

THE ECOLOGY OF *RUPPIA DREPANENSIS* TINEO IN A MEDITERRANEAN  
BRACKISH MARSH (DOÑANA NATIONAL PARK, SW SPAIN)  
*A BASIS FOR THE MANAGEMENT OF SEMIARID FLOODPLAIN WETLANDS*

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The ecology of *Ruppia drepanensis* Tineo  
in a Mediterranean brackish marsh  
(Doñana National Park, SW Spain)  
*A basis for the management of semiarid  
floodplain wetlands*

DISSERTATION

Submitted in fulfilment of the requirements of  
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Hydraulic and Environmental Engineering for the Degree of  
Doctor in the Agricultural and Environmental Sciences  
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by

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*A Carlitos Toyos,  
con mi mejor cariño.*

*Aunque el divino silencio de tu frente  
no lo interrumpa dorada diadema,  
los niños se inclinarán en tu presencia,  
los entusiastas te mirarán atónitos.*

*A tí los días de rutilante sol  
te hilarán rica púrpura y blanco armiño  
y, con pesares y dichas en sus manos,  
de rodillas ante tí estarán las noches.*

*(R.M.R.)*

*A thousand miles journey begins with the first step*

*(Lao Tse)*

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suddenly much smaller, and I believe I grew much bigger. These friends form now a kind of second family, of the type that never ceased to care about the well-being of its members. The listing of their names seems inappropriate to me - as Little Big Canadian said once, 'you know who you are, and my heart and gratitude goes out with you' - for helping me to grow in the realms where reality is not of any help.

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## General abstract

One of the most important features of floodplain wetlands, both from an economical and a purely naturalistic point of view, is their high secondary production, with abundant invertebrates, fish and birds. This production is primarily based on the high productivity of aquatic macrophytes and their associated periphytic algae. Semi-arid floodplain wetlands have a very dynamic character, with yearly recolonization by the fauna and flora as a characteristic phenomenon. It is a consequence of the annual cycle of inundation and subsequent desiccation. The present study concentrates on the understanding of the dynamics of the aquatic vegetation as a research strategy for the management of such wetlands. Aims of the study were to identify potential causes for the decline of the submerged macrophyte populations, and to better understand the factors behind the interannual variation in their development.

The marsh of the Doñana National Park (SW Spain) was selected as a study area. The research concentrated on the growth, photosynthetic performance and reproduction of the dominant macrophyte species in the brackish area of the marsh, *Ruppia drepanensis* Tineo. Working hypotheses were generated from a conceptual model explaining the life cycle and biomass development of the submerged macrophyte populations. In this model, we consider the light climate as the main factor limiting the development of the submerged vegetation. High nutrient concentration is hypothesized as the main factor triggering the decline of the submerged macrophytes, either by an indirect shading effect due to increased growth of periphyton, or by a direct physiological effect on the plant development (toxicity of ammonia) and reproduction (delay of the flowering event caused by high nitrogen availability).

Light intensity and temperature strongly influenced growth and reproduction. The plants showed a strong capacity for photosynthetic acclimation to low light intensities. A weaker acclimation capacity was coupled with a high plasticity with respect to the temperature effect on photosynthesis.

Photoperiod and photosynthetic period did not influence growth, but affected reproduction. A longer photoperiod resulted in earlier flowering, and a longer photosynthetically active period resulted in more flowering and eventually higher seed production. Flowering was triggered by temperatures above 15 °C, and was strongly reduced at 30 °C. Although in all cases some plants were able to flower, the set of characteristics

necessary for a successful reproduction can be defined as a long photosynthetic photoperiod and a range of temperatures above 15 °C but below 30 °C.

Relatively high nitrate concentrations in the water column resulted in postponed flowering both under high and low sediment nutrient concentrations. Under field conditions, this can result in a complete failure of the reproduction, as the wetlands dry up after a 3 to 4 months period suitable for vegetative growth.

High ammonia concentrations in the root zone resulted in a limitation of growth and a failure of the reproduction. Photosynthetic oxygen production was not affected, but respiration increased strongly. The effect was more pronounced at higher temperatures. We conclude that, under hypertrophic conditions, toxicity of the ammonia generated in the anaerobic sediment layer can severely limit the development of the submerged plant populations.

Low bicarbonate levels at the end of the season strongly restricted the photosynthetic production. Plant age also had a significant effect, with low photosynthetic production after the beginning of seed production. Together with the depletion of nutrients and with the effect of high temperatures on photosynthesis, these factors can explain the decline of the vegetation at the end of the season, sometimes before the actual drying up of the bigger wetlands.

Two years of field data confirmed the importance of wind-induced sediment resuspension and periphyton growth in influencing the light climate experienced by the submerged vegetation. Phytoplankton was always scarce in the areas where submerged vegetation was developing. Plant biomass increased exponentially in early spring (March), with steady biomass yields (up to 100 g afdw m<sup>-2</sup>) together with abundant flowering and fruiting in late spring (April-May). Interannual variation was found to be very high, both concerning the abundance and the distribution of the submerged vegetation, mainly because of differences in rainfall which influenced the inundation cycle. Grazing by waterfowl can also account for this effect, as in dry years birds concentrate in the few wetlands still containing water.

For our conceptual model, we conclude that there is experimental evidence for direct physiological effects of high nitrogen concentrations on the decline of submerged macrophyte populations. This finding can complement the general hypothesis of a negative periphyton-mediated effect of high nutrient loads on the aquatic plants during the process of cultural eutrophication. Low light conditions have a strong, negative effect on the development of macrophyte populations. Macrophyte growth tends to improve the underwater light climate by stabilizing the sediment and reducing the attenuation caused by seston particles. Then, shading by periphyton, bicarbonate depletion and low nitrogen and phosphorus levels tend to be the main growth limiting factors. Flowering and seed production are thus quickly followed by plant senescence. Long-term survival of the seed bank and recolonization from seeds from neighbouring wetlands, the role of plant diversity in increasing the stability of the submerged vegetation meadows, the effect of grazing by herbivorous fauna, and the effect on light climate of sediment resuspension by carps, flamingoes and cattle trampling are proposed as elements worthwhile to study in the future.

Finally, some examples are provided for the use of the present conceptual model for the management of semiarid shallow wetlands.

# Chapter 1

## Scope of the research project

L. Santamaría

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## 1. INTRODUCTION

Mediterranean wetlands are often shallow and temporary (Florín *et al.*, 1993), and have brackish or saline water (Comín & Alonso, 1988; Martino, 1988). This applies to endorheic inland lakes, but also to coastal shallow lakes and estuarine floodplain wetlands. Temporary brackish and saline wetlands are characterised by the instability caused by recurrent (and often unpredictable) fluctuations in water regime and (parallel to that) salinity. This results in the presence of highly original communities, composed of opportunistic organisms with very flexible life-cycles and specifically adapted to the summer desiccation and the osmotic stress (Martino, 1988; Williams, 1985).

The fluctuating character of these wetlands is held responsible for their high productivity (Margalef, 1976). The dynamic character of the inhabiting communities makes them able to produce high biomass yields in a very short time (Montes *et al.*, 1982; Duarte *et al.*, 1990). Summer desiccation results in the detritic consumption of these high biomass yields, except in the cases where they are exploited by animals able to move elsewhere during the dry period (mainly migratory birds, but also fishes in the case of floodplain wetlands; Margalef, 1976). Still, an important part of the biomass may remain in the sediment forming the seed bank (here referring to the total pool of plant and animal propagules; Grillas *et al.*, 1992, 1993).

Once substantially altered by human interferences, these ecosystems are not easy to manage. While their conservation and even reconstruction was formerly considered to be relatively easy (Margalef, 1976), the ultimate dependence of these systems on poorly predictable climatic fluctuations makes them difficult to understand, to anticipate and thus to manage. Waterway embankment and drainage to obtain agricultural land are the two main disturbances affecting the estuarine and floodplain temporary wetlands. Through the modification of the spatial and temporal pattern of inundation and salinity, these disturbances may severely upset the diversity and productivity of such wetlands.

Due to their shallow character, a great number of the Spanish temporary wetlands are colonized by dense meadows of submerged vegetation. This vegetation offers shelter, food and physical substrate for the invertebrates and fishes (De Nie, 1987), resulting in a high secondary production. Several of these wetlands (among others, the National Parks of Doñana and Tablas de Daimiel, and the 'lagunas' of La Mancha) are of major international importance due to their role as resting and breeding zones for a large amount of Western European migratory birds (Finlayson & Moser, 1991). The capability of these wetlands to sustain such high numbers of migratory birds relies on the maintenance of a high primary production by the submerged vegetation.

The present study focused on the ecology of the submerged macrophyte communities of a Mediterranean temporary marsh, the Doñana marsh, located in the estuarine floodplain of the Guadalquivir River (SW Spain). In this area, dense meadows of submerged macrophytes grow in a mosaic of fresh to brackish wetlands flooded during winter and spring. *Ruppia drepanensis* Tineo is the most abundant macrophytic species (Duarte *et al.*, 1990; Grillas *et al.*, 1993).

This chapter introduces a research project that lasted from 1990 till 1994. The ultimate goals for the entire research project were to gain insight into the causes behind the decline of the submerged macrophytes in the Doñana marsh, to determine the optimal strategy to redevelop its potential biological diversity, and to generate strategies for the sustainable management of Mediterranean floodplain wetlands. In this PhD study, emphasis

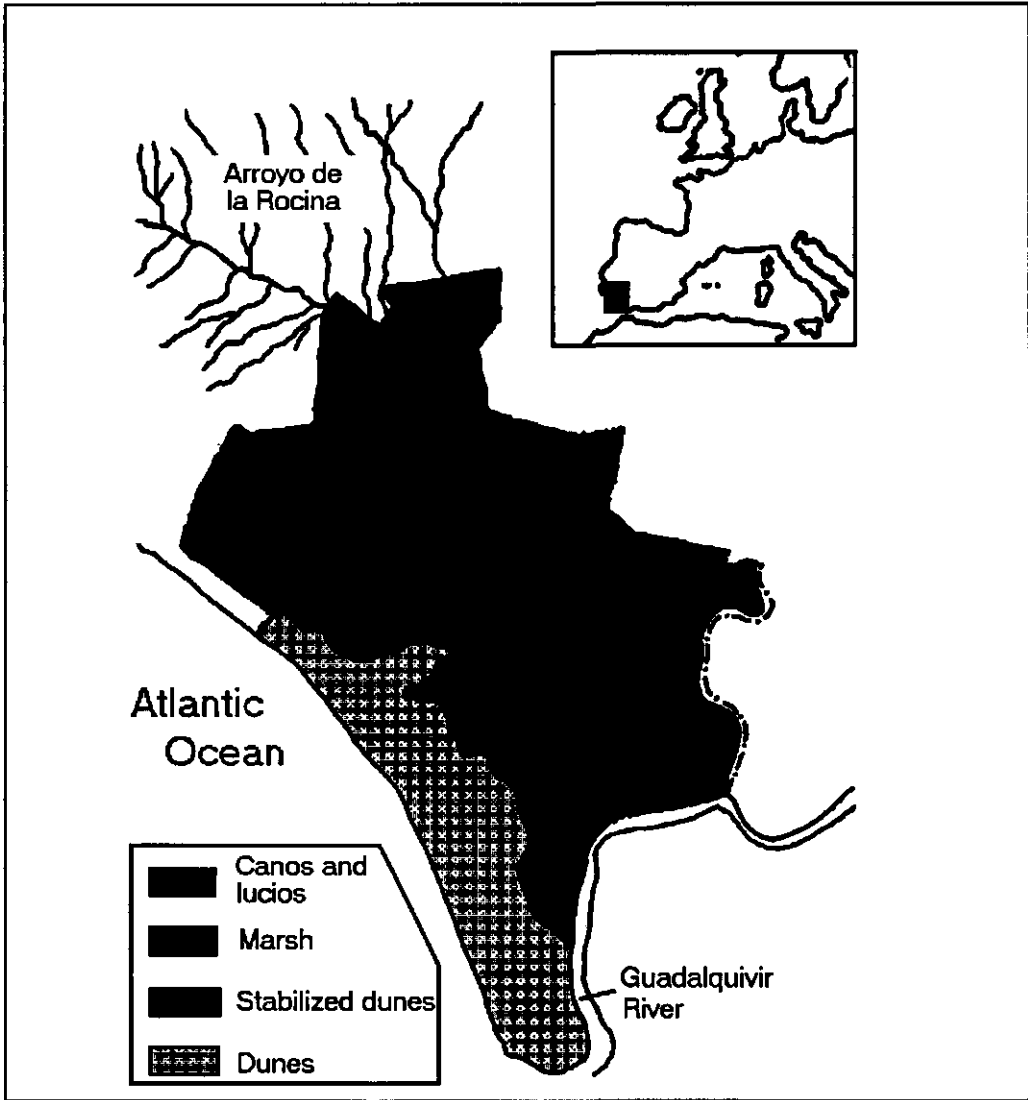


FIG.1: Situation and landscape units of the Doñana national Park. Adapted from Aguilar *et al.* (1986).

was placed on trying to collect as many relevant data as possible within one specific macrophyte community, the one dominated by *R. drepanensis*. It complements other projects carried out in the marsh. These include the population dynamics of the American swamp crayfish (*Procambarus clarkii* (Girard)) and its impact on the macrophyte populations, the relationship between the abundance and distribution of the seed bank and the standing aquatic vegetation (Grillas *et al.*, 1993), and the productivity of the submerged plant communities of the marsh (Geertz-Hansen *et al.*, in prep.).

## 2. DESCRIPTION OF THE STUDY AREA

The Doñana National Park is located in the floodplain of the Guadalquivir River (SW Spain, Atlantic coast; Fig. 1). It is the biggest Spanish National Park, covering 50.000 hectares, and it includes three different landscape units: dunes, Mediterranean forest and bushes on stabilized sand, and the former floodplain marsh of the Guadalquivir River. The Doñana National Park is especially known for its fauna, which combines a high diversity (125 species of birds and 28 of mammals) with the presence of endemic or endangered species. Moreover, its marsh is of major European importance for the wintering and nesting of high numbers of waterfowl and other wetland migratory birds (about 40.000 geese and 150.00 to 200.000 waterfowl overwinter regularly in the marsh; Aguilar *et al.*, 1986). The high production of the submerged vegetation of the marsh (Duarte *et al.*, 1990), and the subsequently high secondary production of invertebrates (Montes *et al.*, 1982) and fishes (Hernando, 1978), is responsible for feeding most of these wetland birds during winter and spring.

The plant and faunal communities of the Doñana National Park are composed of species adapted to the Mediterranean climate, with very dry summers and mild winters. Most of the rainfall occurs in autumn and winter, and most of the wetlands are temporary: they fill with water between October and March, and dry up between May and June. Many species are winter annual for this reason, which means that the highest biological activity in the aquatic habitats is found in winter and spring. This is indeed characteristic for a majority of the inland wetlands of the Iberian Peninsula, 70 % of which are small, shallow lakes with extreme seasonal fluctuations of the water level (Florin *et al.*, 1993).

The marsh of the Doñana National Park constitutes one third of the original area flooded by the Guadalquivir River (Bernues, 1990). Moreover, the two branches of this river which formerly flooded the Doñana marsh have been closed or diverged during the transformation of the rest of the floodplain into agricultural areas, and they do not discharge into the marsh any longer. The only watershed which still feeds the marsh is the local 'arroyo de la Rocina' (Fig. 1). Finally, the construction of a clay wall all along the Southern limit of the marsh, with gates for the control of water interchange through the original outflow channels have severely restricted the inflow of brackish water which used to enter the marsh during high tides. These changes virtually meant the end of the Guadalquivir estuary as an example of a characteristic Southern European floodplain type ecosystem.

At present, thus, the flooding of the different wetlands composing the marsh depends mainly on the direct rainfall and the inflow from the 'arroyo de la Rocina' watershed, plus some surface- and groundwater flowing from the neighbouring dune area (West of the marsh).

Because of the deposits of evaporated salts accumulated in certain parts of the marsh, a variety of habitats differing in salinity regime may be found in the area, following roughly a NW-SE gradient of increasing salinity. In combination with this, four groups of habitats may be distinguished according to their hydrology and water permanence. The 'caños' are the original channels of riverine water circulation, constituting three main courses from the former inflows of (from West to East) 'Madre de las Marismas', 'Guadamar' and 'Travieso'. The 'lucios' are depressions of the floodplain which keep water during a longer period of time than the 'quebradas', very shallow habitats which only keep water when the whole marsh is flooded (Fig. 2).

