*										
<i>Juncus setchuensis</i>	*	*										
<i>Miscanthus sinensis</i>	*											
<i>Monochoria vaginalis</i> var. <i>plantaginea</i>			*									
<i>Sacciolepis indica</i>		*										
<i>Solidago altissima</i>	*											
Floating-leaved plant												
<i>Potamogeton distinctus</i>					*							
Submerged plant												
<i>Potamogeton octandrus</i>			*		*	*	*	*	*	*	*	*
Amphibious plant												
<i>Eriocaulon nakasimanum</i>			*		*							
<i>Eriocharis acicularis</i> var. <i>longiseta</i>			*	*	*							
<i>Isoetes japonica</i>		*	*	*	*							
<i>Lobelia chinensis</i>	*	*										
<i>Ludwigia ovalis</i>	*	*										
<i>Schoenoplectus lineolatus</i>			*	*	*	*	*	*	*			

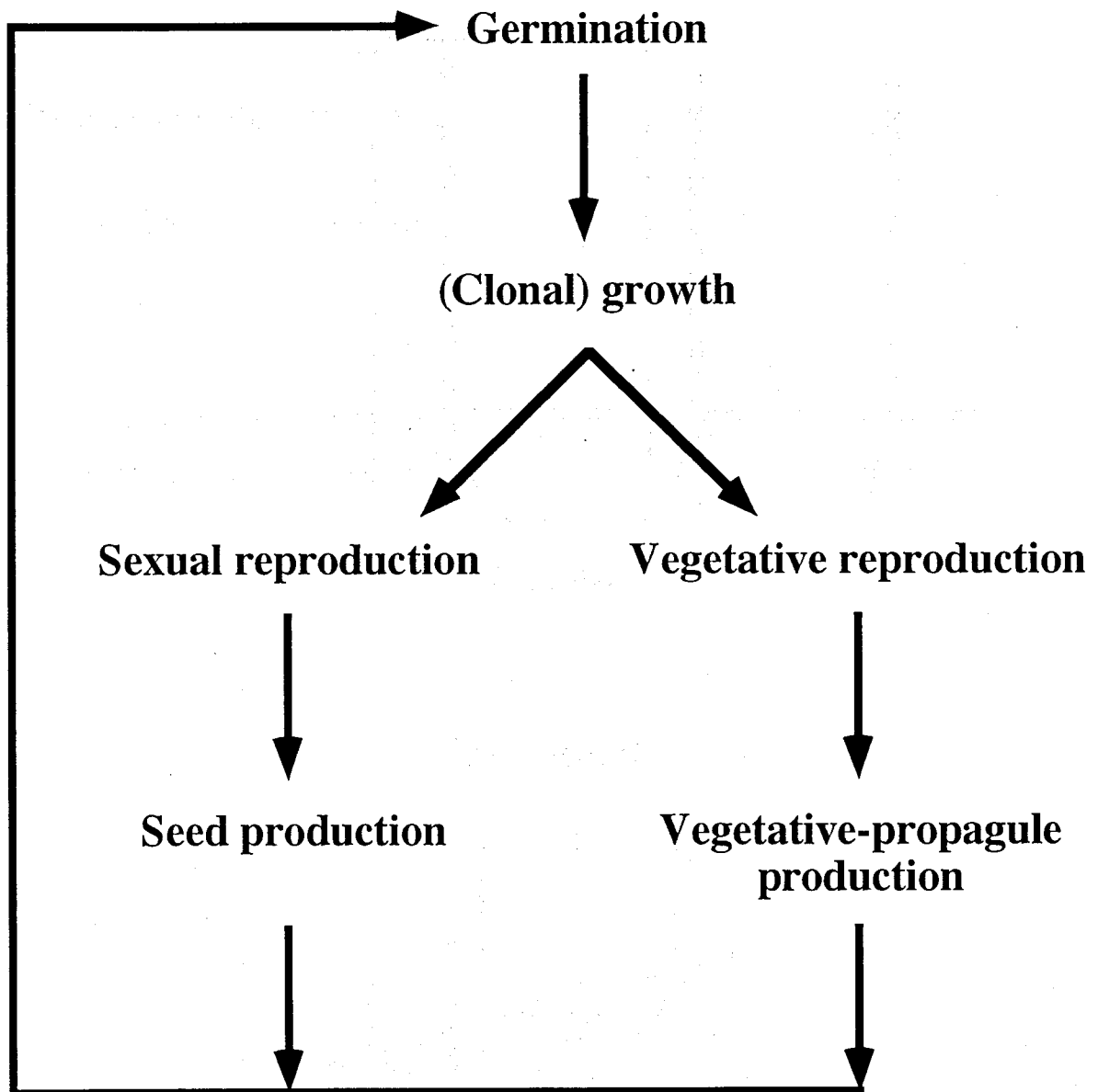


Fig. 1. Diagram of life cycles of vascular aquatic plants with vegetative means of reproduction. The parenthesis shows the case for plants with clonal growth.

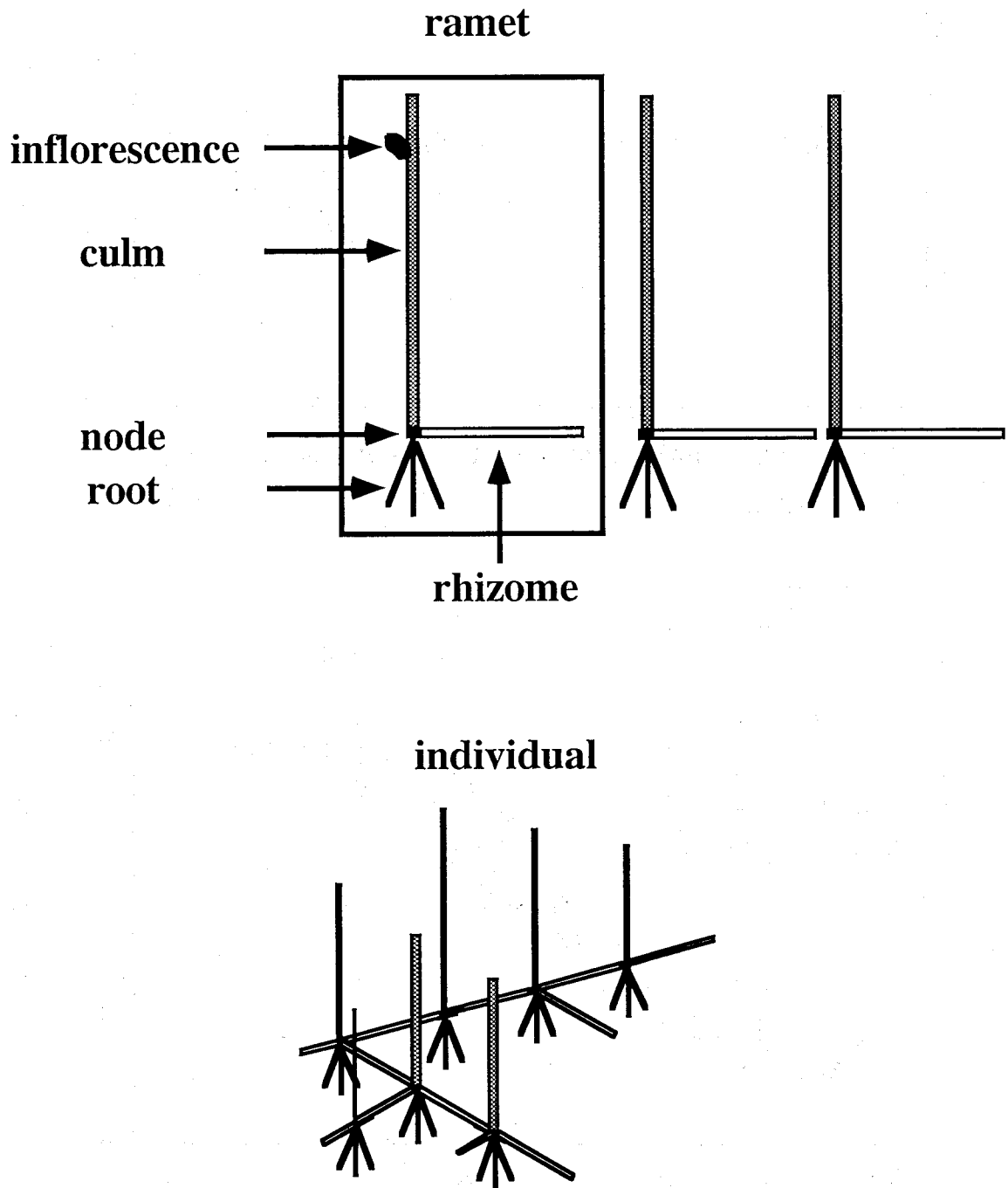


Fig. 2. Diagram of growth form of *Schoenoplectus lineolatus*.

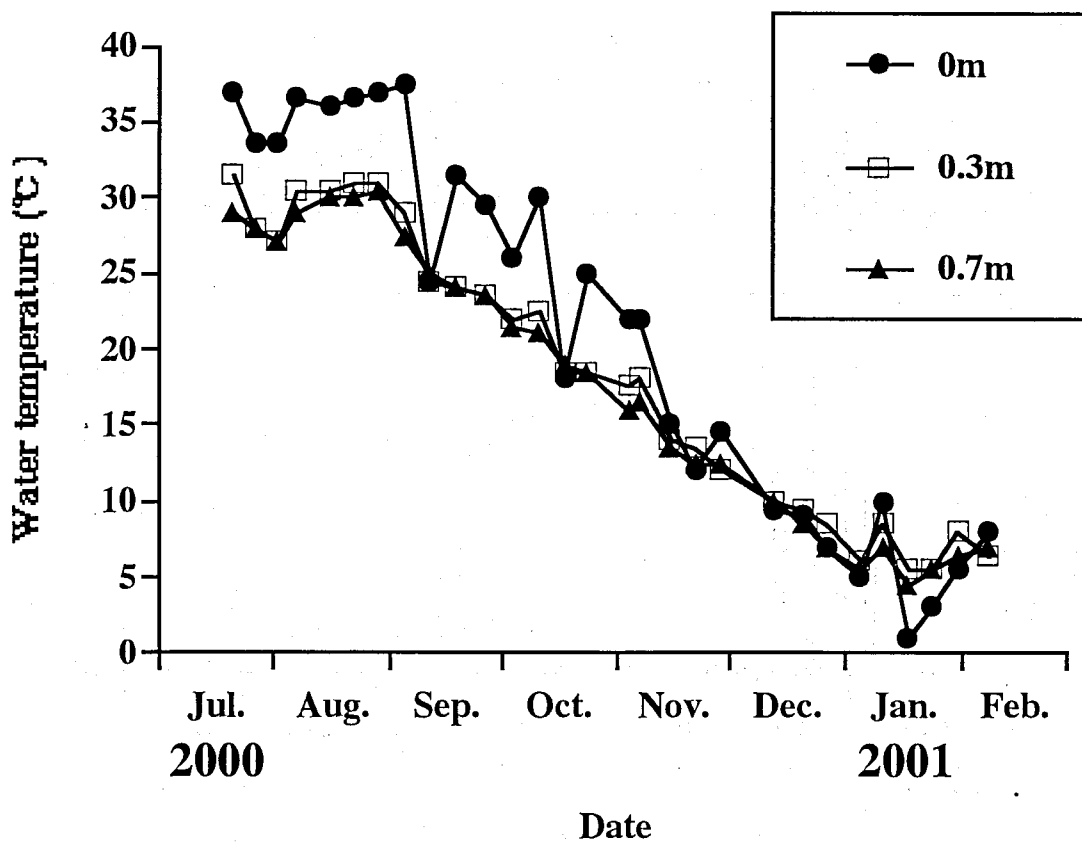
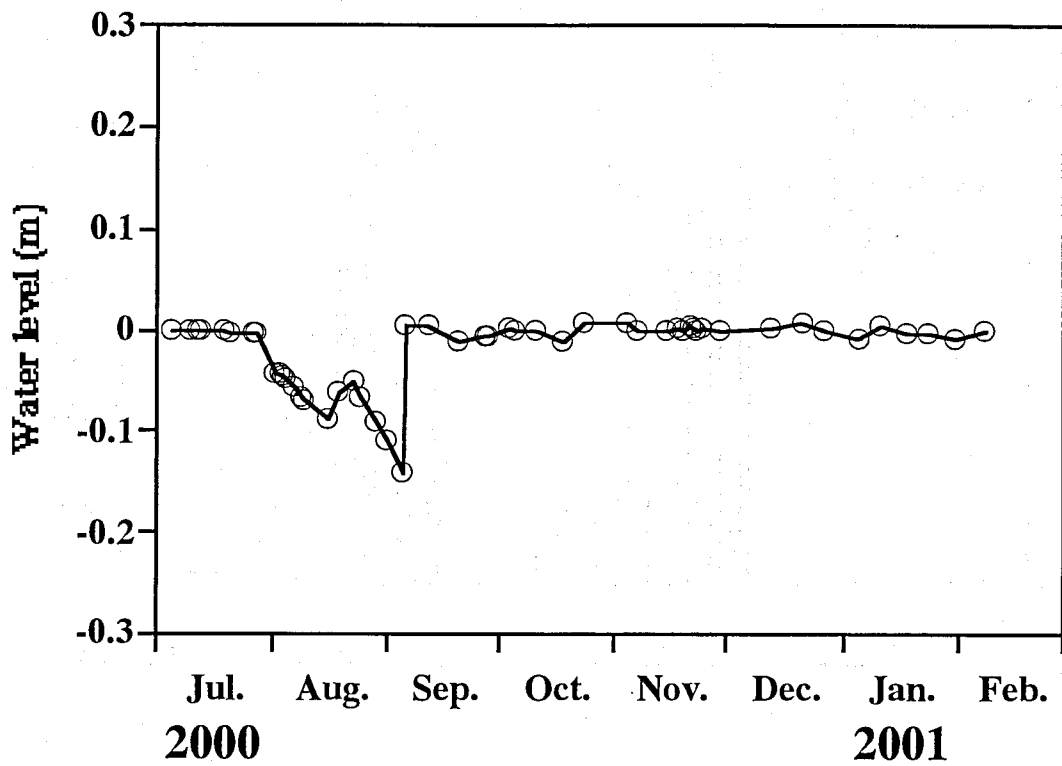