Salinity and water permanence are the two most important habitat characteristics of the Doñana marsh. They are closely linked, in the sense that salinity rise closely precedes

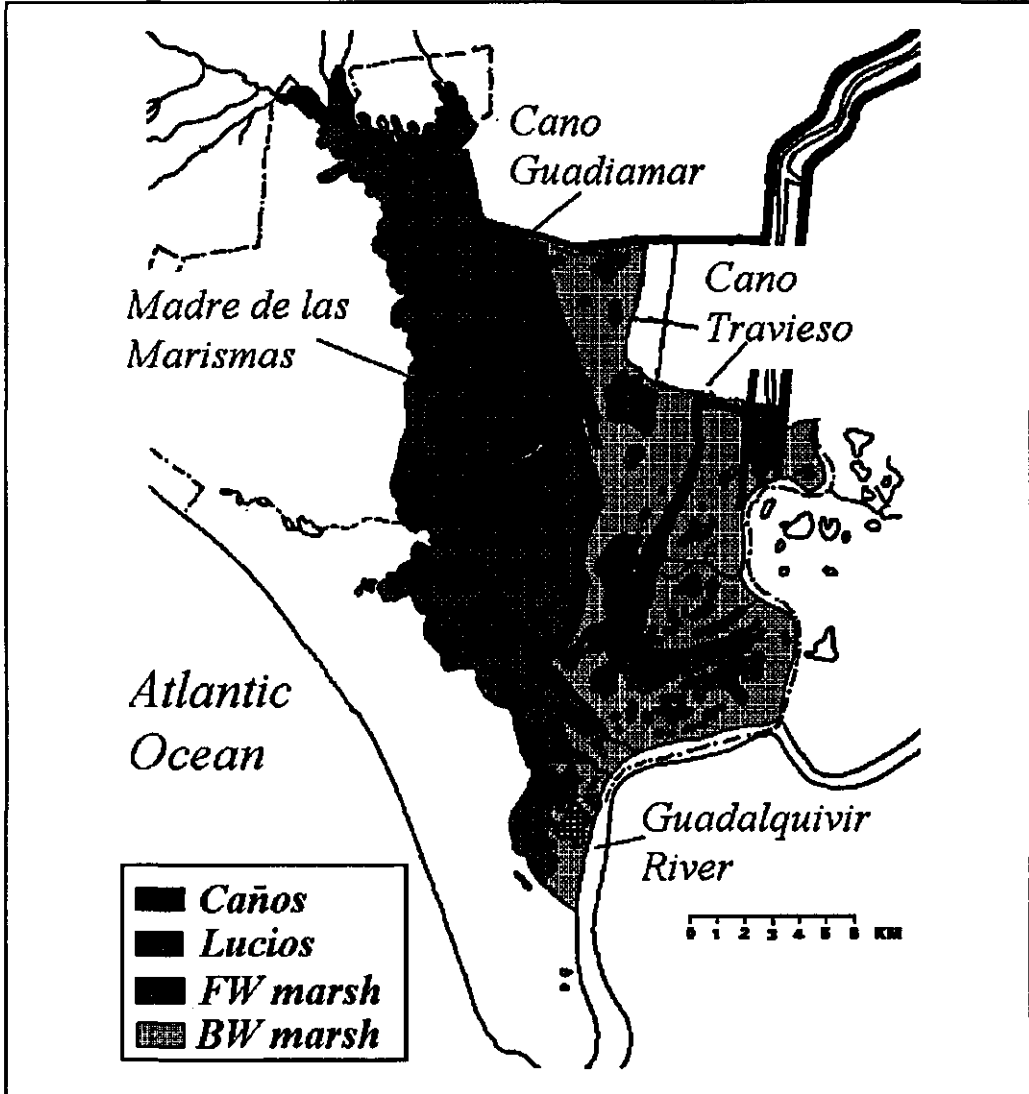


FIG.2: Different types of wetlands in the Doñana marsh. 'Quebradas' are subdivided in freshwater ('FW marsh') and brackish ('BW marsh'). Adapted from Aguilar *et al.* (1986).

desiccation, and that a species with a wider range of salinity tolerance may profit of a longer period suitable for growth and reproduction in the more saline wetlands. I consider both factors as very important because they are vital to the dominant primary producers in such shallow habitats, the aquatic macrophytes.

The characteristics of the brackish habitats of Southern Europe differ essentially from those in Northern or Central Europe. Van Vierssen & Den Hartog (in prep.) suggest that the salinity factor (and specifically its fluctuation) and the drying up of a habitat are such

dominant factors in Southern Europe, that the species composition of the submerged phytocoenoses seems almost completely determined by them. This has an important consequence for the study and management of Mediterranean estuarine floodplains such as the Doñana marsh. Salinity and temporality tend to result in the dominance of one or a few of macrophyte species. Each of these habitats has a low-diversity, but it is the heterogeneity of the wetland habitat mosaic that results in a high biological diversity for the marsh as a whole. Moreover, a certain replacement of species may occur along time.

In contrast, the brackish ecosystems in Northern and Central Europe usually sustain higher-diverse communities, which occur in large areas of the estuarine or floodplain system with minor changes in species composition along the environmental gradient. Adequate management of a fraction of the estuary is in this case enough to protect a representation of the whole system, and the study of its macrophytic component has to deal initially with the interaction between species at the community level. Mediterranean estuarine and floodplain wetlands are more likely to require an appropriate management on their overall extension, due to their 'mosaic' structure and the interannual changes in community distribution following climatic variability. The study of their macrophytic component may be better approached by combining several autecological studies, the interactions between species being probably less important.

Six species of angiosperms (*R. drepanensis*, *Zannichellia obtusifolia* Talavera, García-Murillo & Smit, *Callitriche truncata* Guss., *Ranunculus baudotii* Schank and *Myriophyllum alterniflorum* DC., and *Aithenia orientalis* (Tzvelev) García-Murillo & Talavera) and two genera of charophytes (*Chara* and *Tolypella*) compose the submerged vegetation of the marsh (Duarte *et al.*, 1990; Grillas *et al.*, 1993). Neither paper described the community types, but the two more abundant angiosperms in the marsh (Grillas *et al.*, 1993), *R. drepanensis* and *Z. obtusifolia*, are respectively the dominant species in the two different associations defined by Van Vierssen & Den Hartog (in prep.) as characteristic for the Mediterranean temporary wetlands: *Ruppium drepanensis* (Ruppiaetea: Ruppion), and *Zannichellietum peltatae* (Potametea: Callitricho-Batrachion).

### 3. THE CONCEPTUAL MODEL

I proposed above that salinity and water permanence are the two most important habitat characteristics of the Doñana marsh. The osmotic stress (high salinities) and the drying up of the habitats mean essentially the same for any macrophyte: they restrict the length of the life-cycle. Both the formation of land-forms (as in *R. baudotii*) and the development of a broad range of salinity tolerance (as in *R. drepanensis*, *A. orientalis* and *Tolypella*) result in an extended growing season. But the production of special propagules is still the strategy adopted by all species to survive the summer drought. In habitats which dry up completely during prolonged times, the production of drought resistant seeds seems to be the only relevant survival mechanism. Tubers and turions are drought resistant to a much lesser extent, and have only been found in occasional permanent localities of the marsh (for example, tubers of *Potamogeton pectinatus* L. were found in a lucio where the sediment is subject to groundwater seepage from the nearby dunes).

The winter annual submerged macrophytes from these brackish waters produce abundant seeds (e.g. Verhoeven, 1979; Grillas *et al.*, 1993: seed banks of 1000 to 5500 seeds m<sup>-2</sup>), and they enable early germination and growth when the habitat has water (late



autumn-winter; see e.g. Van Vierssen & Van Wijk, 1982<sup>1</sup> for the germination ecology of *Zannichellia peltata* Bertol.).

The life-cycle of annuals has two essentially different phases (Silvertown, 1982):

1. the **established phase**: plants grow vegetatively.
2. the **regenerative phase**: reproduction occurs. Propagules (seeds, for *R. drepanensis*) are produced, survive and produce new plants.

I will now go into some more detail about these two different phases. In Fig.3, I have summarised my thoughts regarding the interplay of different factors affecting them, together with their sequence along the plant's life-cycle.

### 3.1. The established phase

It is important to define the length of the period which is available for normal vegetative growth. Firstly, differences in salinity tolerance may modify the extension of the period suitable for vegetative growth. Secondly, I postulate that the time a species 'needs' before it can reproduce can be quite different. Therefore, information is needed on the factors regulating the onset of reproduction in these habitats.

I propose vegetative growth to be mainly affected by the following factors:

1. the *light climate*: the light climate experienced by the plant ('received PAR') will determine its photosynthetic production, and may influence the plant's architecture (morphology, chlorophyll concentration) and physiology (e.g. its photosynthetic and respiratory rates) due to acclimation processes (Boardman, 1977). In turn, the received PAR will depend on plant architecture, and on the turbidity of the overlying water column. The latter is mainly caused by (a) sediment resuspension, which may be increased by wind-induced turbulence and by bioturbation (carp feeding habits; trampling by cattle and treading by flamingoes, Duarte *et al.*, 1990), and is expected to be reduced by the growth of the submerged vegetation (due to the stabilization of the sediment particles; Duarte *et al.*, 1990); and (b) on the concentration of phytoplanktonic algae, which depends on the concentration of nutrients in the water and is reduced in the presence of dense macrophyte stands (Van Vierssen & Prins, 1985; Hootsmans, 1994). Finally, periphyton growing on the macrophyte leaves may further attenuate the incoming irradiance.

2. the *availability of C*: through the growing season, especially in brackish water (pH  $\geq$  8), the concentration of free CO<sub>2</sub> may become undetectable, bicarbonate becoming the dominant C species. Further increases in pH may also restrict the availability of bicarbonate. The photosynthesis of the submerged macrophytes will thus directly depend on their ability to use bicarbonate, and on their particular response to a restricted C supply.

3. the *availability of N and P*: main sources of N and P are expected to be found in the sediment for most of the Doñana wetlands. Still, a relatively high release to the water column may be expected due to their high surface-volume ratio. N and P bound to the clayish sediment and coming from the organic matter mineralised during the dry season are

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<sup>1</sup> One of the varieties studied by these authors, *Z. peltata* var. *peltata*, received the species status as *Z. obtusifolia* in Talavera *et al.* (1986).

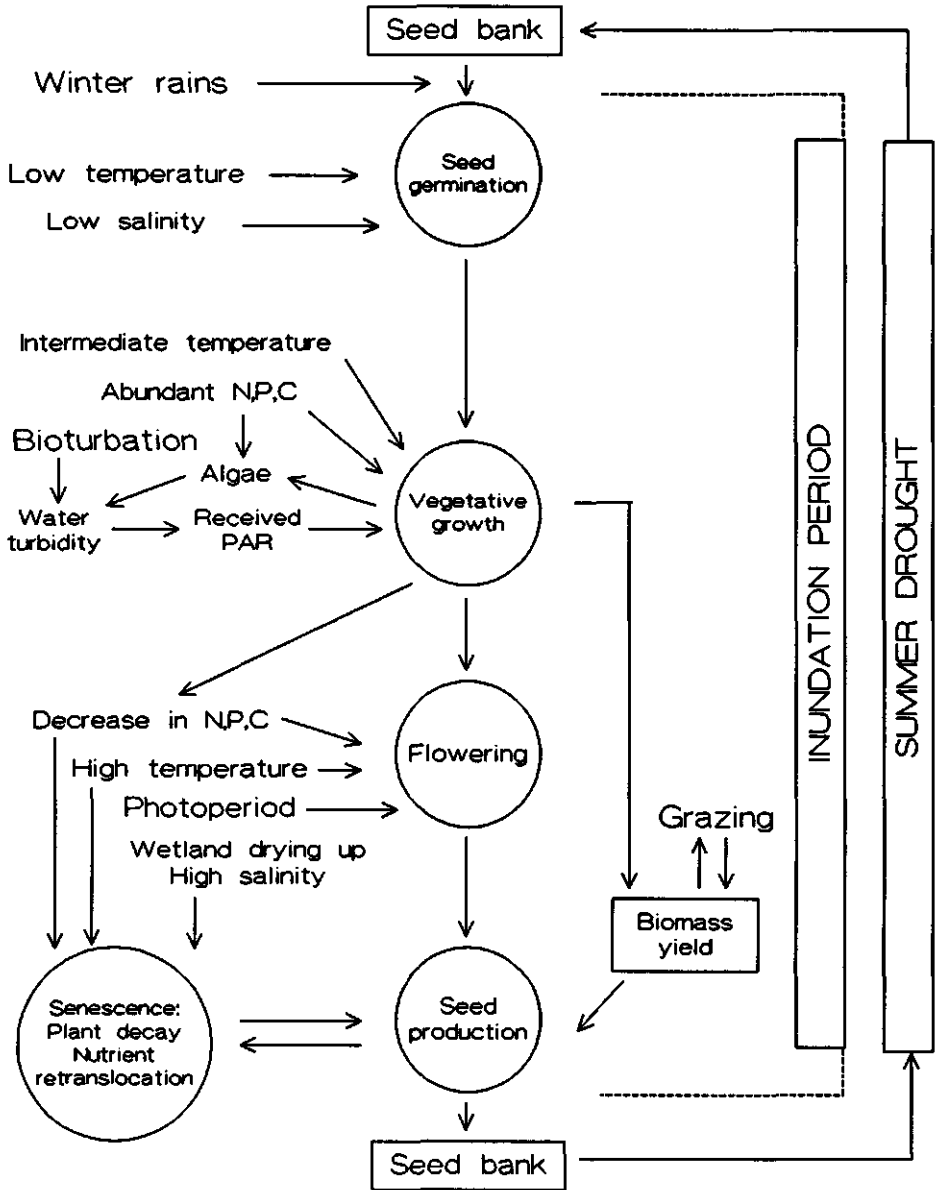


FIG.3: The most important factors determining the development of the submerged macrophyte meadows in the Mediterranean temporary wetlands. Boxes indicate quantities, circles indicate processes.

hypothesised to be high at the beginning of the growing season, while their availability to the plants would decrease strongly following abundant vegetative growth. In that moment, either N or P could become limiting for the growth of the plants.

4. the *temperature*: while low temperatures (8-12 °C) result in optimum germination in *R. drepanensis* and *Z. peltata* (see above), intermediate temperatures (15-20 °C) are expected to result in optimum vegetative growth, and even higher temperatures (20-30 °C) in the induction of reproduction (as reported by Setchell, 1924 and Verhoeven, 1979 for *Ruppia maritima* L. s.l.).

### 3.2. The regenerative phase

This period will be shorter than the total length of the life-cycle, yet it is of vital importance for the year-to-year survival of the population. To know how much time is needed to complete its life-cycle and compare it with the period available in the field, this aspect should be studied in detail. The major environmental cues which are responsible for the production of seeds are the photoperiod s.l., the temperature and the nutrient status of the environment. Besides, the size of the plants may also affect both the timing and the abundance of propagule production.

1. The *photoperiod* has been widely reported to trigger the induction of flowering in many plant species (Salisbury, 1981). Winter annual species such as the aquatic plants from the Doñana marsh are expected to flower in spring, when daylength is quickly increasing. Thus, long-day conditions may be expected to induce propagule production in these species.

2. The *temperature* is hypothesised to be a factor of major importance in triggering the different developmental stages of brackish water plants (Verhoeven, 1979). As mentioned above, relatively high temperatures (20-30 °C) may initiate the reproduction, while still higher temperatures ( $\geq 30^\circ\text{C}$ ) may progressively inhibit the formation of the propagules.

3. The *availability of nitrogen* is hypothesised here to also induce propagule formation. Increased energy expenses due to a shortage in N availability at the end of the growing season would, according to this hypothesis, result in a switch to the allocation of the plant resources to reproduction.

4. The *size of the plant* has been related both with the initiation and with the abundance of propagule production for several aquatic angiosperms (Titus & Hoover, 1991; Olesen, 1993). A fast vegetative growth would thus result in an earlier flowering and a higher abundant seed yield, increasing the chances for next year's survival.

Due to their annual character, Mediterranean brackish water species are likely to allocate all their resources in a quick and abundant production of seeds once reproduction has been triggered. Senescence of the plant closely follows, having the supplementary role of re-allocating all the nutrient stored in the plant tissue to the newly formed propagules.

#### 4. RESEARCH APPROACH

Several research topics were derived from the conceptual model. A submerged macrophyte community dominated by *Ruppia drepanensis* was chosen to act as model system. This species is the most abundant in the Doñana marsh (Duarte *et al.*, 1990; Grillas *et al.*, 1993), in a majority of the Spanish inland temporary lakes (Cirujano & García-Murillo, 1990) and in a great number of Mediterranean temporary wetlands (Cirujano & García-Murillo, 1992).

This project combined several laboratory experiments with the collection of two years of field data in the Doñana brackish marsh. Besides, the distribution of this species was mapped in May 1991 for the totality of the marsh.

A laboratory study was done on the influence of light climate on plant growth and reproduction (chapter 2). Special attention was paid to the ability of the plants to acclimate to low-irradiance regimes, both morphological and physiologically, and to the discrimination of irradiance- and photoperiod-mediated effects on the induction of flowering. The last required a careful discrimination between the photoperiod and the photosynthetically active period experienced by the plants.

The effect of water temperature on plant growth and reproduction was studied in the laboratory (chapter 3). Johnson's mathematical description of the effect of high temperatures for metabolic rates (Johnson *et al.*, 1974) was first applied to literature-reviewed data on submerged macrophyte's photosynthetic performance. This formulation provided a flexible and accurate description of the decrease in photosynthetic activity at high temperatures, based on a simple model of enzymatic reversible thermal inactivation. Two experiments further concentrated on describing the acclimation response of *R. drepanensis* to higher temperatures, and on characterising the effect of temperature on flower induction.

The separate effect of two different forms of nitrogen supply were under consideration in chapter 4 and 5. In chapter 4, the effect of ammonia toxicity was tested by means of a rhizosphere fertilization experiment. After this, a second experiment tried to elucidate the effect of an increased nitrate supply on the timing and abundance of flowering.

A set of field measurements performed in the field in 1991 aimed at achieving a quick evaluation of the interactive effect of carbon limitation, irradiance level and plant reproduction on the photosynthetic performance of intact plants (chapter 6). These measurements were used also to evaluate the comparability of our photosynthesis measurements using plants grown in the laboratory and those performed using field material.

The interplay of different factors in the field was studied during two years in the Doñana marsh. Special attention was paid to the evaluation of the light climate as a critical factor regulating the development of the *R. drepanensis* vegetations (chapter 7). The role of periphyton growth in affecting the underwater light climate was also evaluated. Field data permitted also a quantification of the relationships derived from the laboratory experiments, considered of basic importance to achieve conclusions concerning the functioning of the macrophyte-dominated ecosystems.

The last chapter attempts to integrate the results and assess their implications for the management of the Mediterranean floodplain and temporary wetlands (chapter 8).

5. APPENDIX: TAXONOMY, DISTRIBUTION AND ECOLOGY OF *Ruppia drepanensis*

The genus *Ruppia* L. (Tribe *Helobiae*, Fam. *Potamogetonaceae*) refers to a cosmopolitan group of delicate submerged phanerogams inhabiting coastal brackish waters and inland saline habitats. Typical characters are their narrow linear leaves, strongly branched horizontal and vertical stems, and inflorescences born on a peduncle of variable length. As Verhoeven (1979) pointed out, lack of obvious taxonomically suitable characters and niche overlap of the different taxa made the subdivision of the genus controversial.

Verhoeven (1979) attempted to clarify the taxonomy of the genus *Ruppia* in Western Europe. He combined taxonomical, ecological and physiological work with cytotaxonomical results reviewed from Reese (1962), and concluded that three taxa existed: *R. cirrhosa* (Petagna) Grande, inhabiting larger permanent water bodies of medium-high salinity, *R. maritima* var. *maritima* L., occurring mainly in smaller permanent water bodies with low-medium salinity, and *R. maritima* var. *brevirostris* (Agardh) Aschers. Graebn., characteristic for temporary waters of various sizes.

Unfortunately, Verhoeven's work left out the Italian and Spanish populations of *Ruppia*, where *R. drepanensis* occurs (sometimes referred to as *R. maritima* var. *drepanensis* (Tineo) C.Shum; Cirujano, 1986). After Verhoeven (1979), several authors revised the taxonomy of *Ruppia* in Western Europe by combining taxonomical characters with chromosome counts and ecological features (Marchioni, 1982a, 1982b; Van Vierssen *et al.*, 1981), sometimes also including the germination ecology of different taxa (Van Vierssen *et al.*, 1984). Subsequent work published by Cirujano (1986), Talavera & García-Murillo (1987) and Cirujano & García-Murillo (1990) gave additional support to the discrimination of *R. drepanensis* as a separate species.

Like *R. cirrhosa*, *R. drepanensis* shows long flower peduncles and epihydrophilous pollination, while *R. maritima* has short flower peduncles and hypohydrophilous pollination (Cirujano, 1986). Although in *R. cirrhosa* peduncle length correlated positively with increasing depth (Verhoeven, 1979), a minimum length is probably genetically fixed, since *R. drepanensis* plants growing in very shallow lakes still developed their characteristic long peduncles (Cirujano, 1986). Peduncle length is linked to the pollination mechanism and, as differences in pollination prevent cross-fertilization, it can be an important taxonomic criterion.

*R. drepanensis* has delicate leaves, that are much narrower (0.1-0.3 mm width) than in *R. cirrhosa* (0.5-1 mm width) and lack the two lateral venations. *R. drepanensis* inhabits temporary coastal and inland waters, tolerating high fluctuations in salinity and a broad range of ionic composition (from sodium-chloride to magnesium-sulphate waters; Cirujano, 1986; Martino, 1988). While *R. cirrhosa* has a perennial life-cycle and produces asexual propagules (vegetative stolons and stems; Verhoeven, 1979), *R. drepanensis* is a winter annual depending on the production of sexual propagules (seeds) for surviving the summer dry period. *R. maritima* may show both strategies depending on habitat characteristics, although vigorous seed production probably occurs mainly for dispersal (Verhoeven, 1979).

While *R. maritima* and *R. cirrhosa* are cosmopolitan species, *R. drepanensis* is apparently restricted to the Western Mediterranean: it has been found in Italy, Spain, Portugal, Tunisia, Algeria and Morocco (Cirujano & García-Murillo, 1990). In Europe, *R. maritima* and *R. cirrhosa* show overlapping distributions. Both occur all along the Atlantic and Baltic coasts, in most countries bordering the Mediterranean (Verhoeven, 1979), and are also reported from inland saline lakes in Russia, Rumania, Germany and France (Verhoeven, 1979). *R. maritima* also occurs in inland saline lakes in Spain (Cirujano, 1986).

Reese (1962), working with plant material originating from Germany, found *R. cirrhosa* to have a chromosome number of  $2n=40$  and *R. maritima* to have  $2n=20$ . Van Vierssen *et al.* (1981) reported contrasting numbers for *R. maritima* populations from The Netherlands ( $2n=20$ ) to those from the Camargue, France and Cádiz, Southern Spain ( $2n=40$ ). While Marchioni (1982b) found a  $2n=20$  chromosome number for *R. maritima* from Sicily (Italy), Cirujano (1986) reported  $2n=40$  for *R. maritima* from Central Spain. Talavera & García-Murillo (1987) mentioned *R. cirrhosa* to have  $2n=40$  chromosomes and *R. maritima* to have  $2n=20$  and 40 chromosomes, all their plant material originating from Cádiz (S Spain). On the other hand, Cirujano (1986), Marchioni (1982b) and Talavera & García-Murillo (1987) all obtained identical counts ( $2n=20$ ) for *R. drepanensis* plants from Central Spain, Italy and Southern Spain respectively.

Reese (1962) considered *R. cirrhosa* ( $2n=40$ ) to have originated as a polyploid from *R. maritima* ( $2n=20$ ). However, submerged angiosperm hypohydrophilous species are generally considered to have evolved later than epihydrophilous species (Sculthorpe, 1967). Cirujano (1986) suggested that *R. cirrhosa* could have originated as a polyploid from *R. drepanensis*, switching to a permanent life-cycle while adapting to permanent less-saline waters and thus increasing the relative importance of vegetative reproduction. In this context, it seems

also reasonable to consider *R. maritima* to have evolved from *R. drepanensis* by switching to a hypohydrophilous pollination.

Van Vierssen *et al.* (1984) related differences in optimum germination temperature to the life-cycle and distribution of the European species of *Ruppia* and *Zannichellia*. In general, winter-annual species have a low optimum germination temperature (*R. drepanensis*, *Z. peltata* Bertol.) and occur distributed in Southern Europe. Summer-annuals occurring in Central Europe (from N Spain to Denmark) have relatively high optimum germination temperatures (*R. maritima* var. *maritima* and *Z. pedunculata* Rchb.). *Z. major* Boenn. and *R. cirrhosa*, which are perennials occurring in permanent waters, produce a relatively low number of seeds. After analyzing the germination characteristics of *R. maritima* var. *maritima*, they also concluded that this species shows a rather complicated life-history, allowing it to colonise both permanent and temporary waters as far as the latter do not dry up more often than once every 2 years.

It is noteworthy that *R. maritima* may coexist with *R. drepanensis* in some temporary water bodies, although it clearly tends to colonise permanent habitats. Moreover, several polyploidic populations of *R. maritima* have been reported in Southern Europe. Both facts support the theory proposed by Cirujano (1986) on the evolutionary origin of *R. cirrhosa*. These considerations place *R. drepanensis* in the centre of speciation of the genus *Ruppia* in Europe. Changes in the optimum germination temperature may have led to the evolution of *R. maritima* in more permanent wetlands, while a switch to vegetative reproduction resulted in *R. cirrhosa*. With sexual reproduction losing importance but seed production being still vital, the appearance of hypohydrophilous pollination in *R. maritima* is easily explained. *R. cirrhosa*, in contrast, reproduces mainly vegetatively, so hydrophily did not represent any major advantage.

The ecology of *R. maritima* s.l. and *R. cirrhosa* has been quite thoroughly studied in the USA (see, e.g., Richardson, 1980; Thursby & Harlin, 1984; Dunton, 1990; and Koch & Dawes, 1991a, 1991b) and Europe (e.g. Verhoeven, 1979, 1980a, 1980b; Kjørboe, 1980; Menéndez & Comín, 1989; and Kautsky, 1991). The same applies to the ecology of the Australian *Ruppia* species (e.g. Brock 1982a, 1982b, 1983; Brock & Casanova, 1991; also Mitchell, 1989, from New Zealand). In contrast, work dealing with the ecology, physiology or population dynamics of *R. drepanensis* is virtually lacking. Cirujano (1986) and Martino (1988), while working on the flora of the Spanish saline lakes, gave some indications on the characteristics (temporary saline and brackish lakes), salinity range (up to 80 mS cm<sup>-2</sup>) and ionic composition (from sodium-chlorine to magnesium-sulphate waters) of the wetlands inhabited by this species. Van Vierssen & Martino (unpublished data) studied the influence of temperature, salinity and ionic composition of the water on the germination of *R. drepanensis* seeds. They found maximum germination rates of 75 to 100 % when combining low temperatures (8 to 12 °C) with low salinities (freshwater to 6 g l<sup>-1</sup> NaCl or to 20 g l<sup>-1</sup> MgSO<sub>4</sub>). Finally, García *et al.* (1991) studied the photosynthetic performance of *R. drepanensis*, finding relatively low net photosynthesis rates, a moderate photoinhibition above 695 μE m<sup>-2</sup> s<sup>-1</sup> and high carotenoid concentrations which they attributed to an adaptative response to high irradiance regimes.

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## Chapter 2

# Interactive effect of photoperiod and irradiance on the life cycle of a winter annual waterplant, *Ruppia drepanensis* Tineo.

L. Santamaría and W. van Vierssen

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**ABSTRACT:** In a laboratory experiment, *Ruppia drepanensis* Tineo seedlings from a brackish marsh in Southern Spain were grown in a factorial experiment combining photoperiod (PP: 16 and 10 h), photosynthetic period (PAP: 16 and 10 h) and irradiance level (Photosynthetic Photon Flux Density, PPFD: 285 and 145  $\mu\text{E m}^{-2} \text{s}^{-1}$ ). Additionally, two other irradiances (110 and 50  $\mu\text{E m}^{-2} \text{s}^{-1}$ ) were tested under the 16 h PAP treatment. Several morphometric characters were recorded during the first five weeks of the experiment, and photosynthesis and respiration were measured after 11 and 16 weeks of growth.

Results showed a significant reduction of growth and development with decreasing irradiance level, together with a strong reduction in the seed yield below 145  $\mu\text{E m}^{-2} \text{s}^{-1}$ . Plants showed a limited morphological adaptation to low PPFD, but a strong acclimation effect was found in the photosynthetic response. With decreasing irradiance level, maximum photosynthetic rates ( $P_m$ , estimated non-linearly from hyperbolic photosynthesis-irradiance curves) increased. PP and PAP did not affect growth and development, but both longer PP and PAP resulted in earlier flowering, whereas longer PAP also resulted in a higher seed production.

Regarding the influence of the underwater light climate on *R. drepanensis* populations, we conclude that both the reduced growth due to low irradiance levels and the decreased seed production resulting from the shorter PAP will reduce interactively the survival potential of the vegetation. Consequently, annual waterplant populations such as *R. drepanensis* are predicted to decline and ultimately vanish at underwater irradiance levels ( $\leq 145 \mu\text{E m}^{-2} \text{s}^{-1}$ ) which are still above the light compensation point calculated from the photosynthesis-irradiance relationship.

## 1. INTRODUCTION

### 1.1. *The scope of the study*

Mediterranean wetlands are often shallow and temporary in nature: they usually have no water from late spring to early autumn. Such wetlands may support a high productivity during late spring and early summer (Duarte *et al.*, 1990), but their fauna and flora are obliged to a yearly regrowth from drought resistant propagules. Vigorous wind-induced sediment resuspension may also occur, especially in early spring when there is little vegetation to stabilise the lake bottom (Santamaría *et al.*, 1994a). This strongly reduces the light available to submerged macrophytes. Such an environment functionally resembles turbid eutrophic lakes (De Nie, 1987). Thus, shallow wetlands are particularly sensitive to phytoplankton and periphyton growth due to eutrophication, as negative shading effects on the submerged vegetation may act synergically with sediment resuspension. Yearly dependence on the offspring (seeds) of previous years is likely to accelerate the decline of macrophyte populations under such circumstances.

*Ruppia drepanensis* Tineo is the dominant submerged macrophyte in many Mediterranean saline and brackish waterbodies (Martino, 1988; Cirujano & García Murillo, 1990), and particularly in the Doñana National Park brackish marsh area. It is an annual species producing abundant drought-resistant seeds in late spring to survive summer desiccation. High interannual variation in rainfall distribution makes the hydrological cycle of these wetlands rather unpredictable. Under such circumstances, production of an excess of propagules in favourable years seems to be an effective strategy to create a seed bank able to regenerate the population even after several unfavourable, dry years (Grillas *et al.*, 1993; see also Thompson & Grime, 1979). Thus, factors affecting flowering and seed production may be of major importance for the year-to-year survival of *R. drepanensis* populations.

Seed production depends on a chain of events (Titus & Hoover, 1991). First of all, plants have to achieve a vegetative development sufficient to sustain the production of seeds. Flower production has to be triggered, pollination has to occur, seeds have to be formed and must ripen, and all this should be finalised before the wetland dries up. Time being such an important factor, any delay in flowering and fruiting or in biomass development may strongly restrict seed production.

Several factors are known to affect flower induction. Photoperiod (PP) is often considered as the most important one, and may also affect plant morphology and development (Salisbury, 1981). Plant biomass is reported to affect not only flower abundance and seed production, but sometimes also flower induction (Titus & Hoover, 1991). As irradiance (photosynthetic photon flux density, PPF) and photosynthetic period (PAP; defined as 'time interval during which the irradiance level is sufficient to sustain a positive net photosynthesis' by Chatterton & Silviu, 1979) are known to affect plant morphology and biomass production, they may indirectly affect flower induction, flower abundance and seed production. Moreover, PAP has been reported to directly affect the production of asexual propagules (Van Vierssen & Hootsmans, 1994). Hence, it seems reasonable to hypothesize a possible effect of PAP on flowering and seed production.