Fig. 3. Seasonal changes of water level and water temperatures at three water depths (0, 0.3, 0.7 m) during the experimental period in Shin-ike Pond.

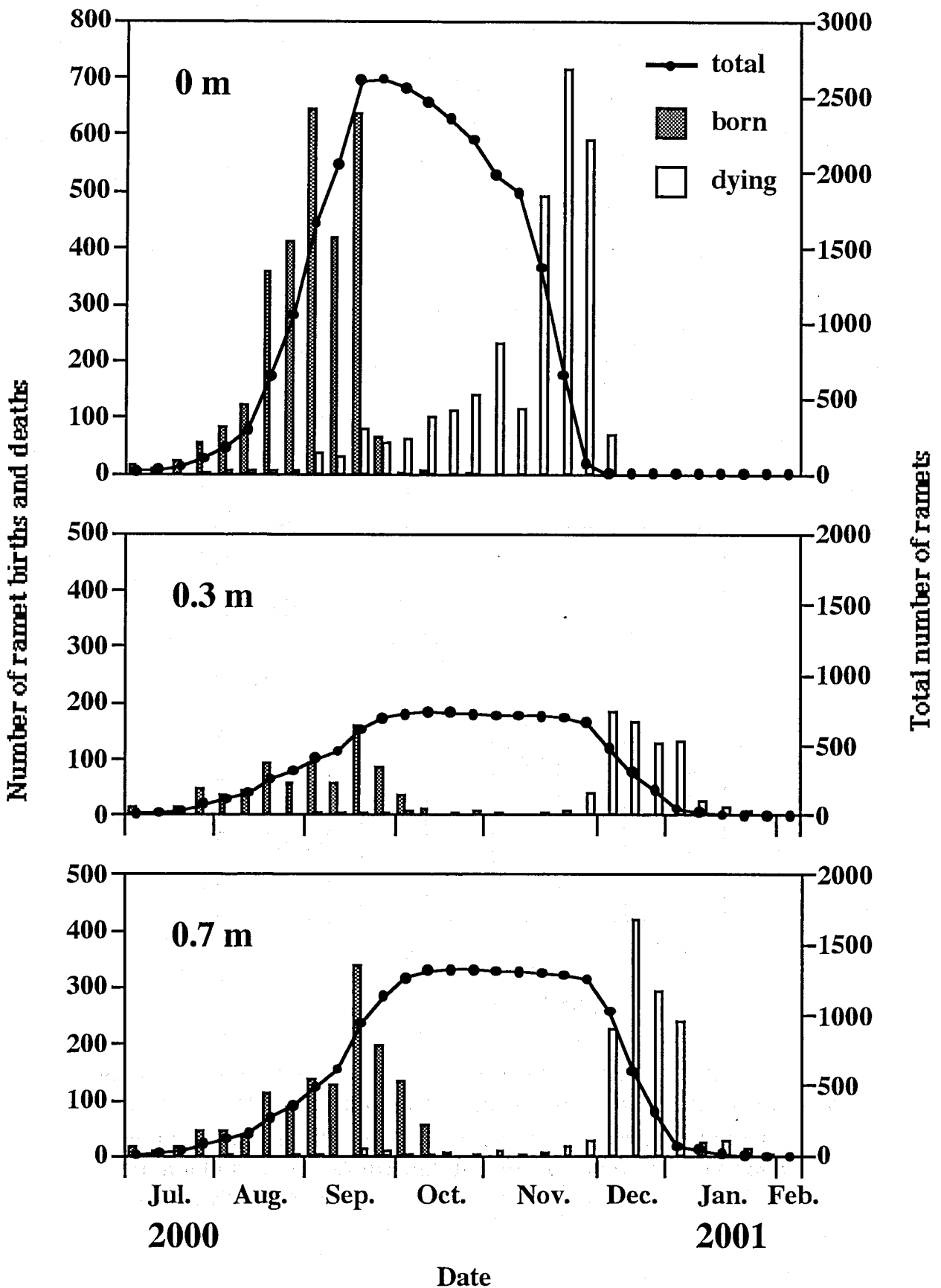


Fig. 4. Number of ramet births and deaths and total number of ramets for *Schoenoplectus lineolatus* at three water depths (0, 0.3, 0.7 m). Values are the sums of eight individuals at each depth. Field survey was performed at least once a week.

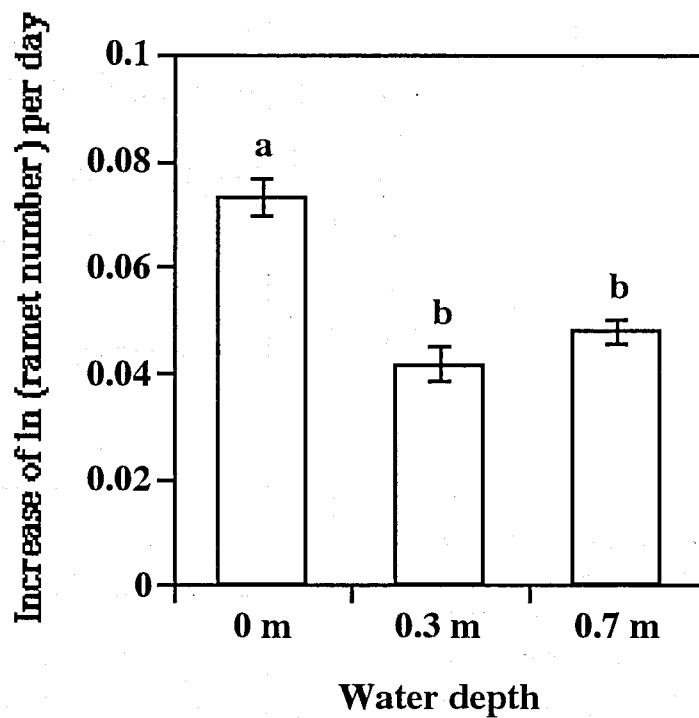


Fig. 5. Clonal growth rate of *Schoenoplectus lineolatus* at three water depths (0, 0.3, 0.7 m). Values are means \pm SE ($n = 8$). The formula by which the clonal growth rate was calculated was shown in text. Different letters show significant differences between water depths due to Tukey's HSD test ($P < 0.05$).

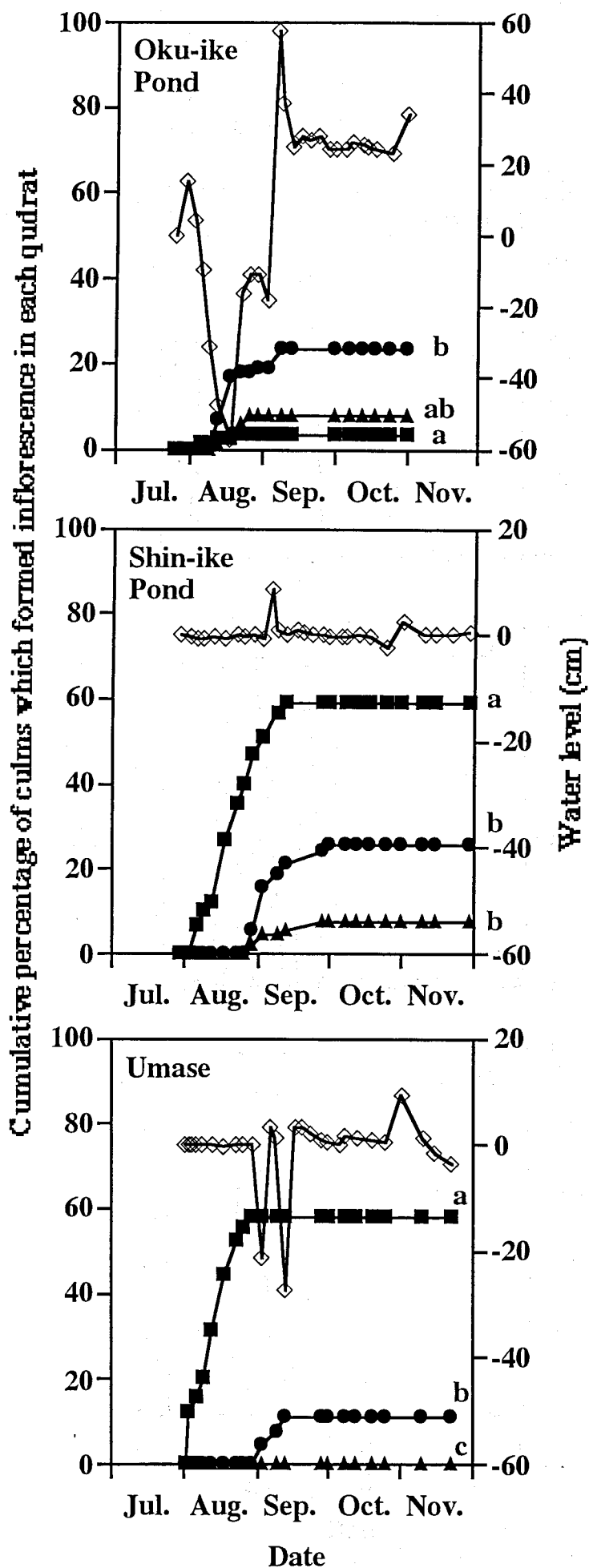


Fig. 6. Cumulative percentages of inflorescence formation (means; untransformed data) in *Schoenoplectus lineolatus* at three water depths ((■) Shallow, (●) Middle, (▲) Deep), and the water level (◇) in three ponds (Oku-ike Pond, Shin-ike Pond and Umase). Significant differences ($P < 0.05$) among the final cumulative percentages of three water depths in each pond are indicated by different letters. Unplanned comparisons are due to Tukey's HSD test and Scheffe's F test, respectively, for in Oku-ike Pond and for both in Shin-ike Pond and in Umase.