As described in Van Vierssen & Hootsmans (1994), shading due to turbid water conditions not only restricts the instantaneous PPF, but also shortens the PAP experienced by the submerged plants. The more turbid the waterlayer is, the shorter the PAP becomes and, PP being hardly affected, the lower the PAP/PP ratio becomes. Increasing depth enhances this effect.

As decreases in irradiance and PAP both result from increasing turbidity, it seems worthwhile to study their combined effect on plant growth and reproduction. Mechanisms allowing acclimation to shading effects are of vital importance, both during the vegetative (morphological or physiological acclimation) and the generative phase (early or more vigorous reproduction). Knowledge of these is needed to enable an accurate prediction of the effect of cultural eutrophication on plant natural populations.

Here we study the interactive effect of irradiance, photoperiod and photosynthetic period on the photosynthesis and growth, as well as on the flowering and seed production, of *R. drepanensis*, and we test the following hypotheses:

- (1) a long photoperiod (PP) enhances flowering and seed production;
- (2) acclimation to reduced PPFD is mainly achieved through environmentally-induced changes in the light response curve;
- (3) a short photosynthetic period (PAP) will promote an acclimation response similar to that due to low irradiances (PPFD);
- (4) flowering and seed production is followed by deterioration of the vegetative apparatus, even in the absence of adverse environmental conditions.

Prior to discussing the experiment to test these hypotheses, separate sections are devoted to (a) the analysis at clonal and genet level, and (b) the mathematical description of the photosynthesis-irradiance relationship. The first is considered necessary because within-plant variability is an important characteristic of obligate annual populations relying on sexual reproduction for the production of propagules, and it may be underestimated if analysis is kept at the clonal level. The second is included as we consider photosynthesis to play a major role on plant acclimation to different light regimes, and the mathematical formulation of the photosynthesis-irradiance has been object of recent controversy (see, e.g., Hootsmans & Vermaat, 1994).

### *1.2. Analysis at clonal or genet level: limitations imposed by the life cycle*

Generalized life-cycle stages as proposed by Verhoeven (1979) for *R. cirrhosa* and *R. maritima* may also be applied to *R. drepanensis*. Due to its (winter) annual character and the relative shortness of the period suitable for plant growth and reproduction (3-4 months), *R. drepanensis* has a rather simple life cycle. After seed germination, seedling emergence is followed by a fast horizontal branching ('horizontal propagation', in Verhoeven's (1979) terminology). After that vertical shoots with erect stems develop ('vertical propagation'; Verhoeven, 1979), and subsequently the flowering and seed production from bundles located in vertical shoots. We never observed winter quiescence and budding, and summer secondary horizontal propagation as described by Verhoeven (1979) for other *Ruppia* species.

When attempting to analyze morphology and population dynamics, several different study levels may be adopted (such as, for example, the population, genet, clone and ramet levels). We consider the shoot complex ('set of modular units comprising horizontal and vertical, sexual and asexual modules', Wiegleb & Brux, 1991) to be the appropriate level of study for *R. drepanensis*. In this species this level coincides with the 'genet' and the 'patch' level due to its annual character. Nevertheless, significant morphological adaptations may be expected to appear at the vertical shoot level. Studies at this level may thus be recommendable.

Wiegleb & Brux (1991) discouraged the use of the genet, clone and population levels for the study of *Potamogeton* species due to their low degree of tangibility. However, when dealing with species inhabiting temporary wetlands, all these three levels become clearly differentiated. We may clarify this using the genus *Ruppia*. Under temporary conditions, only sexual propagules are used to survive the dry summer. In such populations, every shoot complex is equivalent to a different genet. In contrast, under stable, permanent conditions, asexual propagules are used to survive the adverse season, while sexual propagules are used for (long-distance) dispersal and to establish a seed bank assuring survivorship after catastrophic events. In the latter habitats, different genets will spread and mix in the population, each one consisting of several clonal shoot complexes. Tangibility of genet and clone will now be very low.

In consequence, the importance of independent clonal units strongly decreases from permanent, undisturbed habitats to temporary, disturbed ones. In temporary habitats, each genet is a clearly discernable, discrete unit in the population of slightly different strategies tried by the plant population every year using genetic recombination. Under such circumstances, to concentrate on the lower clonal level would reduce our perception of the variance at the basis of the survival strategy of the species under study, and may in addition result in problems of pseudoreplication (see Hulbert, 1984) if units being clones of different genets are pooled as replicates.

In this laboratory experiment, individual seeds were used as starting material, hence measurements on the clonal level could always be pooled for each genet, the average value per genet then being considered as a single replicate value for the population. The difficulty of applying this approach in dense populations in the field is obvious, but it may be solved in temporary habitats just by making sure that each unit chosen as replicate is part of a different genet, physical distance being a practical criterion. Erroneous estimation of the actual variance endowed to genetic plasticity is inevitable when working in permanent habitats, as far as the identity and abundance of the different genets would remain unknown.

### 1.3. Curve fitting of the PI curves: may we obtain transcendental parameter estimates?

Photosynthesis-Irradiance curves (PI curves) are graphs or equations describing the characteristic dependence of the instantaneous rate of photosynthesis on the incident irradiance. During the last decades the use of such models has become an important component of plant physiology and production ecology, and many different equations have been proposed (see review by Lederman & Tett, 1981).

Hereafter, 'irradiance' will be used as a measure of the photosynthetically active radiation (PAR; McCree, 1972). Irradiance is the radiometric term for the radiant flux incident on the receiving surface from all directions. As McCree (1981) clearly points out, 'irradiance is the correct radiometer term for the property that is commonly referred to as the "light intensity".' In fact, 'the use of the word "light" (radiation that is visible to humans) is inappropriate in plant research.' Still following McCree (1981), we will express irradiance as incident photosynthetic photon flux density (PPFD, in  $\mu\text{E m}^{-2} \text{s}^{-1}$ ), as he concluded PPF to be clearly superior to photosynthetic irradiance (PI, in  $\text{W m}^{-2}$ ) as a measure of the PAR (the use of PPF instead of PI reduced measurements errors from 19% to 8%).

PI curves all exhibit comparable shapes. Over a range of low irradiances, the rate of photosynthesis rises almost linearly with increased irradiance. At higher irradiances, the rate

increases more slowly and eventually reaches a maximum rate at light saturation. It is generally assumed that the initial slope of the curve is a function of the light reactions of the photosynthesis. The curve levels off as availability of carbon becomes limiting (Wareing *et al.*, 1968). At yet higher irradiances, the rate generally declines due to 'photoinhibition'. Photoinhibition is attributed to an excess of excitation energy coming from the exposure to high irradiances, causing the destruction or reorientation of photosynthetic organelles and/or pigments (Drew, 1979; Ögren *et al.*, 1984). Nevertheless, photoinhibition is frequently absent from measured PI datasets. The three above-described features of PI curves are apparently independent of each other. As Lederman & Tett (1981) pointed out, the principle of parsimony argues that we should not need more than 2 parameters for an adequate description of a PI curve lacking photoinhibition, while 3 parameters should be enough to include the effect of photoinhibition. This has important consequences regarding the search for a general model describing both curves with and without photoinhibition (see below).

Jassby & Platt (1976) attempted to identify a general mathematical description for the PI curves by comparing the abilities of eight 2-parameter models to describe the same set of data. They concluded that a best model could be found and that, for data which did not show photoinhibition, it was their own hyperbolic tangent function. Subsequently, Lederman & Tett (1981) pointed out that the fitting technique used by Jassby & Platt (1976) was statistically inadequate, as it did not provide with objective (simultaneous and independent) estimations of the different parameter of the curves. Both the use of linearization methods and the use of sequential estimation give results that are biased, and thus cannot be subjected to a statistical test. The same objection holds for the works of Chalker (1980) and Orr *et al.* (1988), which both supported the conclusions of Jassby & Platt (1976). Lederman & Tett's (1981) re-analysis of the data of Jassby & Platt (1976) using a direct, simultaneous estimation method showed that the fit of several models could not be distinguished from that of the hyperbolic tangent. They conclude that with the presently available methods of measurement and parameter estimation, several simple models are equally good. Although the use of several criteria (parsimony, simplicity, catholicism and conservatism) is suggested to select a model, they stress that 'once modellers have used the objective RSS (Residual Sum of Squares) criterion to eliminate badly-fitting models, then the choice of a model may be made according to whatever criterion seems more relevant'.

Hootsmans & Vermaat (1994) compared two different models, the rectangular hyperbola (Michaelis-Menten model) and the hyperbolic tangent functions. After fitting both functions to 86 PI datasets measured in the submerged macrophytes *Potamogeton pectinatus* L. and *Potamogeton perfoliatus* L., the two models did not differ significantly in their average goodness-of-fit (RSS). Moreover, they used the RSS values reported by Iwakuma & Yasuno (1983) to compare the fits of several different equations by the latter authors, showing that although these authors reported both the rectangular hyperbola and the hyperbolic tangent as poorer-fitting models, in fact only the linear model resulted in a statistically significant worse fit. Cossby *et al.* (1984) inferred similar conclusions after applying a different discriminating method, the extended Kalman filter, to several models (including the rectangular hyperbola and the hyperbolic tangent) using oxygen data from a macrophyte-dominated Danish stream. All these results strongly support the conclusions of Lederman & Tett (1976) concerning the choice of a best model.

A core aspect in the problem of selecting an adequate model for describing the photosynthesis dependence on irradiance lays in the meaning of the parameter estimates. Lederman & Tett (1976) distinguished between what they called the 'idealistic position', stating that some parameters have physiological reality (are 'transcendental') and so their

meaning and value is the same in all different equations, and the 'pragmatist position', stating that the parameters of the curves are just symbols, the curves themselves not being more than purely geometrical descriptions of the shape of that particular PI dataset.

A clear distinction of both positions seems important. Although Gallegos & Platt (1981) emphasized that the hyperbolic tangent equation proposed in Jassby & Platt (1976) was not more than a purely 'pragmatist' empirical description, Lederman & Tett (1981) suggested that the conclusions drawn by Jassby & Platt (1976) were probably a consequence of their non-explicitly stated 'idealistic position'. Moreover, Gallegos & Platt (1981) referred to Chalker (1980) to provide a link between their empirical description and the underlying physiological reality. Chalker (1980) showed that the hyperbolic tangent model is the mathematical solution to the description of the change of P and I as a quadratic power series

$$\frac{dP}{dI} = a_0 + a_1 P + a_2 P^2 \quad (1)$$

under certain conditions considered to be typical of every PI curve. Unfortunately, Chalker (1980) gave no physiological justification for the description of  $dP/dI$  as an expanded power series.

Lederman & Tett (1981) arrived to a reconciliation of the 'idealistic' and the 'pragmatic' positions by 'viewing the values of  $\alpha$  and  $P_m$  ... as statistics estimating population parameters that are functions of the 'real' physiological variables low-light efficiency and maximum photosynthetic rate.' According to their point of view, 'sample values of  $\alpha$  and  $P_m$  may be used as ... estimates of the 'real' parameters (and thus predictively ..) in the equation in which they were derived, but not transcendently.' What they consider estimates of a real physiological parameter may not be used as transcendental because when several models are fitted to the same dataset, the resulting parameter estimates are those that minimize the RSS for each model, and thus several mathematically different models are likely to use differing parameter estimates to describe the same curvature (Hootsmans & Vermaat, 1994).

Hence, comparing the results of PI curves provided by different authors becomes quite difficult if they have used different mathematical models. The best solution is re-fitting their datapairs to a common model before performing any comparison. Unfortunately, the actual data values are not provided in most of the cases. The only solution then is maintaining a critical point of view, keeping in mind that observed differences in parameter estimates may be a mathematical artifact due to the use of different equations.

Megard *et al.* (1984) provided a mechanistic basis for the description of PI curves of oxygenic photosynthesis. The equation is derived from a kinetic analysis of plausible reactions between photons and plant pigments, and it predicts the photoinhibitory onset of photosynthesis as an intrinsic property of these reactions. According to their equation, photoinhibition is the result of a reversible inactivation of photosynthetic pigments because of the absorption of extra quanta. An important feature of the proposed mechanism is that the inhibition component affects oxygenic photosynthesis at all irradiances, although its effect does not become obvious unless irradiance is very high. The equation is as follows:

$$P(I) = \frac{P_m * I}{K_1 + I + \frac{I^2}{K_2}} \quad (2)$$

where  $P_m$  is the theoretical maximum rate of production,  $K_1$  is a constant depending on the photosynthetic response to low irradiance, and  $K_2$  is a inverse measure of the degree of

photoinhibition at high irradiance. The maximum observed production is always less than the theoretical maximum  $P_m$  for finite values of  $K_2$ , and it occurs at a unique optimal irradiance  $I_{opt} = (K_1 * K_2)^{1/2}$ . The equation of Megard *et al.* becomes an equation for a rectangular hyperbola when  $K_2$  is infinite. Even when  $K_2$  is much larger than  $K_1$ , the photoinhibition effect may not be obvious but it is also responsible for the plateau of the curve. Thus, rates measured at all irradiances contain an inhibition component.

We may consider the fact that Megard's model provides us with an estimation of the theoretical maximum rate of production as a disadvantage, since other models produce the 'actual' maximum rate of gross production  $P_m$ . But, for any other continuous model not incorporating a photoinhibition term, we can observe that  $P_m$  is a maximum value obtained for infinite  $I$ . Megard's model just brings  $I_{opt}$  from infinity to  $(K_1 * K_2)^{1/2}$ .

We foresee one single problem when using this model for fitting PI curves in a range of irradiances in which photoinhibition is not apparent. The model then requires three parameters to fit a curve showing only two independent features. This has a mechanistic support, but when running the fitting techniques, great variations of  $K_2$  produce very small variations of RSS (as the 'effect' of  $K_2$  is only strongly 'felt' outside the range of the actual observations), and often results in non-sense parameter estimation for  $K_2$  (high negative values or values above  $10^4$ ). In such cases, and due to what might be considered a restriction enforced by the available dataset, we may be obliged to use the simplification  $K_2 \rightarrow \infty$ , utilising the rectangular hyperbola equation. The mechanistic description of the oxygenic photosynthesis remains still the same, but the constant accounting for photoinhibition is considered of minor importance for the limited range of irradiances under consideration.

The theoretical background of Megard's model requires the use of curves of oxygenic photosynthesis in their chlorophyll-based form. But for ecological works, it is often of importance to calculate the production in a biomass-based form. In fact, the comparison of PI curves from analogous experimental conditions expressed both in biomass-related and in chlorophyll-related form allows for a discrimination of the effect of chlorophyll concentration and the effect of changes in the concentration or in the activity of the enzymes affecting carbon metabolism. An extra reason for doing such is Hootsmans & Vermaat's (1994) remark: the extrapolation of chlorophyll-related results to the whole plant is difficult and often inaccurate.

In the present work, we will model our PI curves using the rectangular hyperbola, a simplification of the Megards model. As we modelled net photosynthetic oxygen production, the equation had an extra term accounting for the dark respiration ( $R$ ), as follows:

$$P = \frac{P_m * I}{K_m + I} - R \quad (3)$$

Two different fittings are used, one for biomass-related datapoints, providing us with growth predictions of use in the understanding of the population dynamics, and another one for chlorophyll-related datapoints, providing us with parameter estimations of use when discussing plant physiology. As transformation of data affects RSS estimation, separate fits were necessary for the biomass- and chlorophyll-related curves.



## 2. MATERIALS AND METHODS

### 2.1. Experimental setup: plant material cultivation

Seeds attached to plant material were collected in an old channel of the Salinas de San Isidoro (Doñana National Park, SW Spain), at the end of the growing season (June 1990). A mixed population of *R. drepanensis* and *Althenia orientalis* (Tzevelev) García-Murillo & Talavera occurs in this locality. Seeds and wet plant material were stored together in plastic bags at 4 °C, until August 1990.

The seeds were germinated in tap water, in the dark and at room temperature (20 °C). After three days, germinated seedlings were selected and transferred to containers with brackish water (1 g l<sup>-1</sup> artificial sea salt), where they stayed for one week under low irradiance conditions (10 to 20  $\mu\text{E m}^{-2} \text{s}^{-1}$ ) and at room temperature. Previous experience had shown that this treatment resulted in the best survival percentage. After one week the seedlings, with a size between 3 and 5 cm, were randomly distributed over the treatments and subsequently planted.

Each treatment consisted of one aquarium (60 x 40 x 40 cm<sup>3</sup>) containing 39 plastic cups (8 cm height) filled with 100 ml of a mixture of sand and clay (3:1 by weight) and covered by 1 cm of washed sand. Space among the cups was also filled with washed sand. The remaining volume of the aquaria was filled with tap water one week before planting the seedlings. Each aquarium had its own system of two pumps with independent thermostats, one connected to a cooling system and one equipped with a heating system. Temperature was maintained at 20  $\pm$  0.5 °C. To ensure that the water was homogeneously mixed, the heating pump was working continuously, although not constantly heating. Tap water was used to replenish the aquaria when necessary.

As light source fluorescent light tubes (Philips, colour 84, 36 W) were used while neutral density filters created the different irradiance levels. Irradiance was measured at the beginning and at the end of the experiment, always at 1 cm below the water surface, by means of a LICOR LI-192S sensor.

Two replicate water samples were taken in acid-rinsed polyethylene flasks for the analysis of dissolved nitrate (weekly) and phosphorus (fortnightly). Both nitrate and phosphate were determined by ion chromatography (DIONEX 4500i and integrator SHIMADZU C-R5A).

Two simultaneous experiments were carried out:

- *Experiment 1: effect of photoperiod and photosynthetic period.* Two irradiances (PPFD = 285 and 145  $\mu\text{E m}^{-2} \text{s}^{-1}$ ) and three different light periods (resulting from the combination of two photoperiods with two photosynthetic periods, both of 10 and 16 hours) were combined in six treatments. A summary of the resulting 6 treatments is provided in Table 1.

- *Experiment 2: effect of low irradiance levels.* Two additional irradiances were created under the LL photoperiod. In consequence, a total of 4 irradiance treatments were conducted under the LL photoperiod: 285, 145, 110 and 55  $\mu\text{E m}^{-2} \text{s}^{-1}$ .

TABLE 1: Summary of the experimental light climate in experiments 1 and 2. D = darkness, L = light. EPAR: total photosynthetically active radiation received per day.

Code	Light regime	Photoperiod	PAP	Irradiance ( $\mu\text{E m}^{-2} \text{s}^{-1}$ )	EPAR ( $\text{E m}^{-2}\text{day}^{-1}$ )
<i>Experiment 1:</i>					
LL1	long-day (4D-16L-4D)	long	long	0-285-0	16.5
LL2	long-day (4D-16L-4D)	long	long	0-145-0	8.0
LS1	dim (4D-3dim-10L-3dim-4D)	long	short	0-8-285-8-0	10.5
LS2	dim (4D-3dim-10L-3dim-4D)	long	short	0-8-145-8-0	5.5
SS1	short-day (7D-10L-7D)	short	short	0-285-0	10.3
SS2	short-day (7D-10L-7D)	short	short	0-145-0	5.3
<i>Experiment 2:</i>					
LL1	long-day (4D-16L-4D)	long	long	0-285-0	16.5
LL2				0-145-0	8.0
LL3				0-110-0	6.4
LL4				0-55-0	3.0

## 2.2. Characterization of plant material

During the first six weeks of the experiment the following morphometrical parameters were recorded in 18-20 randomly-selected plants (=genets):

- total number of shoots, bundles and leaves per plant;
- number of leaves and length of the longest leaf per bundle;
- number of vertical shoots, number of leaves allocated into vertical shoots, and internodal length of the vertical shoots;
- number of inflorescences per plant.

The same plants were measured each week. After 11 weeks, these plants were collected for photosynthesis measurements and subsequently used for final morphometrical measurements (as described above), nutrient tissue content and biomass determination. The same procedure was applied to the remaining 19 plants, used for photosynthesis measurements in the 16th week.

For chlorophyll determination, a sample of 5-10 leaves per plant was taken. Fresh weight (fw) of this leaf sample and of the remaining plant was measured after blotting twice with tissue paper, and the leaf samples were temporarily stored at  $-20^{\circ}\text{C}$ . Subsequently, the remaining plant material was dried for 24 h at  $70^{\circ}\text{C}$ . A number of dried plants was used for nutrient determination and stored at room temperature until further analysis. The rest of the plants was dried ( $105^{\circ}\text{C}$ ) and ashed ( $520^{\circ}\text{C}$ ) to obtain dry weight (dw) and ash free dry weight (afdwt). Treatment- and age- specific linear regressions between the fw and  $70^{\circ}\text{C}$ -dw values, and the afdwt values from the 'biomass' subsamples were used to calculate the afdwt of the 'nutrient' and the 'chlorophyll' subsamples.

Chlorophyll- and phaeopigments-*a* and *b* were determined according to Wintermans & De Mots (1965). Phaeopigment-*a* and -*b* concentrations were always very low, and only in a few treatments they were significantly different from zero, so they will not be presented in the results section. Total N and P content of the plant material was determined spectrophotometrically with a Technicon multi-analyzer after digestion of about 300 mg dw

with a mixture of sulphuric acid, salicylic acid, selenium and hydrogen peroxide (Novozamsky *et al.*, 1983). A separate fraction was made up from the seeds of the 16-weeks old plants. However, it was not always possible to obtain reliable nutrient content determinations due to the low biomass of the seed samples.

### 2.3. Experimental set-up for photosynthesis measurements

All photosynthesis measurements were done in a 96 l aquarium connected to a cooling system which kept the temperature during the incubation at  $20 \pm 1$  °C. The temperature selected was the same at which the were cultured. The aquarium was filled with tap water and 20 g  $\text{NaHCO}_3$  was added to arrive at inorganic carbon levels considered saturating for several other macrophyte species (3.7 mM  $\text{HCO}_3^-$ ; Sand-Jensen, 1983). Nitrogen was bubbled through the aquarium prior to the oxygen production measurements, in order to reduce the initial concentration of dissolved oxygen.

Three independent, replicate systems were used. Each one consisted of an electrode chamber and a 5 cm diameter perspex tube interconnected with pvc tubing. A peristaltic pump (Watson Marlow 504U) was used to circulate the water. Temperature and dissolved oxygen were recorded every 10 seconds with a Campbell 21X datalogging set connected to three WTW EO-196 oxygen electrodes and WTW OXY-196 electrode meters. Light was provided by a Philips 400 W HPIT metal halide lamp. Different irradiance levels were created by varying the distance between lamp and water surface and by using a neutral density net. A shallow perspex flow-trough waterbath was suspended beneath the lamp to absorb the infrared radiation.

Flow rate was  $0.75 \text{ l min}^{-1}$  (equivalent to  $6.5 \text{ mm s}^{-1}$  in the perspex tube). This is about half of the flow rate used by Hootsmans & Vermaat (1994) and Sand-Jensen (1983). Nevertheless, since in a comparable incubation chamber Westlake (1967) found that rates of photosynthesis of *P. pectinatus* did not increase further above flow rates of  $0.4 \text{ mm s}^{-1}$ , we expect that the used flow rate had not appreciable limiting effect on photosynthesis.

Five to six randomly-selected, intact plants were used per tube. Plants were carefully washed out of the sediment, and kept overnight in the photosynthesis set-up in darkness in order to permit acclimation and to deplete lacunar oxygen reserves. Next morning, dark respiration of the plant material was measured during 30 to 45 minutes. Subsequently, the tubes were exposed to the different irradiance levels (15 to 45 min, until at least a  $0.5 \text{ mg l}^{-1}$  increase in dissolved oxygen concentration was reached), starting with the lowest intensity and ending with the highest. Between each measurement the tubes were opened and the medium inside was completely replenished with the surrounding water.

As the light field could not be kept homogeneous for the irradiance levels above  $150 \mu\text{E m}^{-2} \text{ s}^{-1}$ , we measured the irradiance in three points along each tube and used the average intensity per tube for further calculations. Irradiances at 1 cm below the water surface outside the tubes were on average 25, 50, 75, 95, 150, 185, 300, 425 and  $495 \mu\text{E m}^{-2} \text{ s}^{-1}$  and 30, 55, 80, 100, 150, 195, 295, 395 and  $505 \mu\text{E m}^{-2} \text{ s}^{-1}$  for the 11th and the 16th week measurements, respectively.

## 2.4. Calculations and statistical analysis

### 2.4.1. Water chemistry

Nitrate values from each treatment were fitted using an inverse logistic function,

$$\text{Nit}_t = K * \left( 1 - \frac{1}{1 + q * e^{-r * t}} \right) \quad (4)$$

where  $\text{Nit}_t$  is nitrate concentration at time  $t$ ,  $K$  is the (initial) asymptotic maximum value,  $q$  is an integration constant,  $r$  is the 'unrestricted' rate of nitrate decrease, and  $t$  is the time in days. This function was considered to be the best after analyzing the shape of the scatter plots. Multiple comparisons among fitted curves were performed using an F statistic as described in Vermaat & Hootsmans (1994a), the zero hypothesis being that both sets of datapairs compared could be described equally well with a common regression equation.

### 2.4.2. Morphometrical data

Statistical analysis of the morphometrical data followed Vermaat & Hootsmans (1994b), with some modifications, and were done with the SAS statistical package (SAS Institute Inc., 1988). A logistic curve was fitted for the morphometric variables using a non-linear iterative technique based on the Marquardt algorithm (Conway *et al.*, 1970). A separate curve was fitted for each of the 18 to 20 individual plants measured in every treatment.

Parameters of the curves were compared by means of Two- and Three-Way Analysis of Variance (ANOVA) followed by multiple comparisons among treatments. ANOVA tests were done with the General Linear Models (GLM) procedure in SAS, after  $\log_{10}$  or arcsin square root transformation if the residuals were not normally distributed or the residual variances were not homogeneous. The latter was checked with a plot of predicted versus residual values resulting from the ANOVA. For multiple comparisons, we used the LSMEANS option in SAS. Comparisonwise error rates (CER) for each comparison were adjusted to maintain an experimentwise error rate (EER) of 0.05.

Differences in flower induction were tested using  $\chi^2$  tests (Steel & Torrie, 1981), the null hypothesis being that the relative frequency of flowering plants in all the treatments under comparison could be described by a common binomial probability function. Interaction between PPF and LP was tested using an n-way classification  $\chi^2$  test as described in Steel & Torrie (1981).

### 2.4.3. Photosynthesis data

Each data set relating oxygen concentration to time for a particular irradiance level was checked for measurement errors, and lag phases were excluded. Oxygen exchange rates were calculated by linear regression and expressed in  $\mu\text{g}$  per g afdw of plant tissue per minute ( $\mu\text{g O}_2 \text{ g afdw}^{-1} \text{ min}^{-1}$ ) and in  $\mu\text{g}$  per mg total chlorophyll per minute ( $\mu\text{g O}_2 \text{ mg Chl}^{-1} \text{ min}^{-1}$ ).

A total of 48 light-response (PI) curves was obtained from the experiment (3 replicate curves times 8 light history treatments measured at 2 different ages). The resulting data set

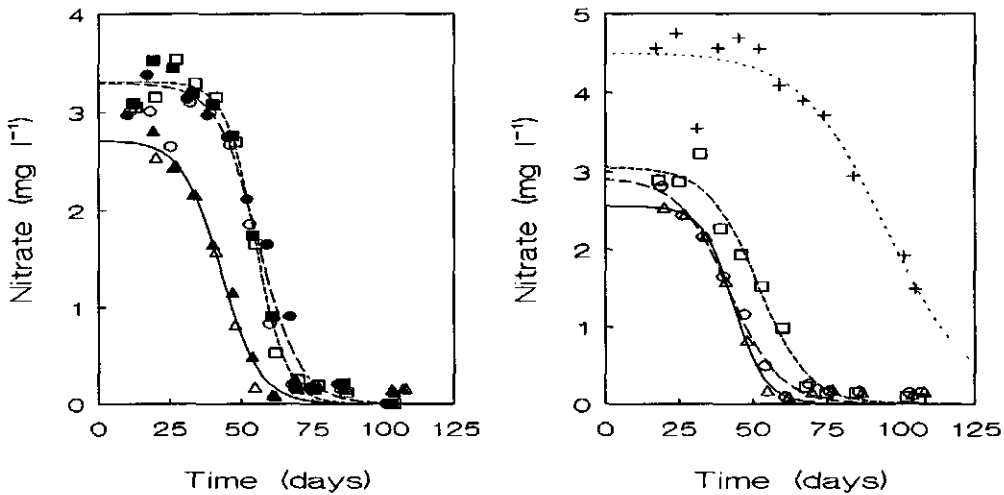


FIG. 1: Dissolved nitrate concentration in the water column of the aquaria from the different treatments. Fitted inverse logistic curves are also provided.

Left: experiment 1. Triangles and continuous line: long-day treatment. Circles and frequently broken line: dim treatment. Squares and broken line: short-day treatment. Filled symbols refer to the LL1, LS1 and SS1 treatments, open ones to the LL2, LS2 and SS2 treatments. Fitted lines are the same for both PPF treatments within each light period treatment (separate fits were not significantly different).

Right: experiment 2. Triangles and continuous line: 285  $\mu\text{E m}^{-2} \text{s}^{-1}$ . Circles and broken line: 145  $\mu\text{E m}^{-2} \text{s}^{-1}$ . Squares and frequently broken line: 110  $\mu\text{E m}^{-2} \text{s}^{-1}$ . Crosses and dotted line: 55  $\mu\text{E m}^{-2} \text{s}^{-1}$ .

for each treatment replica consisted of the experimental irradiance levels and the corresponding  $\text{O}_2$  exchange rates. A rectangular hyperbola (Michaelis-Menten, or MM model) was fitted to each data set using the non-linear regression (NLIN) procedure of the SAS statistical package (SAS Institute Inc., 1988). The resulting model parameter estimates were used for ANOVA and multiple comparisons (as above), together with two derived parameters, the affinity constant ( $\alpha = P_m/K_m$ ; Hootsmans & Vermaat, 1994) and the light compensation point (LCP). Estimated net oxygen production rates at the irradiances at which the plants from the different treatments did respectively grow (hereafter referred to as 'actual photosynthetic rate',  $P_{a,c}$ ) were also calculated and tested for a light acclimation effect.

### 3. RESULTS

#### 3.1. Water chemistry

Orthophosphate concentration in the water column was very low in all treatments from both experiment 1 and 2, being always close to or under the detection limit of the analytical method (maximum measured value: 0.4 mg P l<sup>-1</sup>; detection limit: 0.1 mg P l<sup>-1</sup>). Variation of values along time did not follow any defined pattern. No statistical comparison was performed between treatments.

On the other hand, nitrate concentration decreased with time in all treatments, from

TABLE 2: Results of the ANOVAs for the effect of light period (LP), irradiance (PPFD) and age on the total biomass, chlorophyll and phaeopigment concentration in the leaves, and nutrient concentration (total N and total P) in the tissue of *R. drepanensis*. \*, \*\* and \*\*\* indicate respectively significance levels of 0.05, 0.01 and 0.001. NS=non-significant ( $p > 0.05$ ).

	Total afdW	Chl.tot	Chl-a	Chl-b	Phaco.a	Phaco.b	Chl.-b Chl.(a+b)	TotalN	TotalP
<i>Experiment 1:</i>									
L.Period	.0498*	.0003***	.0001***	.0172*	.0001***	.0001***	.0001***	.0001***	.0006***
PPFD	.0001***	.0001***	.0001***	.0001***	.0001***	.33 <sup>NS</sup>	.95 <sup>NS</sup>	.28 <sup>NS</sup>	.0001***
Age	.0001***	.0001***	.0001***	.0001***	.0001***	.0001***	.0001***	.0001***	.0001***
LP*PPFD	.92 <sup>NS</sup>	.0001***	.0001***	.0076**	.0344*	.23 <sup>NS</sup>	.0001***	.10 <sup>NS</sup>	.0005***
PPFD*Age	.33 <sup>NS</sup>	.0002***	.0001***	.91 <sup>NS</sup>	.0002***	.09 <sup>NS</sup>	.0001***	.38 <sup>NS</sup>	.03*
LP*Age	.31 <sup>NS</sup>	.002**	.0001***	.12 <sup>NS</sup>	.0001***	.0006***	.0001***	.19 <sup>NS</sup>	.74 <sup>NS</sup>
LP*PPFD*Age	.80 <sup>NS</sup>	.0001***	.0001***	.0016**	.012*	.18 <sup>NS</sup>	.0001***	.48 <sup>NS</sup>	.17 <sup>NS</sup>
<i>Experiment 2:</i>									
PPFD	.0001***	.0001***	.0001***	.0001***	.64 <sup>NS</sup>	.91 <sup>NS</sup>	.0001***	.0001***	.0001***
Age	.0001***	.0001***	.0001***	.0001***	.26 <sup>NS</sup>	.41 <sup>NS</sup>	.0001***	.0073***	.0007***
PPFD*Age	.0001***	.0001***	.0001***	.0001***	.27 <sup>NS</sup>	.06 <sup>NS</sup>	.0001***	.75 <sup>NS</sup>	.03*

maximum initial values between 3 and 5 mg N l<sup>-1</sup>. As it may be appreciated in Fig. 1, curves fitted remarkably well the datapair sets. Considerable differences are observed between treatments (Fig.1). Multiple comparisons resulted in significant differences for all comparisons but LL1-LL2 from experiment 2. Data from experiment 1 resulted in significant differences between the long-day treatment and both LS and SS treatments (which did not differ significantly among each other), for both PPFD levels. Different PPFD treatments within each light period treatment showed non-significantly different nitrate concentration fits. Overall, nitrate concentration decreased with increasing irradiance (experiment 2) and was lower under long-day conditions (experiment 1).