(A) Oku-ike Pond

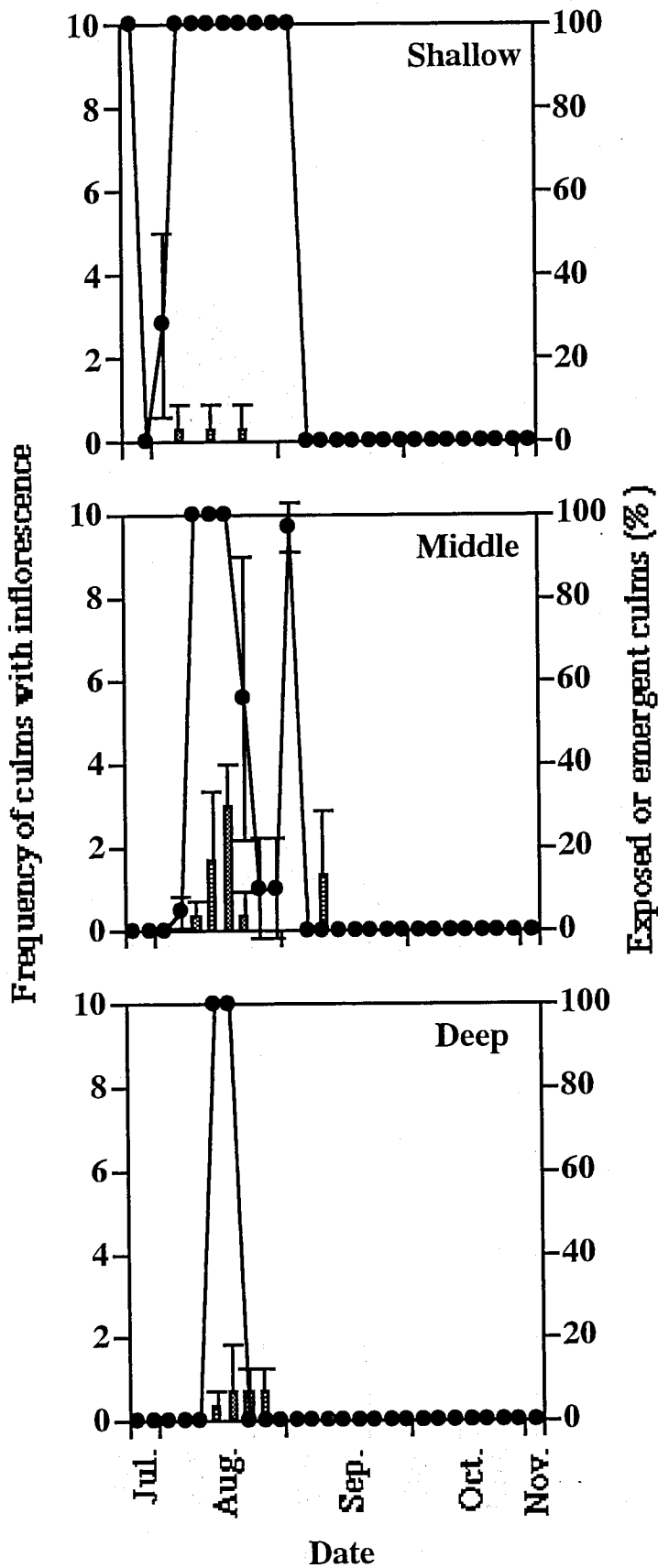


Fig. 7. The relationship between the frequency of inflorescence formation (bar) and the percentage of exposed or emergent culms (symbol) at three water depths (shallow, middle and deep) in three ponds, (A) Oku-ike Pond, (B) Shin-ike Pond and (C) Umase, in *Schoenoplectus lineolatus*. All data are shown as means \pm SD.

(B) Shin-ike Pond

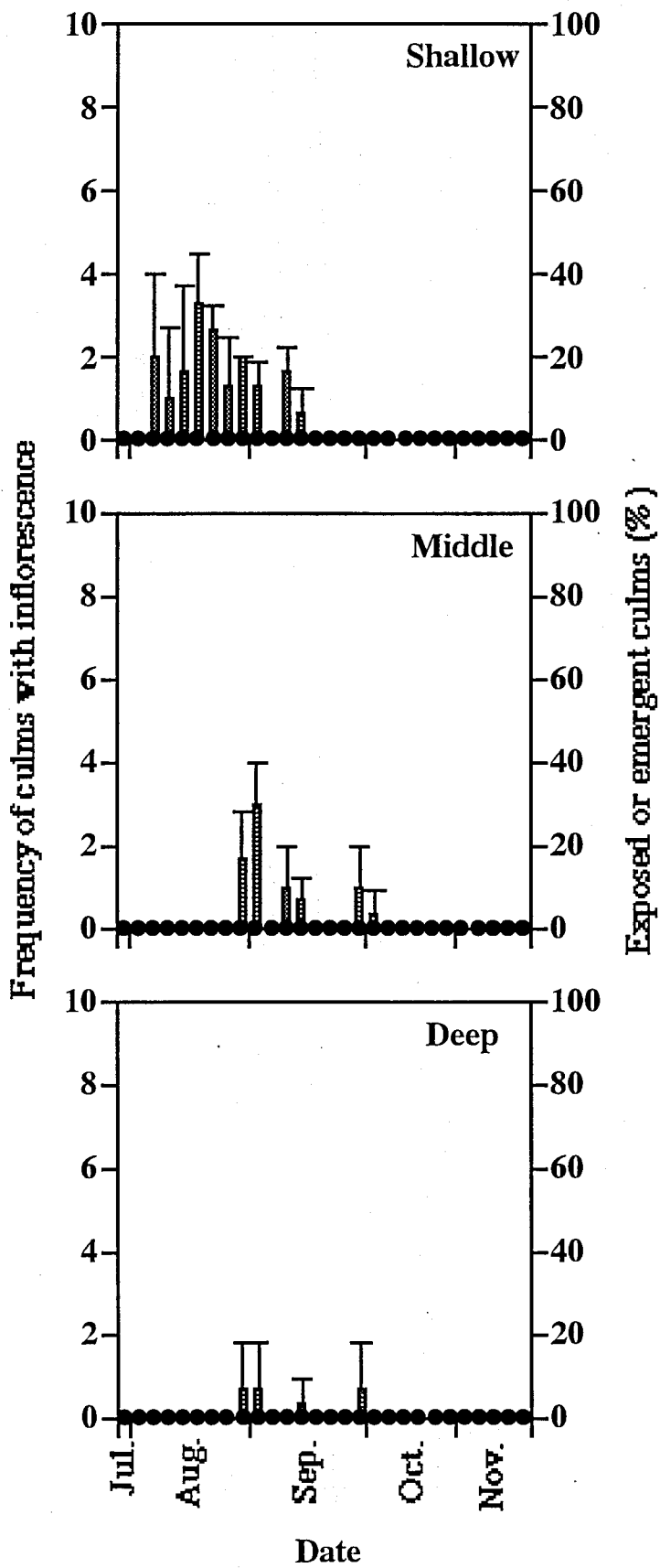


Fig. 7. Continued.

(C) Umase

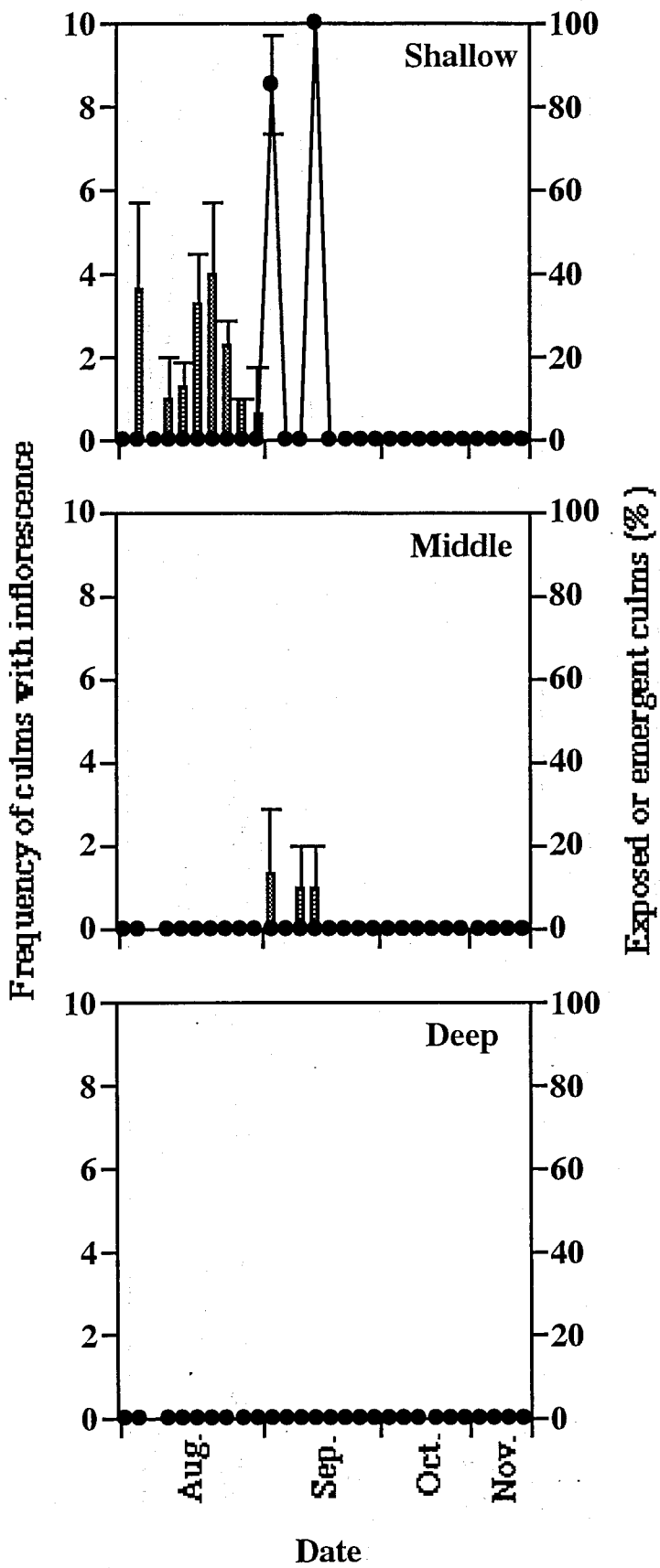


Fig. 7. Continued.

(A) Oku-ike Pond

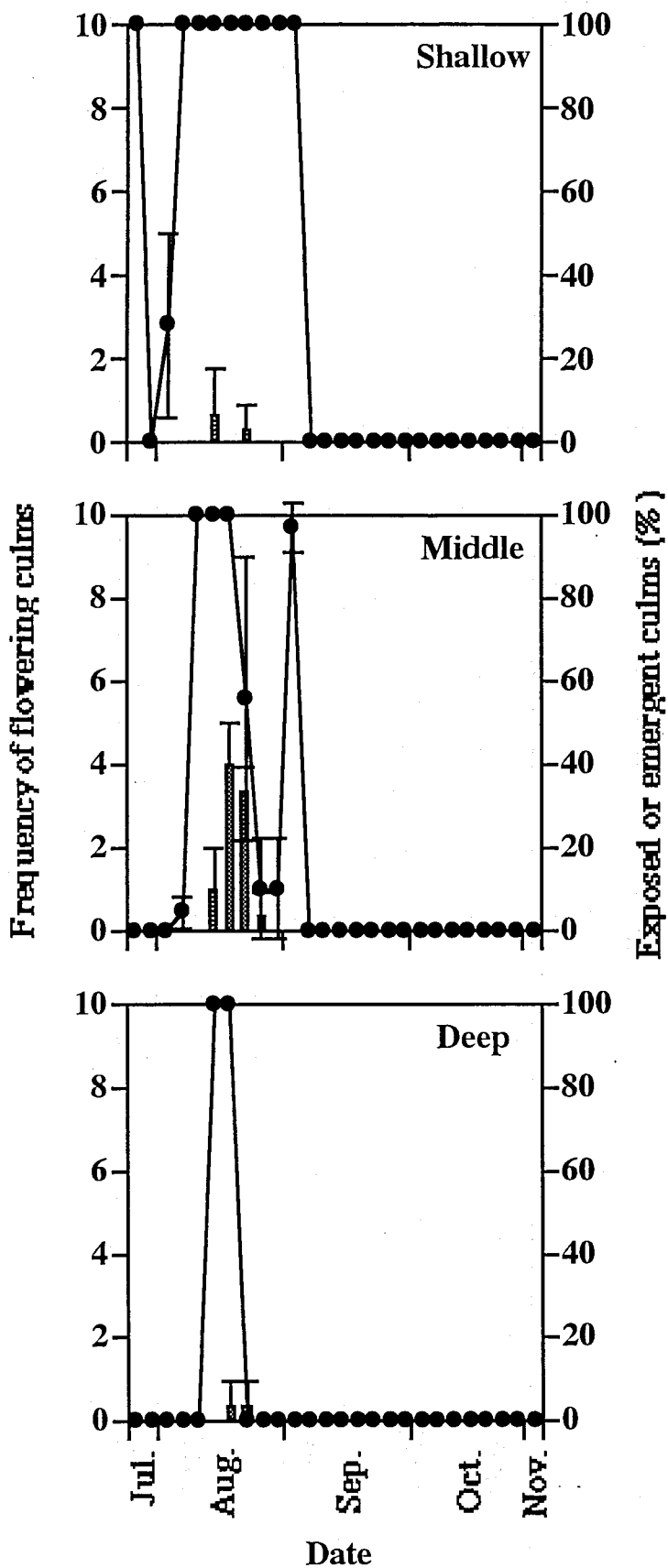


Fig. 8. The relationships between the frequency of flowering (bar) and the percentage of exposed or emergent culms (symbol) at three water depths (shallow, middle and deep) in three ponds, (A) Oku-ike Pond, (B) Shin-ike Pond and (C) Umase, in *Schoenoplectus lineolatus*. All data are shown as means \pm SD.

(B) Shin-ike Pond

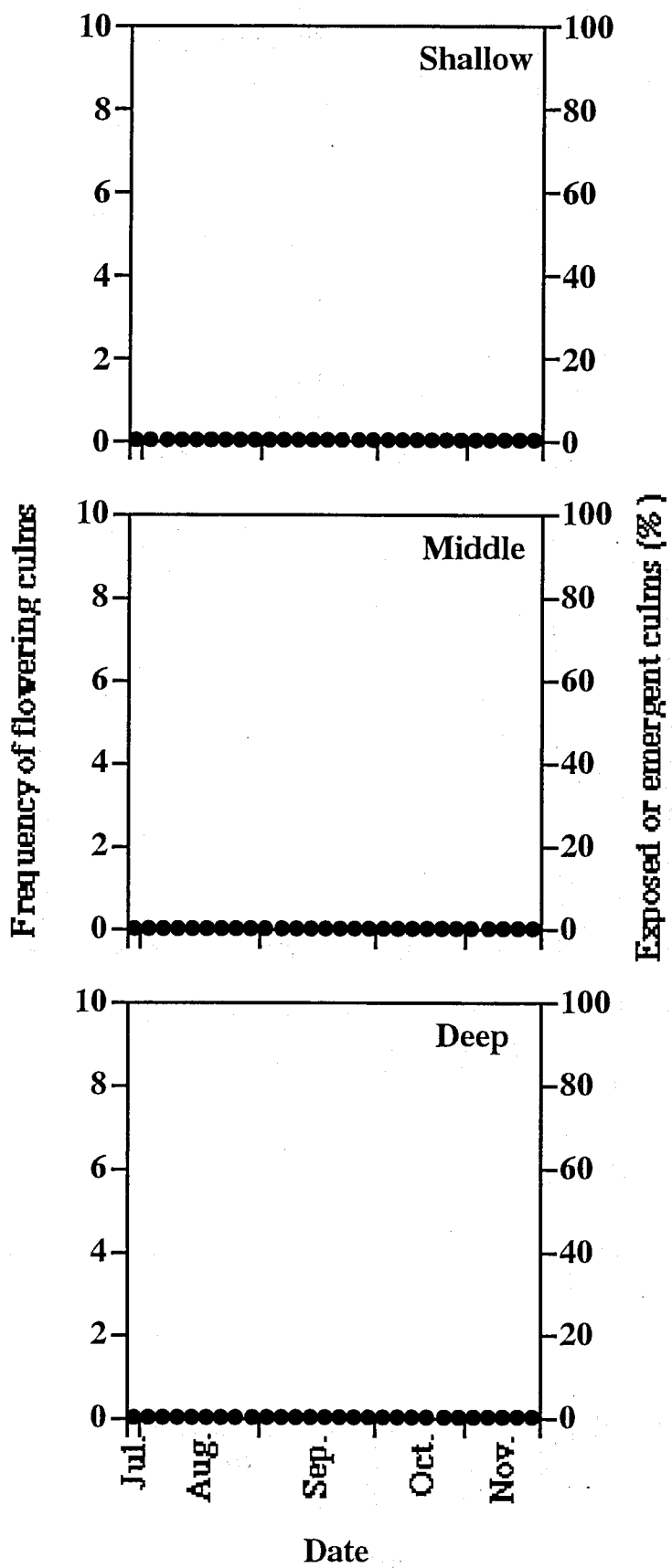


Fig. 8. Continued.

(C) Umase

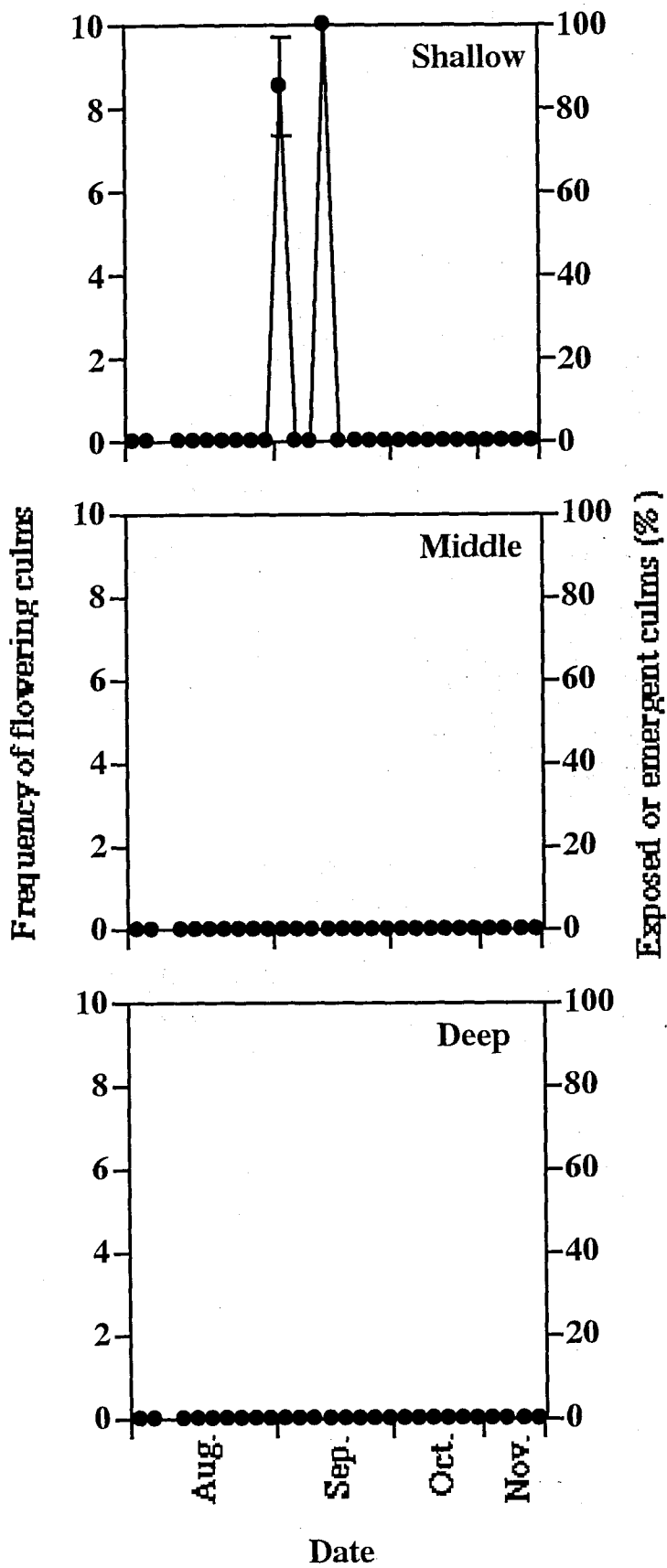


Fig. 8. Continued.

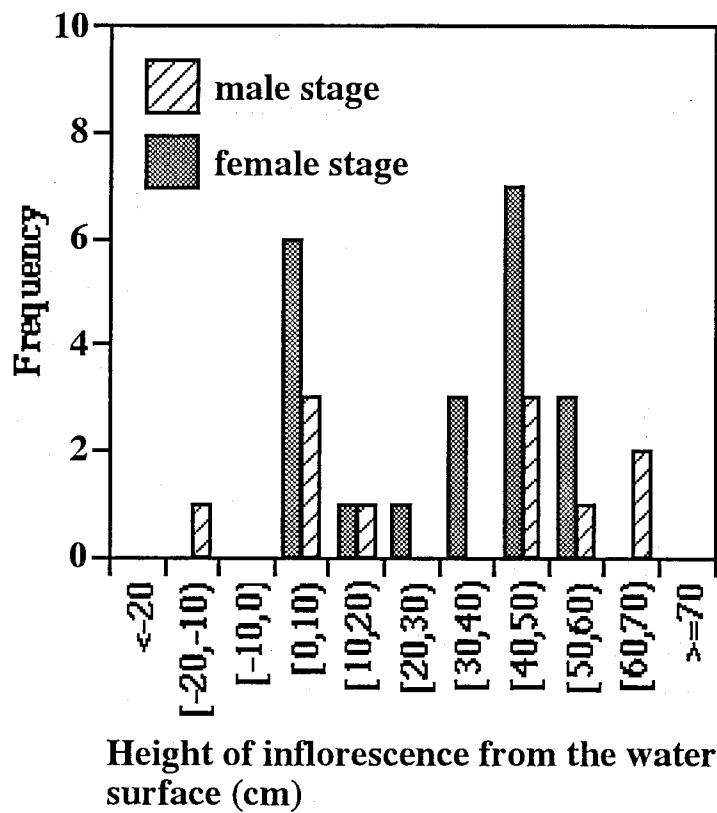


Fig. 9. Height of inflorescence of *Schoenoplectus lineolatus* from the water surface when flowering for male and female stages in Oku-ike Pond.