### 3.2. Characterization of plant material

#### 3.2.1. Plant biomass

*Experiment 1.* Light period (LP), irradiance (PPFD) and plant age (11 versus 16 weeks) all significantly affected the total biomass of the plants (Table 2; Fig.2). However, when performing multiple comparisons, only one comparison between different PPFD resulted in significant differences (LL1 vs. LL2), and none of the comparisons between different LP yielded significant results. All comparisons between different ages (within each combination of LP and PPFD) resulted in significant differences.

As a general trend, total biomass decreased with decreasing irradiance and increased with increasing age. Effect of LP was more confusing, short PPs (LS and SS) yielding higher biomass after 11 weeks of growth, and long PPs (LL2 and LS2) yielding higher biomass 5 weeks later. The dim (LS) treatment always yielded the highest biomass. Overall, differences in biomass were not very pronounced.

*Experiment 2.* As found in the experiment 1, a reduction in the irradiance strongly reduced the total biomass yield (Fig.3). Both PPFD and age and their interaction resulted in highly

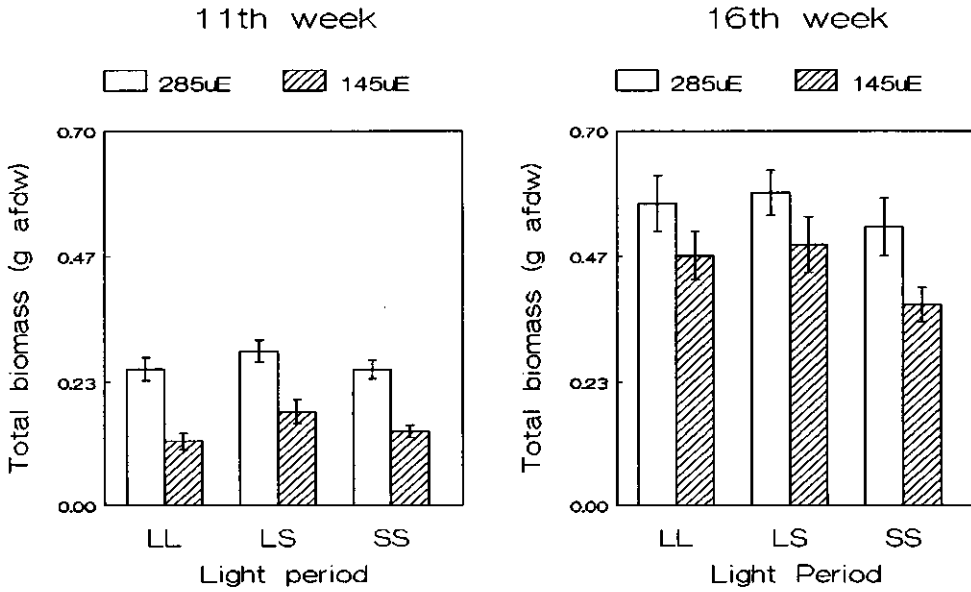


FIG.2: Individual plant biomass (average  $\pm$  standard error,  $n=18$ ) after 11 and 16 weeks of growth under three different photoperiod-photosynthetic period treatment combinations and two different irradiances (experiment 1). LL, LS, SS: see table 1. 285µE and 145µE indicate the two irradiances levels in  $\mu\text{E m}^{-2} \text{s}^{-1}$ .

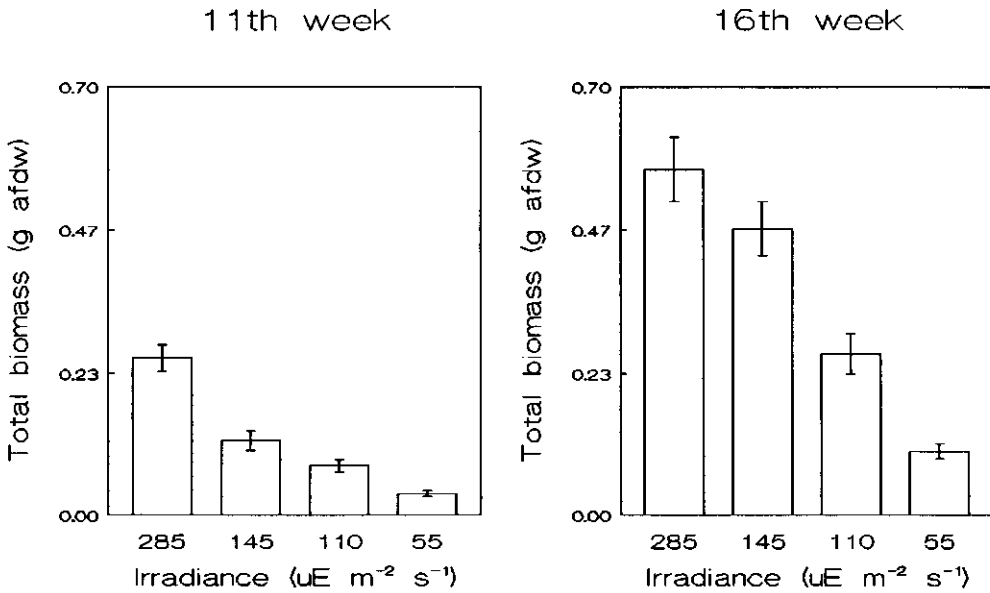


FIG.3: Individual plant biomass (average  $\pm$  standard error,  $n=18$ ) after 11 and 16 weeks of growth under four different irradiances (experiment 2). Photoperiod was 16 hours for all treatments.

TABLE 3: Results of the ANOVAs for the effect of light period (LP, including PP and PAP) and irradiance (PPFD) on the development of several morphometric variables in *R. drepanensis* plants. r: exponential growth rate of the fitted logistic curve; K: maximum asymptotic value of the fitted logistic curve; NLE77, NBU77: measured number of leaves and bundles on the 77th day of growth; LGL35: measured length of the longest leaf on the 35th day of growth; LGL77: estimated length of the longest leaf for the 77th day of growth. <sup>1</sup> refers to the ANOVAs for which the lowest PPFD level has been removed in the experiment 2 analysis. \*, \*\* and \*\*\* indicate respectively significance levels of 0.05, 0.01 and 0.001. NS=non-significant ( $p > 0.05$ ).

	Number of leaves			Number of bundles				Length of the longest leaf		
	r	K	NLE77	r	K	NBU77	r	K <sup>1</sup>	LGL35	LGL77 <sup>1</sup>
<i>Experiment 1:</i>										
L.Period	.0001***	.67 <sup>NS</sup>	.015*	.32 <sup>NS</sup>	.86 <sup>NS</sup>	.07 <sup>NS</sup>	.08 <sup>NS</sup>	.0005***	.005***	.0001***
PPFD	.0001***	.0001***	.0001***	.0001***	.0004***	.0003***	.0003***	.0001***	.0001***	.0001***
LP*PPFD	.45 <sup>NS</sup>	.45 <sup>NS</sup>	.78 <sup>NS</sup>	.94 <sup>NS</sup>	.67 <sup>NS</sup>	.08 <sup>NS</sup>	.08 <sup>NS</sup>	.88 <sup>NS</sup>	.71 <sup>NS</sup>	.87 <sup>NS</sup>
<i>Experiment 2:</i>										
Light	.0001***	.0001***	.0001***	.0001***	.0001***	.0001***	.0007***	.04*	.0001***	.0001***

significant effects in the ANOVA of the total biomass samples (Table 2).

Multiple comparisons resulted in significant differences for all age comparisons within the PPFD treatments, total biomass increasing with increasing age. After 11 weeks of growth, plants grown under  $285 \mu\text{E m}^{-2} \text{s}^{-1}$  had a significantly higher biomass than all other PPFD treatments. After 16 weeks of growth, all treatments except the two highest PPFDs (LL1 and LL2) yielded biomass values significantly different from each other.

### 3.2.2. Plant morphology

*Experiment 1.* Growth curves for both experiments are shown in Fig.4 a-b. Two-way ANOVA performed using the parameter estimates of the fitted curves plus the actual value of the variables after 77 days of growth are summarized in Table 3. Irradiance (PPFD) had a significant effect on all parameters for all the three variables under study. Light period (LP) had a less constant effect: only the exponential growth rate of the total number of leaves (NLE) and the asymptotic maximum value of the length of the longest leaf (LGL) yielded significant results. Values of the measured NLE and estimated LGL on the 77th day of growth were also significantly affected. The interaction of both factors was significant for none of the parameters of any of the three variables NLE, NBU (total number of bundles) and LGL.

As a general trend, both the exponential growth rate (r) and the asymptotic maximum value (K) of NLE and NBU decreased significantly when decreasing the PPFD from  $285 \mu\text{E m}^{-2} \text{s}^{-1}$  (Fig.5a), resulting in much lower values (about half) after 77 days of growth (Fig.6). LGL followed the opposite trend, r and K increasing with decreasing irradiance, which resulted in 30 % longer leaf lengths after 35 days of growth and higher LGL estimations after 77 days of growth (Figs. 5b and 5c). The effect of LP is weaker, with a trend to produce less leaves and shorter leaves under the LL conditions (Figs. 5a and 6).

Subsequent multiple comparisons are summarised in Table 4. Concerning the effect of PPFD on r, comparisons resulted in significant differences within all different LPs for the



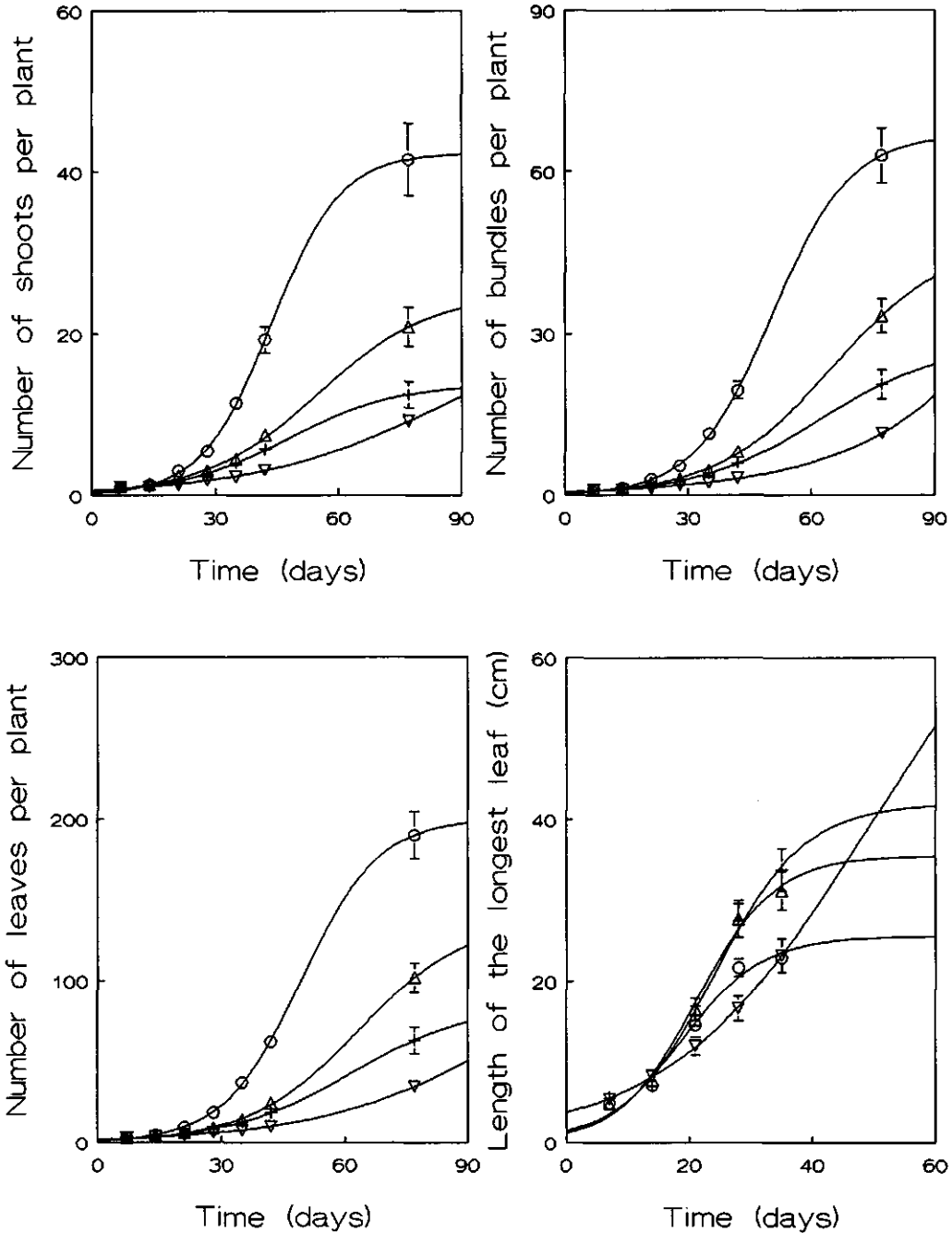


FIG.4a: Effect of the PPFD on the development of the total number of shoots, total number of bundles, total number of leaves and length of the longest leaf of *R. drepanensis*. Circles: 285  $\mu\text{E m}^{-2} \text{s}^{-1}$ ; triangles: 145  $\mu\text{E m}^{-2} \text{s}^{-1}$ ; crosses: 110  $\mu\text{E m}^{-2} \text{s}^{-1}$ ; inverted triangles: 55  $\mu\text{E m}^{-2} \text{s}^{-1}$ . Note different scales in the X-axes.