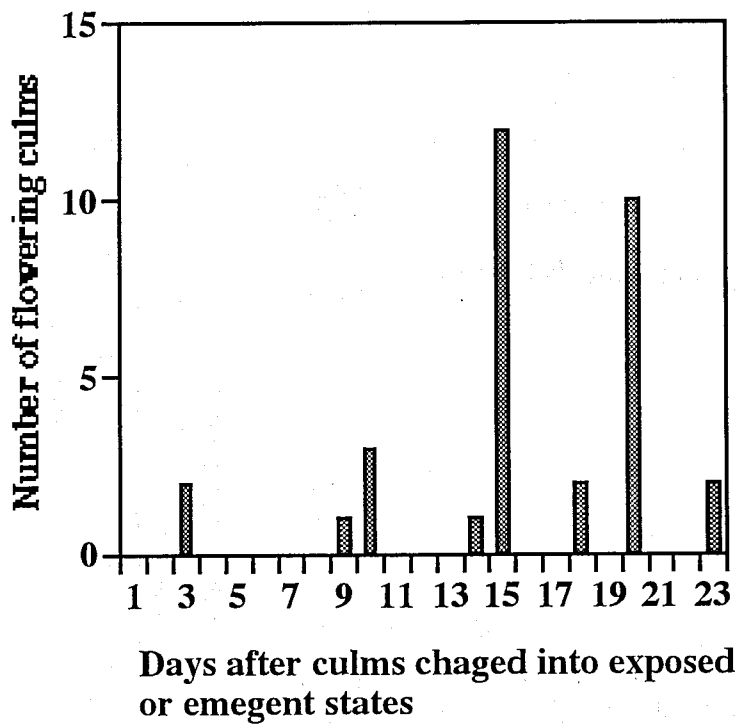


Fig. 10. Days to flowering in *Schoenoplectus lineolatus* after emergence of culms into the air in Oku-ike Pond.

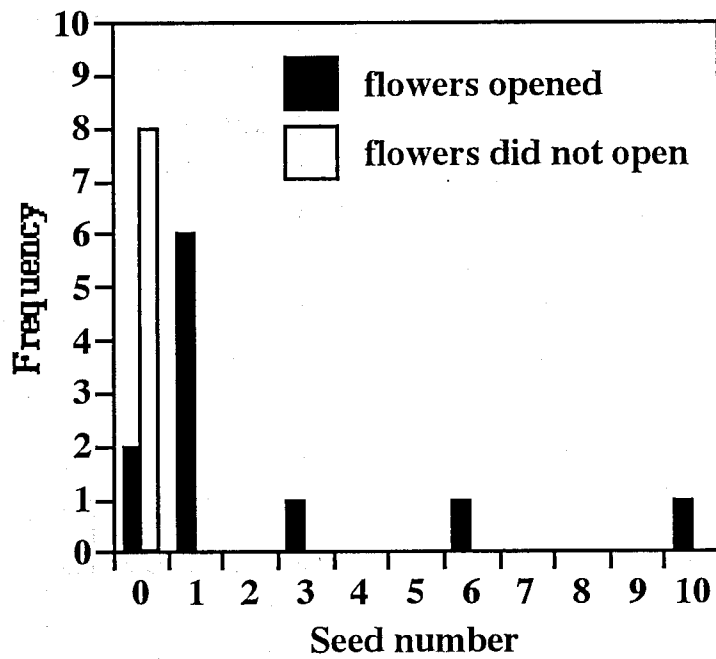


Fig. 11. Frequency of the number of seeds in inflorescences whose flowers opened or not for *Schoenoplectus lineolatus* in Oku-ike Pond.

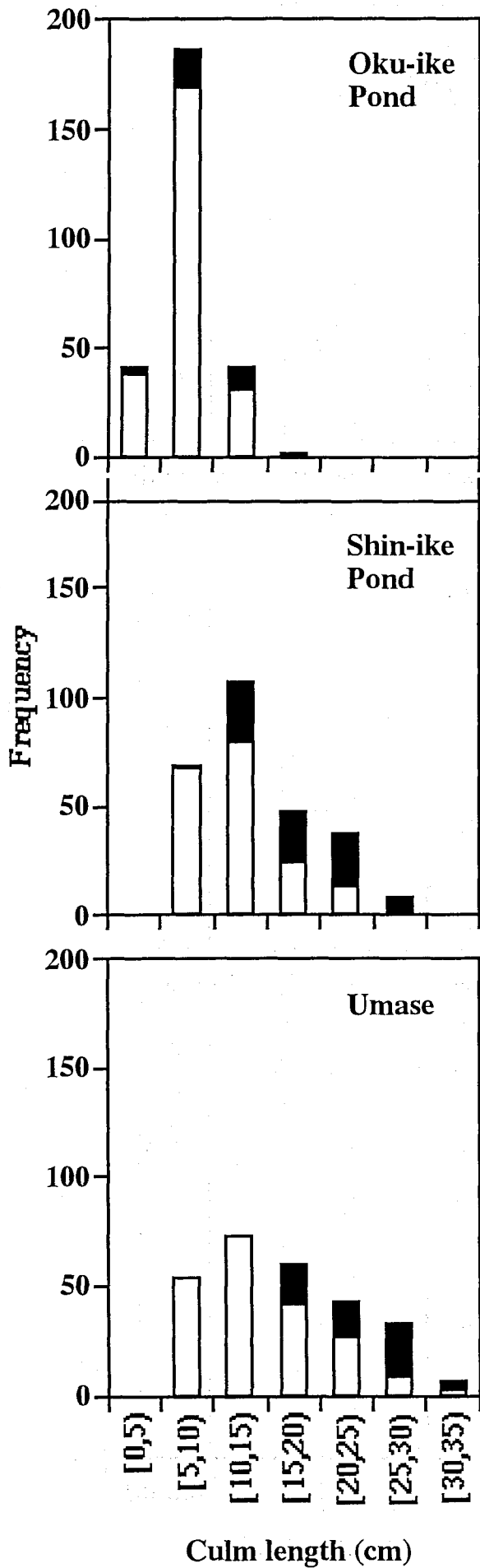


Fig. 12. Frequency distribution of length of culm with (closed bar) and without (open bar) inflorescence of *Schoenoplectus lineolatus* in three ponds (Oku-ike Pond, Shin-ike Pond and Umase). Data were pooled for three water depths. Culms with inflorescence were significantly longer than ones without inflorescence in each pond (see text).

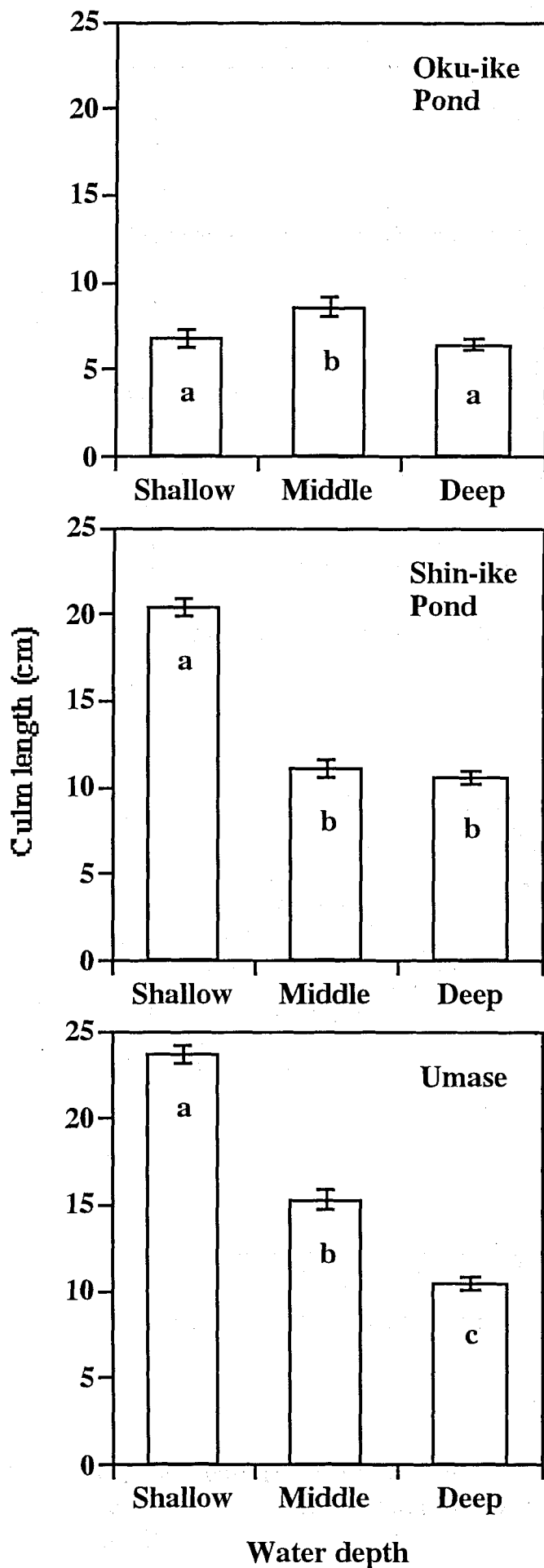
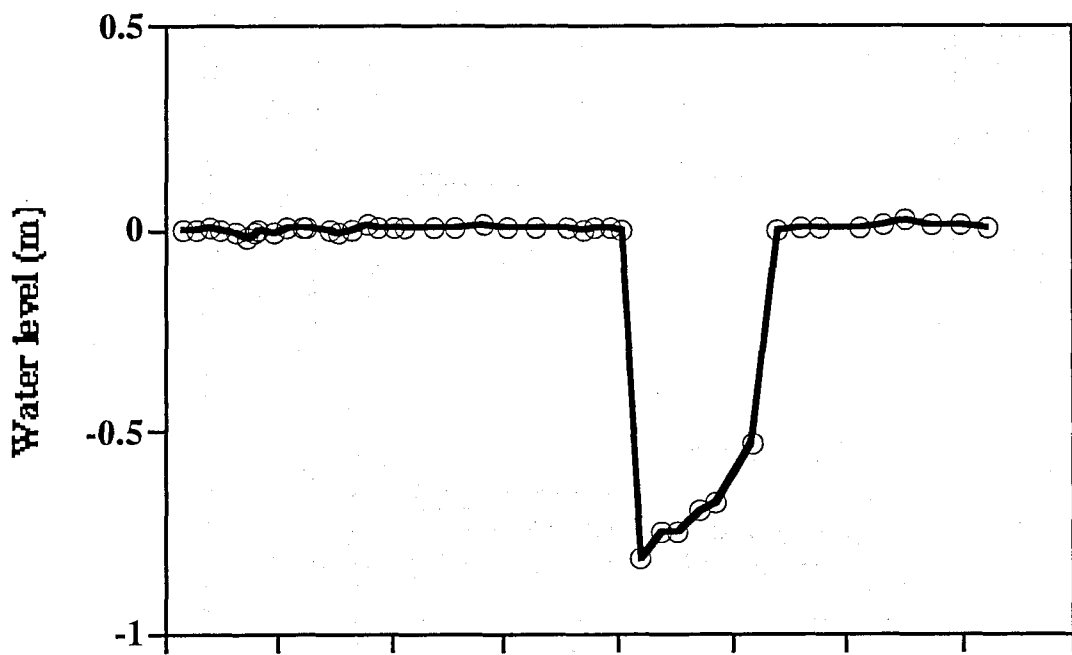
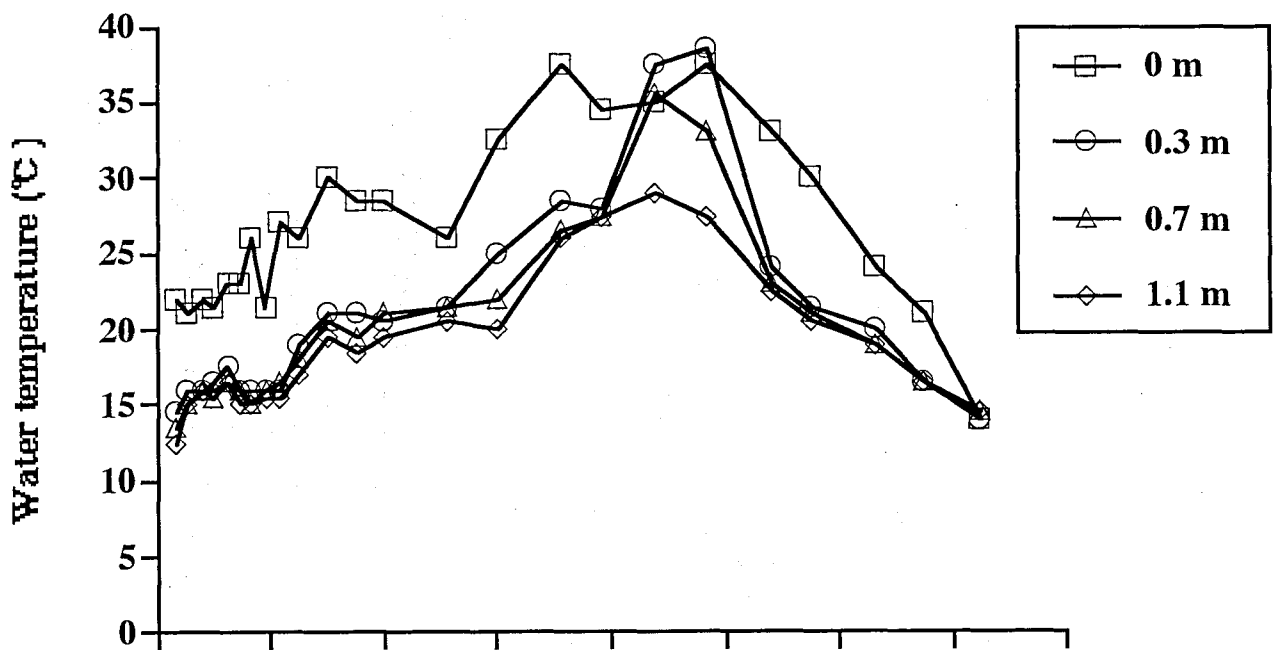


Fig. 13. Culm length of *Schoenoplectus lineolatus* at the end of anthesis at three water depths (shallow, middle and deep) in three ponds (Oku-ike Pond, Shin-ike Pond and Umase). Values are the means and 95% confidence limits. All values are back transformed. Different letters show significant differences between water depths due to Scheffe's *F* test for Oku-ike Pond and Tukey's HSD test for Shin-ike Pond and Umase ($P < 0.05$).



Apr. May Jun. Jul. Aug. Sep. Oct. Nov.



Apr. May Jun. Jul. Aug. Sep. Oct. Nov.

Date

Fig. 14. Seasonal changes of water level and water temperatures at four water depths (0, 0.3, 0.7, 1.1 m) during the experimental period in Shin-ike Pond in 2001.

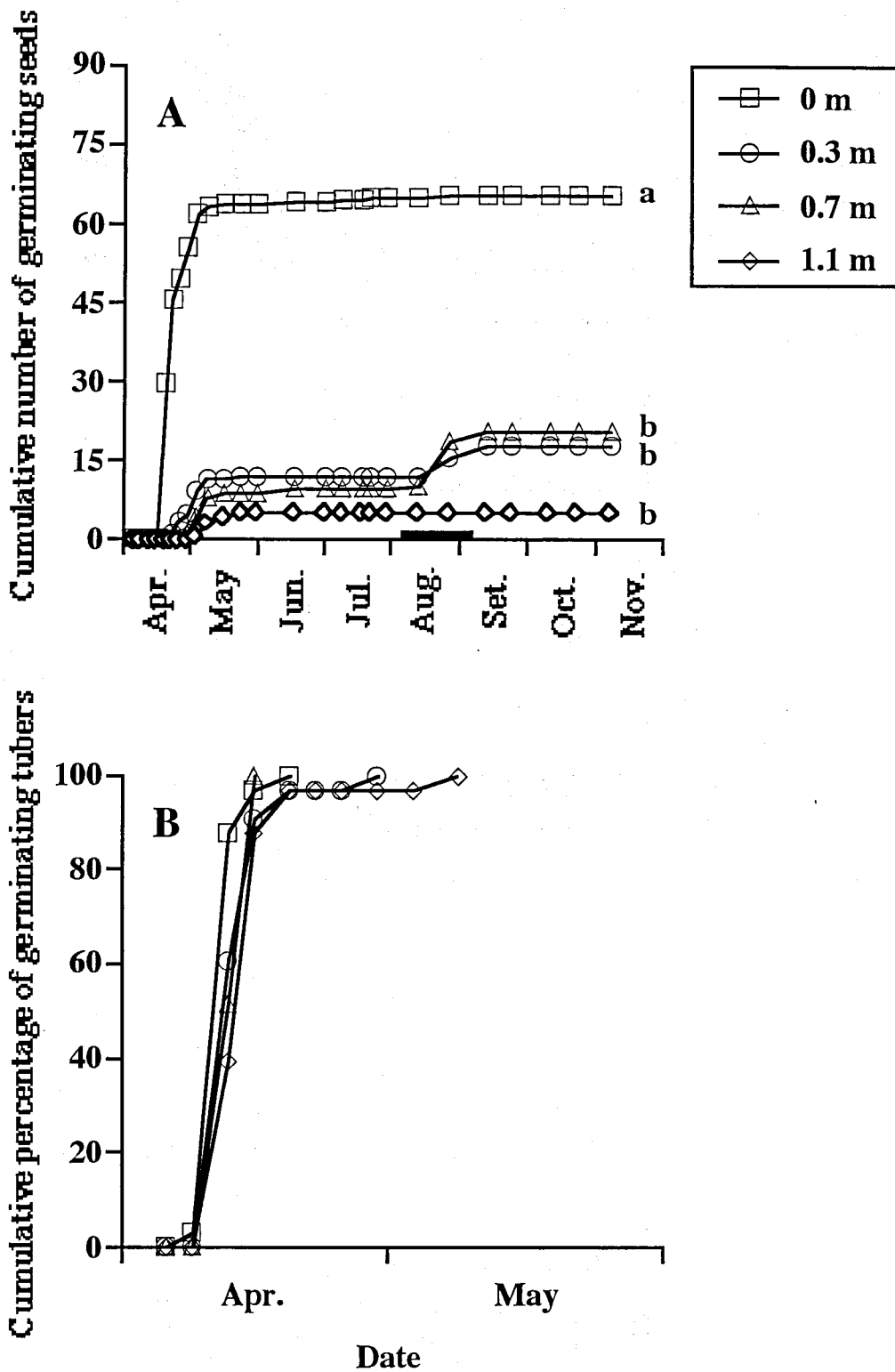


Fig. 15. Seed (A) and tuber (B) germination of *Schoenoplectus lineolatus* at four water depths (0, 0.3, 0.7, 1.1 m) in Shin-ike Pond. Plots describe means of three trays (repetitions) at each water depth. A bold horizontal bar shows the exposed period at 0.3 and 0.7 m, and this exposure stimulated seed germination at these depths. Different letters show significant differences between water depths due to Scheffe's F test ($P < 0.05$).

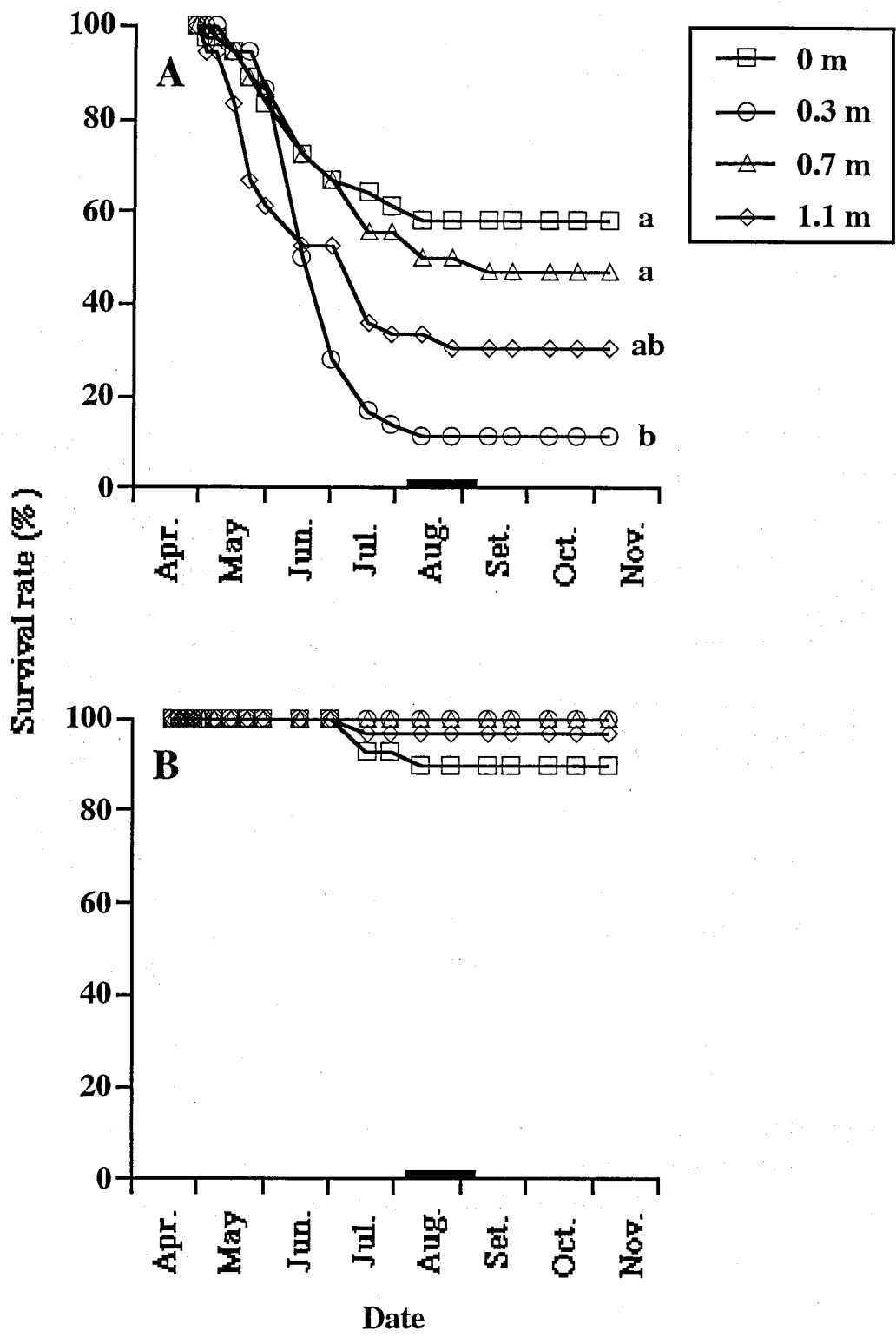


Fig. 16. Survivorship of individuals derived from seeds (A) and tubers (B) of *Schoenoplectus lineolatus* at four water depths (0, 0.3, 0.7, 1.1 m) in Shin-ike Pond. Other things are the same as in Fig. 15. Different letters show significant differences between water depths due to Tukey's HSD test ($P < 0.05$).