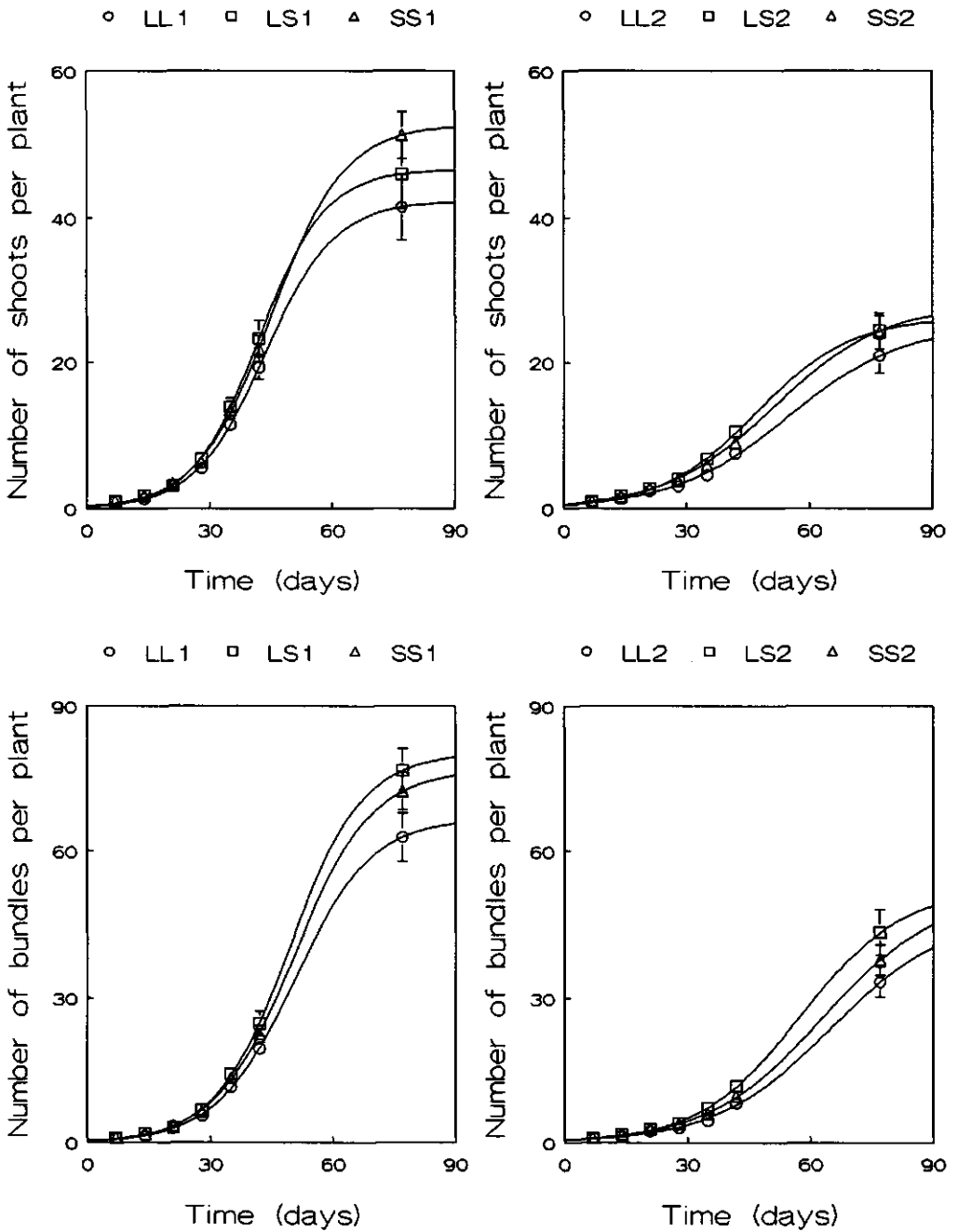


Fig. 4b: Effect of the light period (LP) on the development of the total number of shoots and total number of bundles of *R. drepanensis*. Note the different X-scale in the two last graphs.

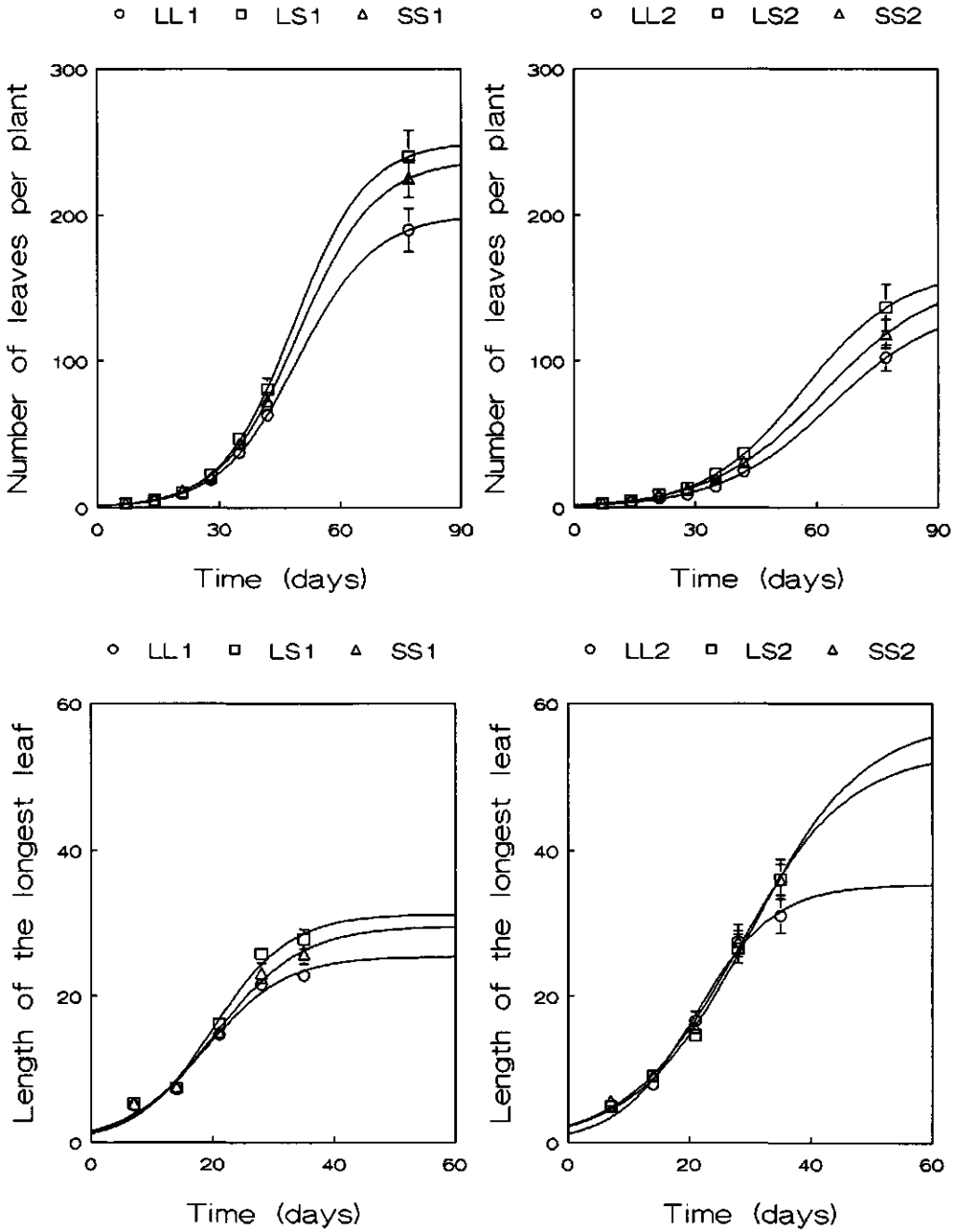


FIG. 4c: Effect of the light period (LP) on the development of the total number of leaves and length of the longest leaf of *R. drepanensis*. Note the different X-scale in the two last graphs.

TABLE 4: Multiple comparisons between different irradiance treatments (PPFD) for several morphometric parameters of *R. drepanensis* plants. Different letters indicate significantly different values for an EER=0.05. 'a' indicates the smallest value, 'b' to 'd' progressively increasing values. Legend as in Table 3.

PPFD ( $\mu\text{Em}^{-2}\text{s}^{-1}$ )	Number of leaves			Number of bundles			Length of the longest leaf			
	r	K	NLE77	r	K	NBU77	r	K <sup>1</sup>	LGL35	LGL77 <sup>1</sup>
285	c	b	c	c	b	d	b	a	a	a
145	b	b	b	b	b	c	b	a	b	b
110	b	a	ba	b	a	b	ba	a	b	b
55	a	a	a	a	a	a	a	a	a	a

variables NLE and NBU, and only one comparison (under short-day conditions, SS) showed significant differences for LGL. Concerning the effect of PPFD on K, all comparisons yielded significant results for the variable LGL, both comparisons under short PAP (LS1 vs. LS2, and SS1 vs. SS2) resulted in significant differences for NLE, and only the comparison under short PP (SS1 vs. SS2) showed significant differences for NBU. In conclusion, PPFD seems to affect mainly the exponential growth rates of the total number of leaves and total number of bundles, which increase with increasing PPFD, and the asymptotic maximum value of the length of the longest leaf, which increases with decreasing irradiance. Nevertheless, the maximum value of NLE and NBU is also affected by PPFD under short LP treatments.

Concerning the effect of LP, much less comparisons yielded significant results, although the trend of r being affected for NLE and NBU and K being affected for LGL was maintained. The dim treatment resulted in significantly higher NLE growth rates at low irradiance, NLE-r estimations being not significantly different when comparing the LL and the SS light regimes. On the other hand, comparisons between K estimations for LGL resulted in only one significant difference, between LL1 and LS1. K was higher for LS, the value for SS being intermediate between both LS and LL.

Multiple comparisons using the actually measured value after 77 days of growth (35 days for LGL) showed significant differences for all comparisons between the two PPFD levels (at all three LPs), and no significant difference for any of the comparisons between different LPs (at both PPFDs). For the estimated value of LGL after 77 days of growth, the result was the same as for the K estimation for LGL, but extended to both PPFD: the K estimation was significantly higher for the dim treatment (LS) than for LL, values for SS being intermediate between both of them and not significantly different from any of them.

Although a trend to allocate more leaves to vertical shoots under low PPFD regimes may be observed for the LL and SS treatments in Fig.7, neither PPFD nor LP nor their interaction significantly affected the fraction of leaves allocated in vertical shoots as measured in the 11th week of growth.

*Experiment 2:* LGL of a majority of the plants growing under the lowest PPFD ( $55 \mu\text{E m}^{-2} \text{s}^{-2}$ ) did not reach the point of the logistic curve where growth levels off. As a consequence, most of the estimated asymptotic maximum values (K) and the estimated LGL for the 77th day of growth (LGL77) resulted in outliers, and this treatment had to be excluded from the ANOVA of these two parameters.

Results of the ANOVA are summarised in Table 3. PPFD had a highly significant effect on all parameter estimates and on the NBU77, NLE77 and LGL35 values. ANOVA

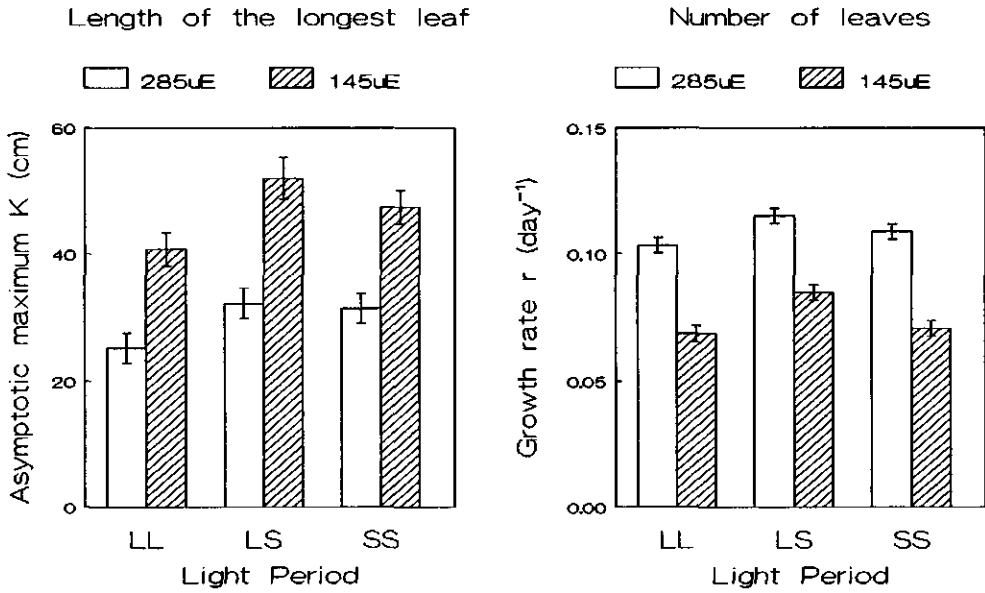


FIG.5a: Effect of the light period (PP and PAP) on the exponential growth rate ( $r$ ) and the asymptotic maximum value ( $K$ ) of the logistic curves describing the development of the longest leaf length and the total number of leaves per plant in *R. drepanensis*.

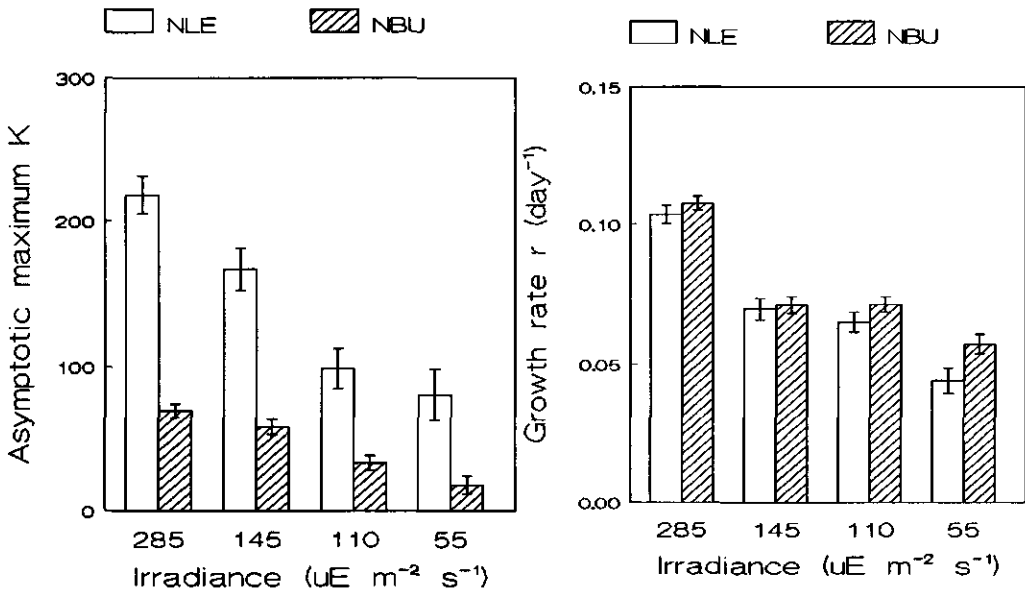


FIG.5b: Effect of the irradiance on the exponential growth rate ( $r$ ) and the asymptotic maximum value ( $K$ ) of the logistic curves describing the development of the total number of leaves (NLE) and total number of bundles (NBU) per plant in *R. drepanensis*.

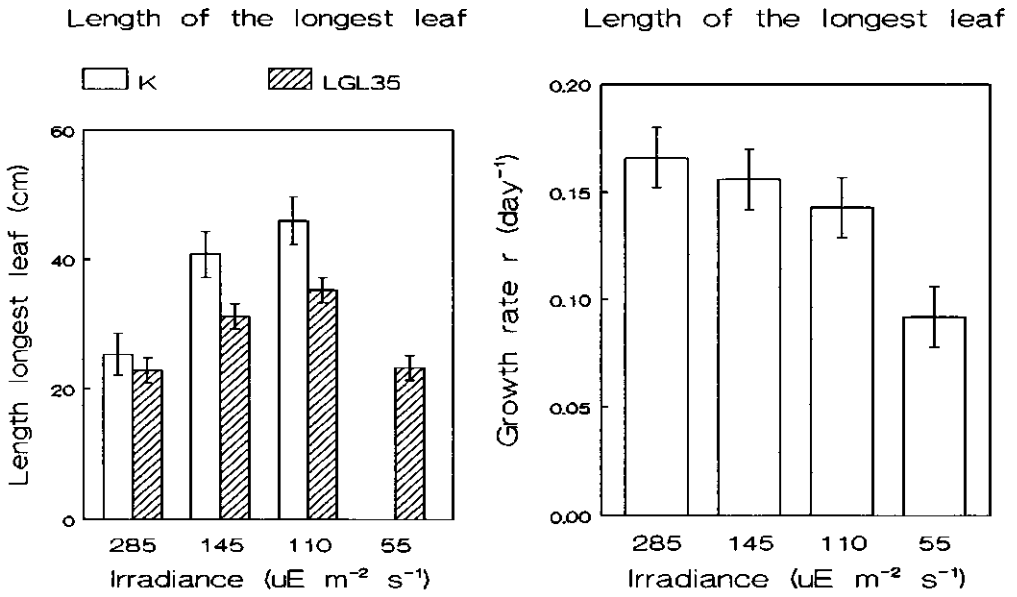


FIG.5c: Effect of the irradiance on the asymptotic maximum value (K) and the actual length of the longest leaf per plant measured after 35 days of growth (LGL35) in *R. drepanensis*.