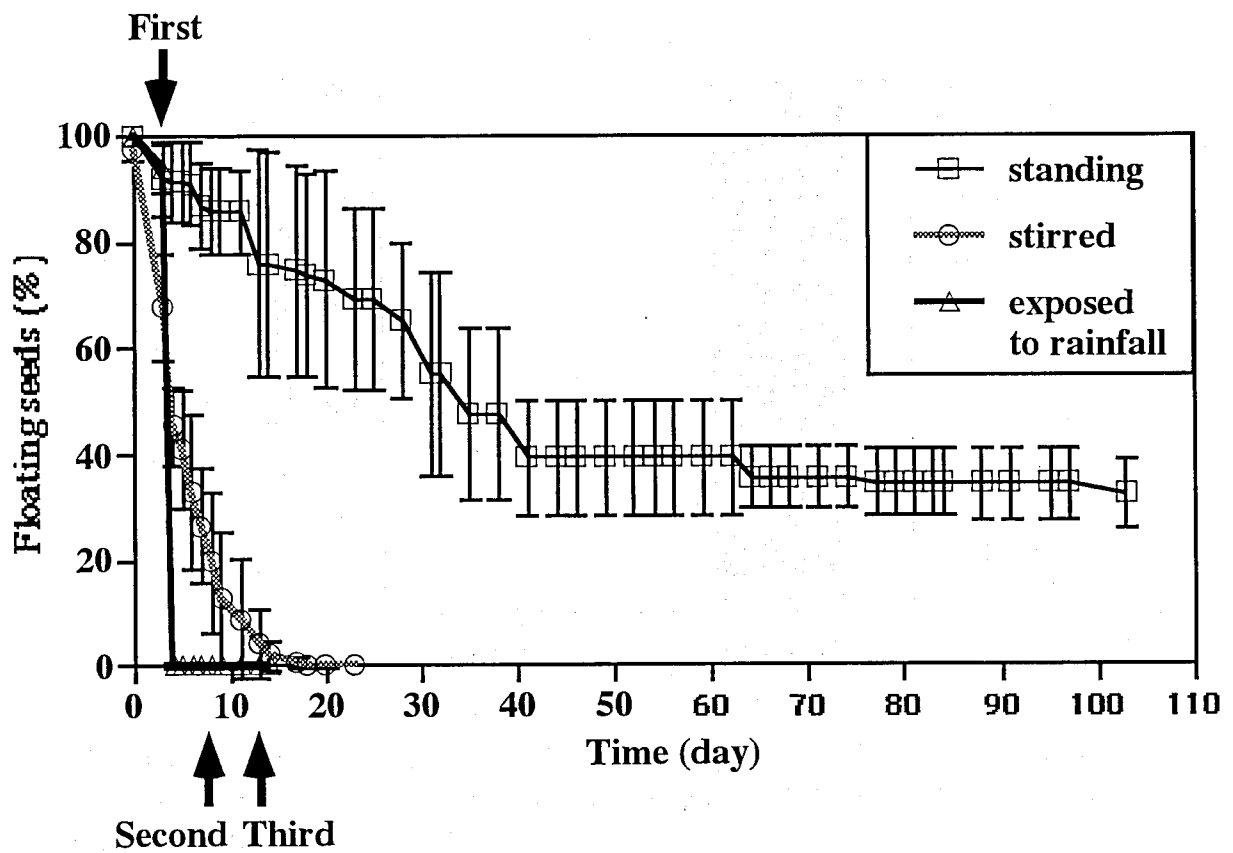


Fig. 17. Seed buoyancy of *Schoenoplectus lineolatus* in three treatments: (□) standing, (○) stirred at every measurement, (△) exposed to rainfall. Plots and vertical bars show means \pm SD. Arrows in the figure show times of exposure to rainfall (see Table 7).

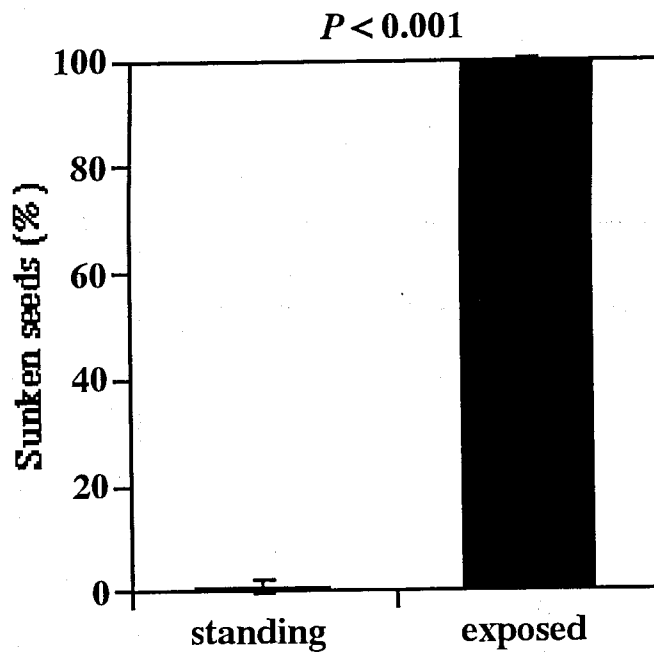


Fig. 18. Effects of first rainfall to seed buoyancy in *Schoenoplectus lineolatus*. Seeds in "exposed" were exposed to rainfall. See Table 7 for the precipitation. Value are means \pm SD.

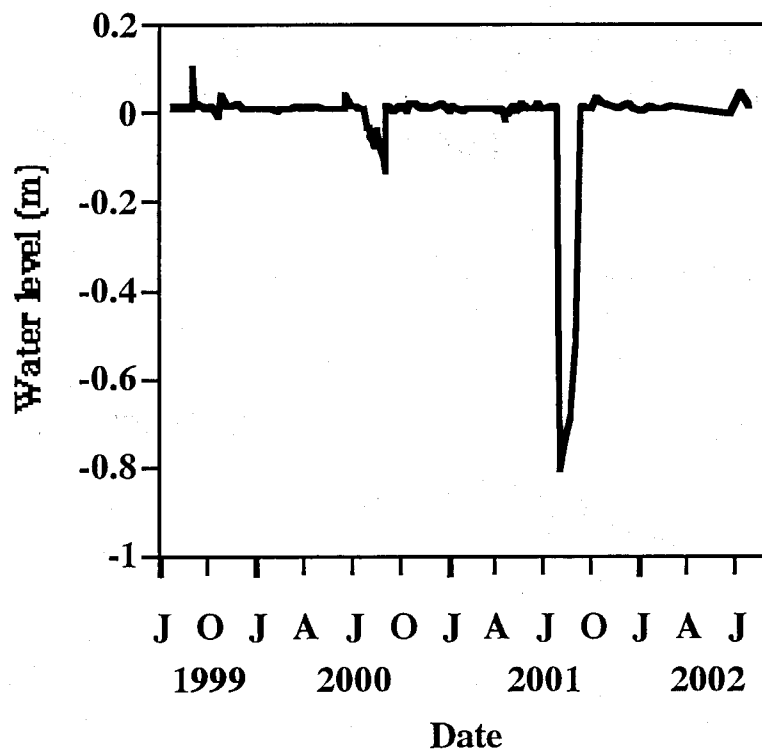


Fig. 19. The water level from 30th July 1999 to 2nd August 2002 in Shin-ike Pond. The water level is described on the basis of 0 m of shoreline at the beginning of the field examination.

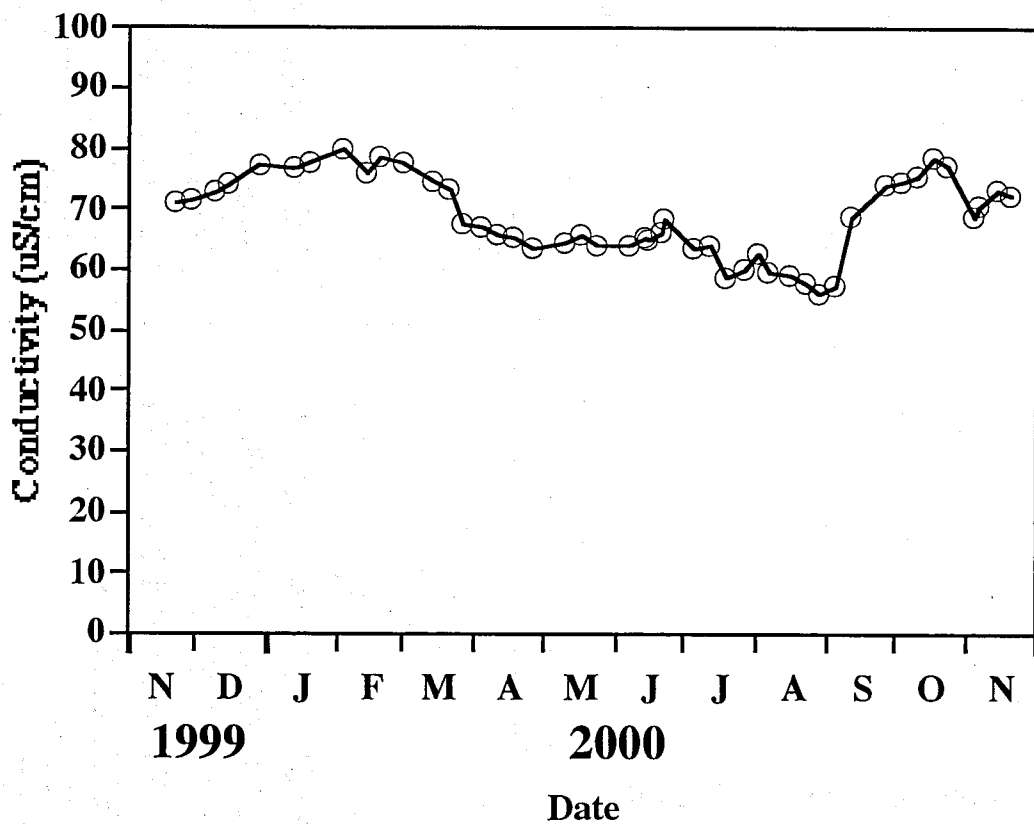
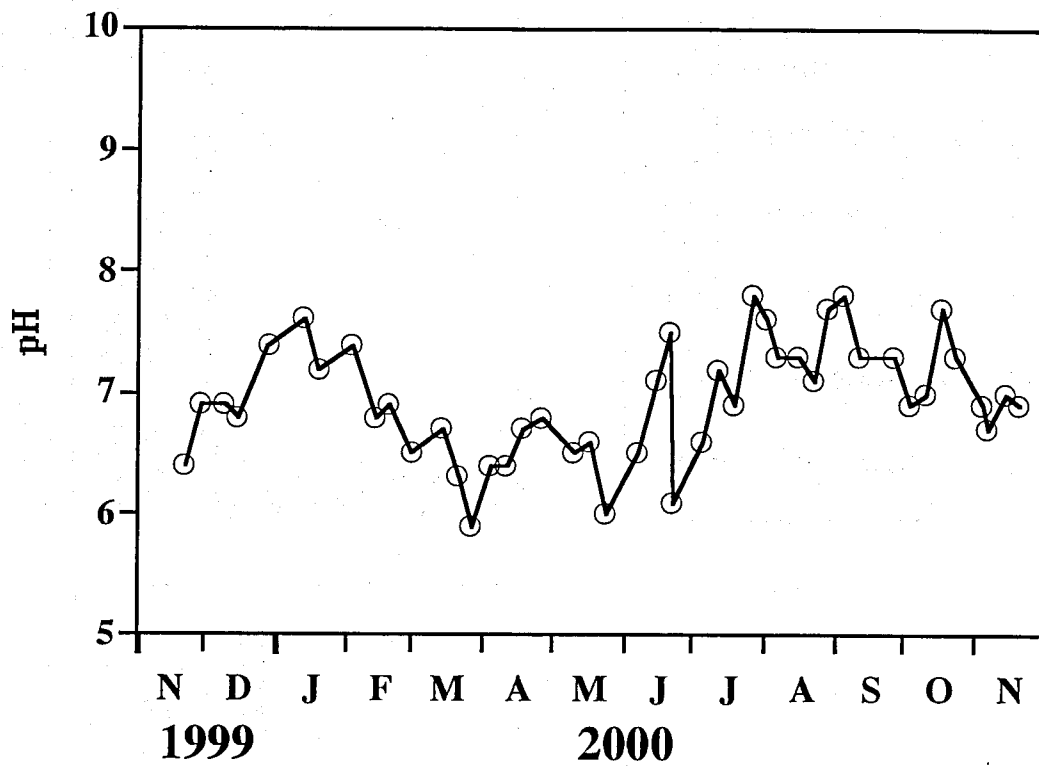


Fig. 20. Seasonal changes of pH and conductivity from 22nd November 1999 to 21st November 2000 in Shin-ike Pond.

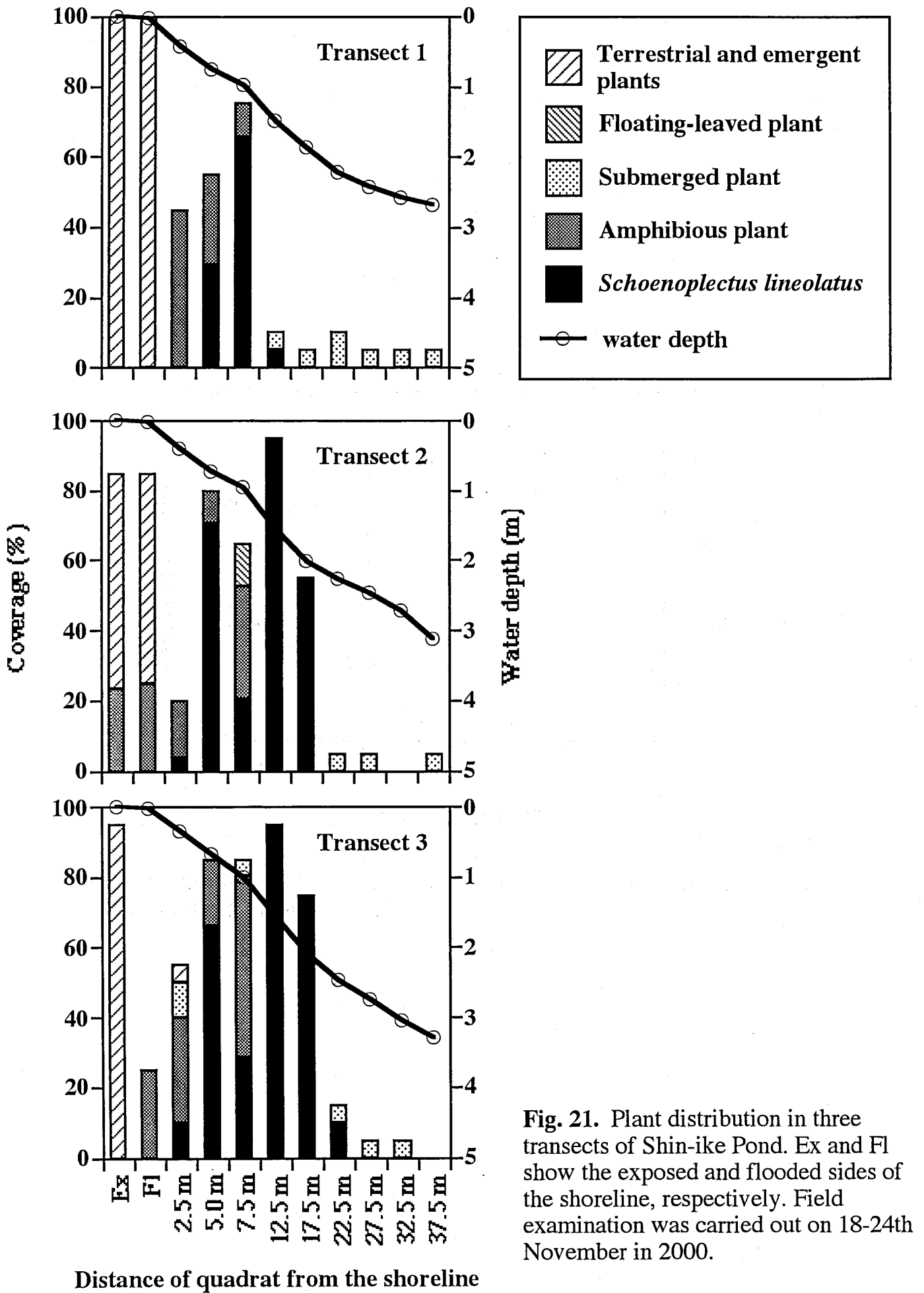


Fig. 21. Plant distribution in three transects of Shin-ike Pond. Ex and Fl show the exposed and flooded sides of the shoreline, respectively. Field examination was carried out on 18-24th November in 2000.

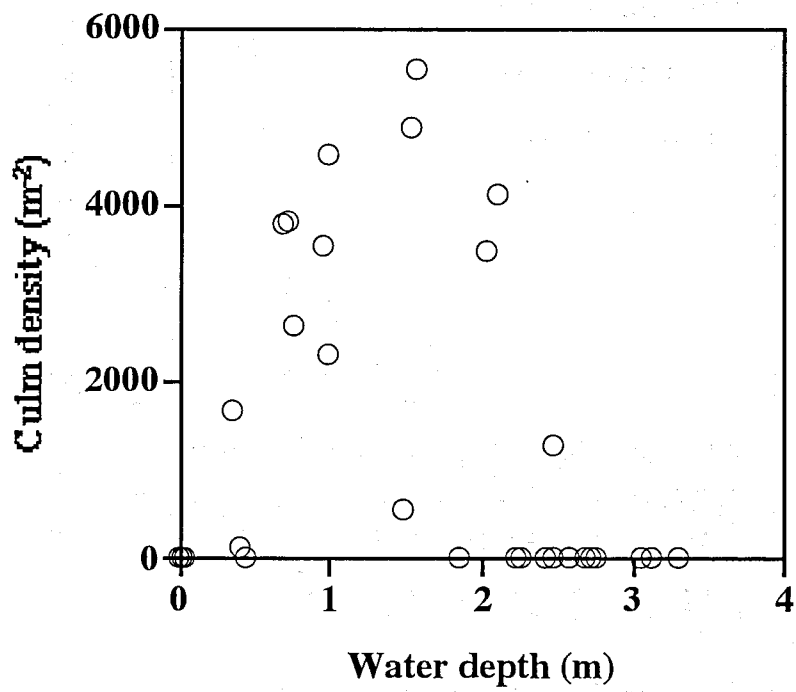


Fig. 22. Relationships between culm density of *Schoenoplectus lineolatus* and water depth in Shin-ike Pond.

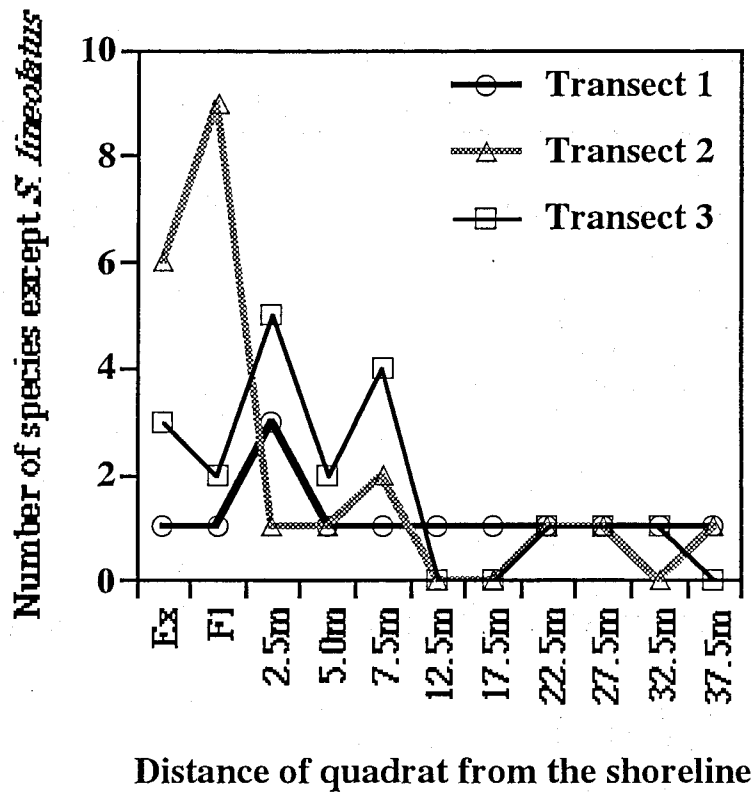


Fig. 23. Number of species except *Schoenoplectus lineolatus* in three transects.

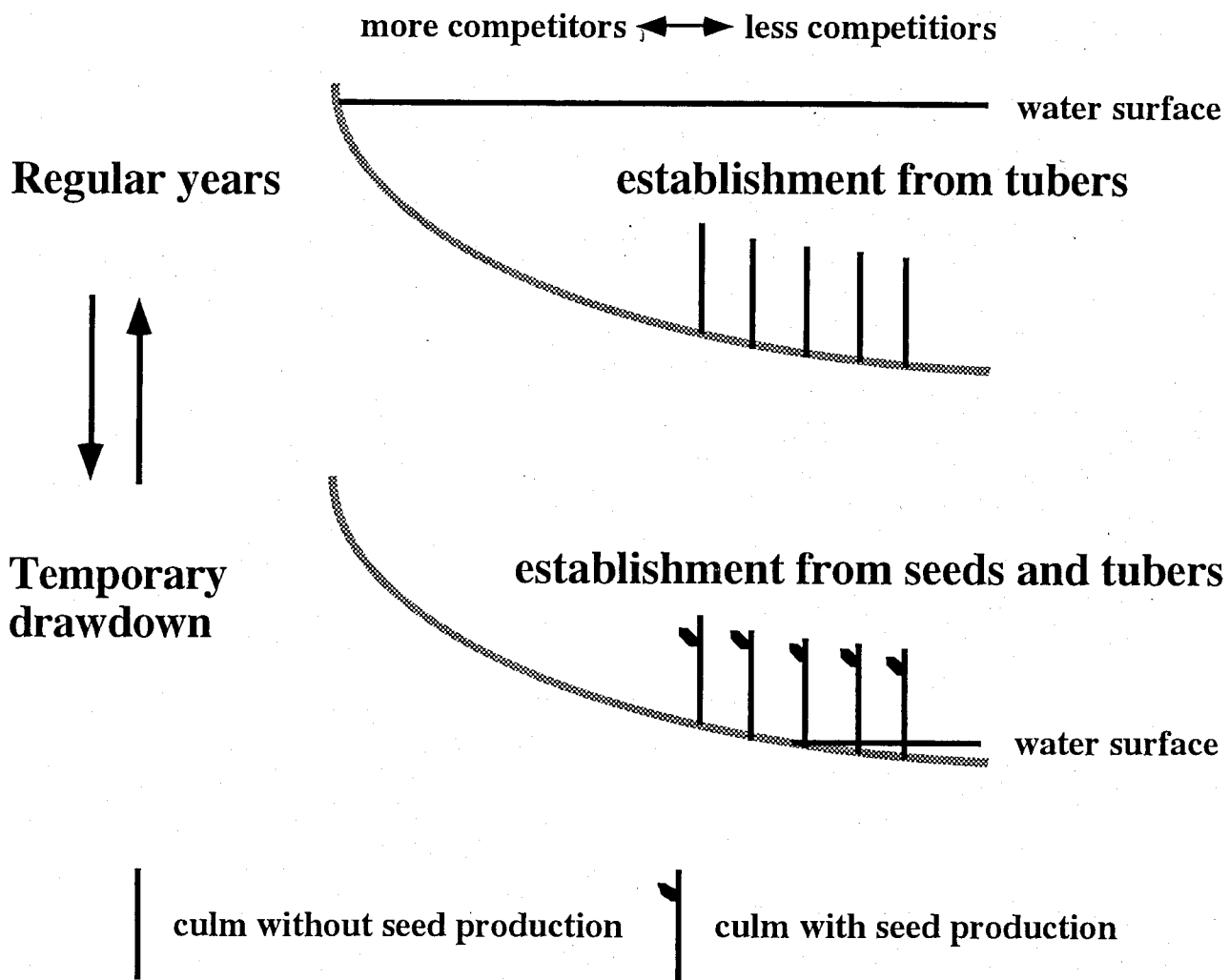


Fig. 24. Diagram of the mechanism for maintaining the persistent population of *Schoenoplectus lineolatus* in Shin-ike Pond.