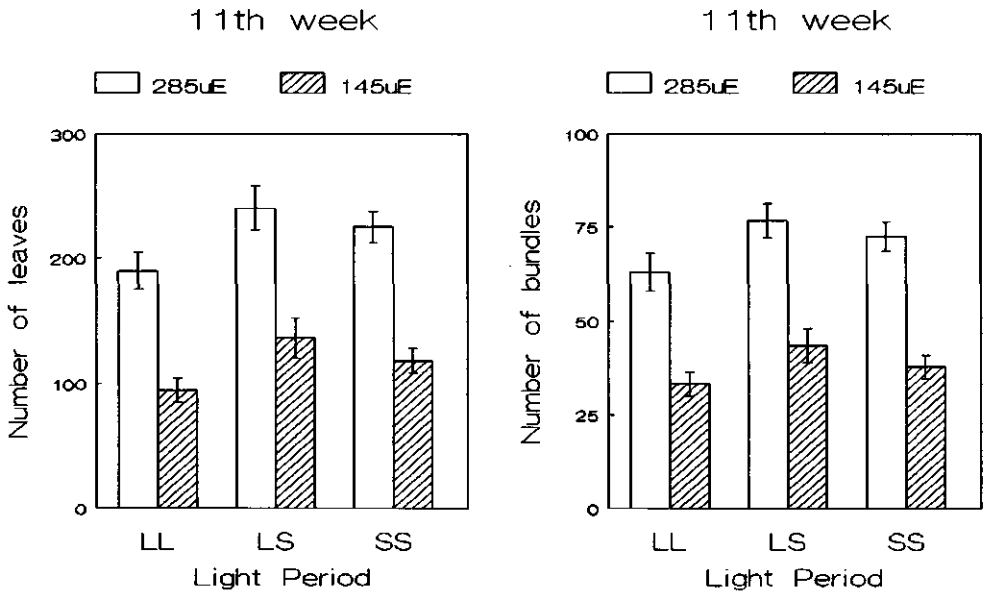


FIG.6: Effect of the light period and the PPFD on the number of leaves (left) and number of bundles (right) per individual plant after 11 weeks of growth. Average  $\pm$  standard error,  $n=18$ .

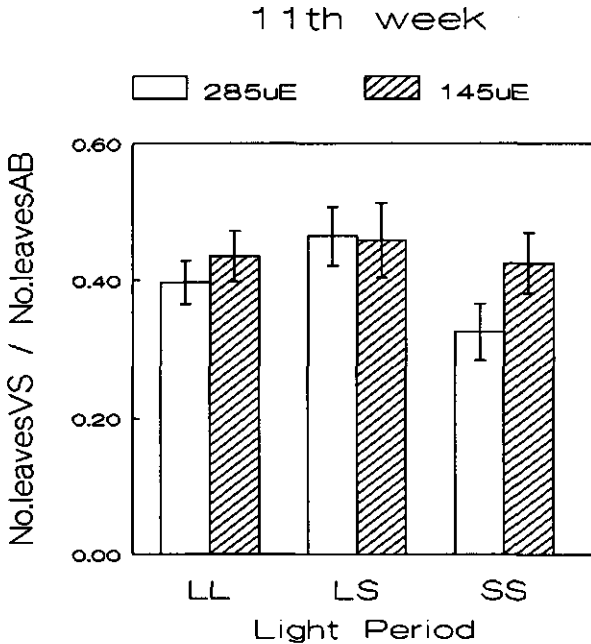


FIG.7: Effect of the light period and the irradiance on the proportion of leaves placed on vertical shoots after 11 weeks of growth. Average  $\pm$  standard error,  $n=18$ .

for the estimated LGL on the 77th day of growth also yielded significant results ( $p < 0.001$ ).

Multiple comparisons showed similar results for the variables NLE and NBU. Concerning the exponential growth rate, all PPFs differed significantly except the two intermediate ones (LL2 and LL3). For the asymptotic maximum value, the two highest PPFs were significantly different from the two lowest ones. All treatments differed significantly both for the NLE and the NBU on the 77th day of growth.

Multiple comparisons for the LGL excluding LL4 resulted in not significant differences for any of the comparisons concerning K; for the 77th day value estimate, LL1 differed significantly from LL2 and LL3. In the multiple comparisons for the estimate of  $r$  and for the 35th day value, which included treatment LL4, the lowest and the highest PPF (LL1 and LL4) differed significantly from the two intermediate PPF (LL2 and LL3).

As a general trend, the parameter estimations K and  $r$  of both the NLE and the NBU decreased with decreasing PPF (Fig.5b), as did the measured NLE and NBU on the 77th day of growth (Fig.8). Growth rate of the LGL increased with decreasing irradiance, resulting in longer leaves after 35 and 77 days of growth (Fig.5c), but decreased again at the lowest PPF, although growth did not saturate and probably leaves tend to become still longer than at higher PPF (see fitted curve in Fig.4a).

Plants tended to allocate a higher fraction of leaves to their vertical shoots with decreasing PPF (Fig.8), this trend being truncated at the lowest PPF. The One-Way ANOVA yielded significant results for the factor PPF, although only one individual comparison showed a significant difference (LL3 vs. LL4).

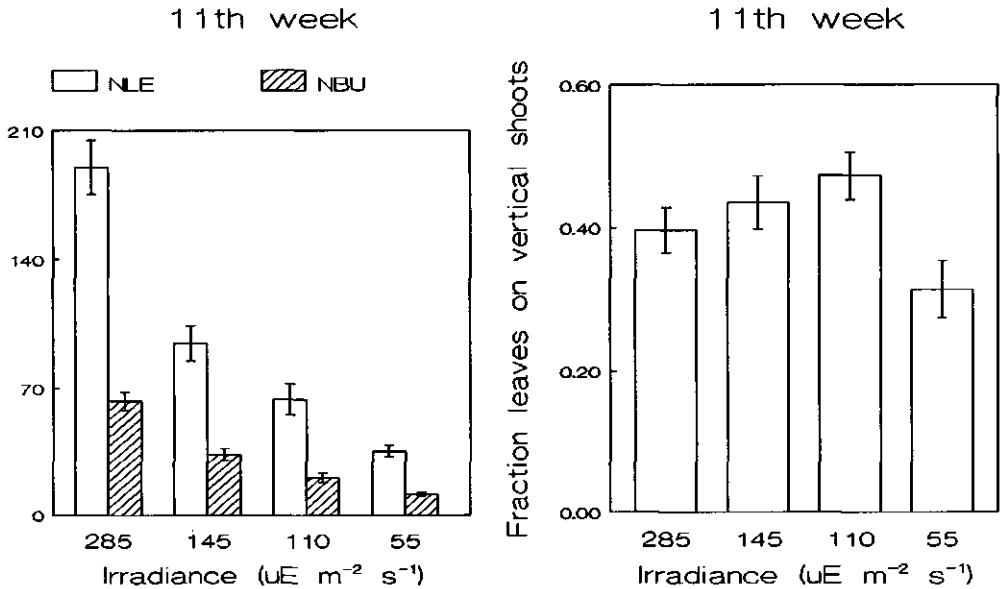


FIG.8: Effect of the PPFD (under a 16 hours photoperiod) on the number of leaves (NLE) and number of bundles (NBU) per individual plant (left) and on the fraction of leaves growing on vertical shoots (right) after 11 weeks of growth. Average  $\pm$  standard error,  $n=18$ .

### 3.2.3. Flowering and seed production

*Experiment 1:* Long photoperiod treatments in combination with a high PPFD (LL1 and LS1) resulted in an earlier induction of flowering (Fig.9). About 50 % of the plants flowered in these two treatments by the 11th week, while only 25 % plants grown under short photoperiod (SS1) or under low PPFD conditions (LL2, LS2 and SS2) had flowers. Five weeks later, all treatments had a similar amount of plants with flowers (75 %), apart the dim treatment (LS1) which had a slightly higher percentage (90%).

ANOVA performed on the number of inflorescences per plant yielded significant results only for PPFD and age. Thus, light period did not influence flower abundance, while a higher PPFD resulted in more per plant (Fig.9). Although no individual comparison concerning PPFD yielded significant results, the number of inflorescences per plant increased significantly from the 11th to the 16th week only for the high PPFD treatments (LL1, LS1 and SS1).

The frequency of flowering plants differed significantly between different light periods at 285  $\mu\text{E m}^{-2} \text{s}^{-1}$  ( $\chi^2=7.07$ ,  $p<0.05$ ), but not at 145  $\mu\text{E m}^{-2} \text{s}^{-1}$  ( $\chi^2=0.23$ ,  $p>0.05$ ). Significant differences between both PPFDs were found only under the long-day conditions ( $\chi^2=5.59$ ,  $p<0.01$ ). Separate  $\chi^2$  tests were performed between the treatments LS1 vs. SS1 (effect of PP;  $\chi^2=4.5$ ,  $p<0.05$ ) and LL1 vs. LS1 (effect of PAP;  $\chi^2=0.45$ ,  $p>0.05$ ), showing that the effect of light period may be attributed exclusively to significant differences caused by photoperiod. An n-way classification  $\chi^2$  test yielded non-significant results ( $\chi^2=1.19$ ,  $p>0.05$ ) for the interactive effect of PPFD and LP.



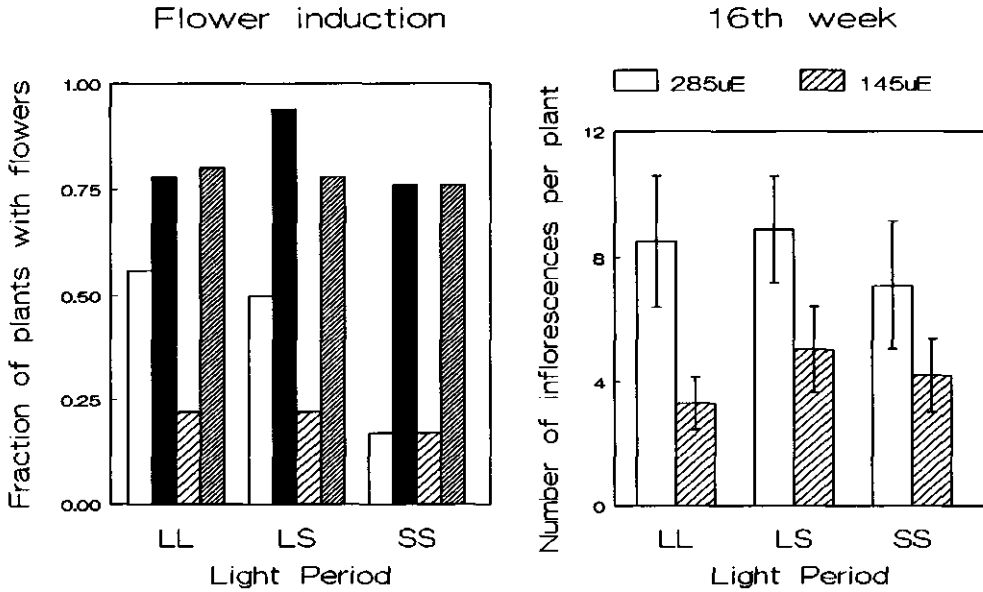


FIG.9: Effect of the light period on the initiation of flowering (left) and the flower production (right). Left graph: Open bars: PPF $D=285 \mu E m^{-2} s^{-1}$ , 11th week. Filled bars: PPF $D=285 \mu E m^{-2} s^{-1}$ , 16th week. Striped bars: PPF $D=145 \mu E m^{-2} s^{-1}$ , 11th week. Densely striped bars: PPF $D=145 \mu E m^{-2} s^{-1}$ , 16th week. Right graph: 285 $\mu E$  and 145 $\mu E$  indicate the irradiance in  $\mu E m^{-2} s^{-1}$ .

Although the final flower abundance was not significantly different for different LPs, the earlier flower initiation of the long PP treatments resulted in a higher seed biomass yield as measured in the 16th week (Fig.10). Moreover, the LL treatment doubled the seed production of the LS plants, under both PPF $D$ . Decreasing PPF $D$  also resulted in a much lower seed biomass yield, under all photoperiods.

As seed production could not be measured separately for each plant replicate, Two-Way ANOVA could only be performed excluding the effect of one-way interactions. Neither PPF $D$  nor LP showed significant effects on the ANOVA on seed biomass. Nevertheless, result of such ANOVA should be consider with some precaution due to the lack of replicates; indeed, overall ANOVA F-value was not significant.

**Experiment 2:** As in experiment 1, reduced irradiance resulted in a postponed flower induction. After 16 weeks only a 25 % of the plants did flower in the LL3 and LL4 treatments, *versus* the 75 % achieved by the LL1 and LL2 treatments. The differences in relative frequencies of flowering plants between different PPF $D$  treatments were highly significant ( $\chi^2=21.18$ ,  $p<0.001$ ).

Consequently, the average number of inflorescences per plant was strongly affected by PPF $D$  (Fig.11), multiple comparisons discriminating the couples of treatments LL1-LL2 from LL3-LL4 for the 16th week of growth. A significant increase in the flower production with age occurred only in the LL1 and LL2 treatments.

As expected, seed production decreased dramatically with decreasing PPF $D$ : seed

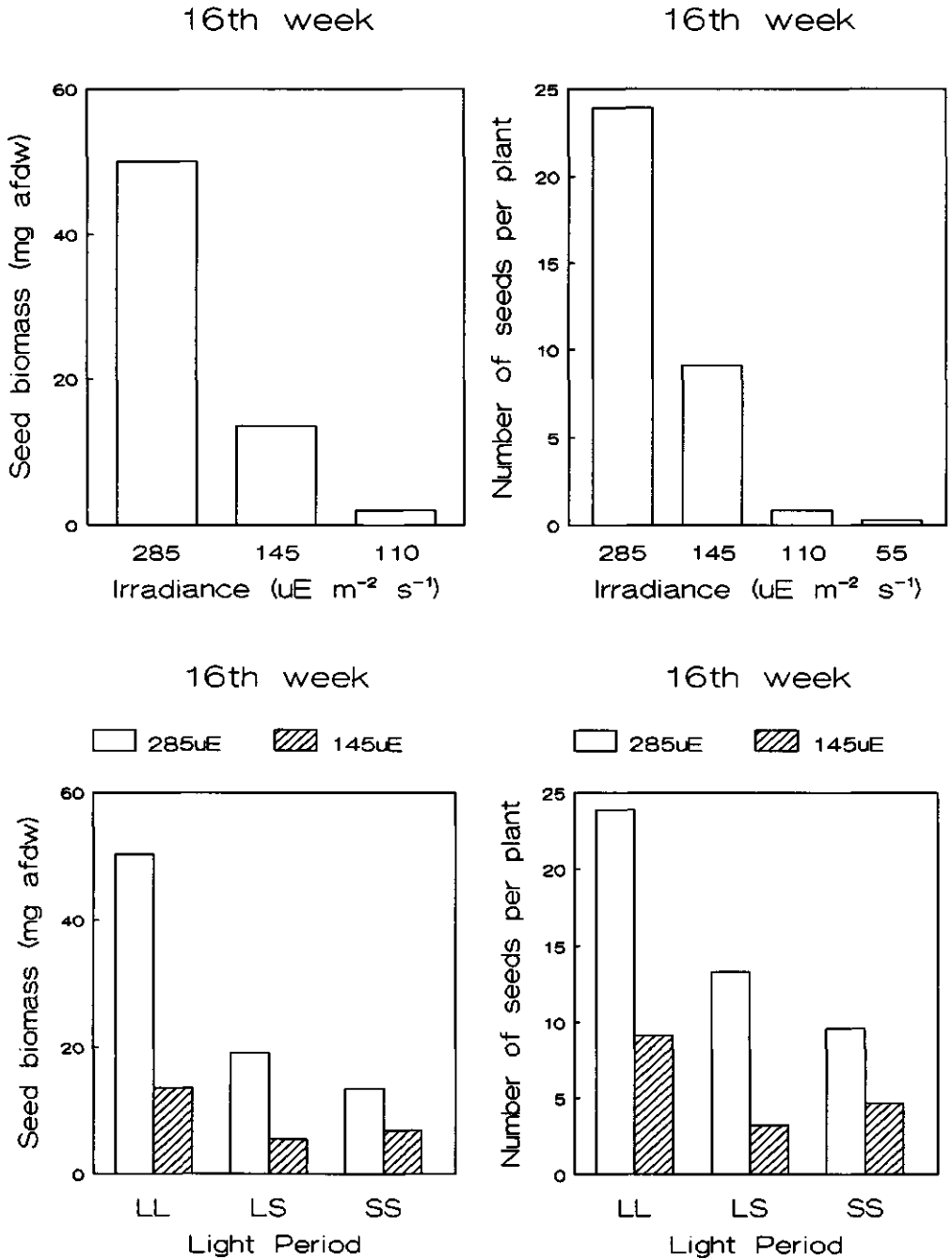


FIG. 10: Effect of the PPFD (under a 16 hours photoperiod; above) and the light period (below) on the average seed biomass and average number of seeds per plant after 16 weeks of growth ( $n=18$ ). Legend as previous figures.

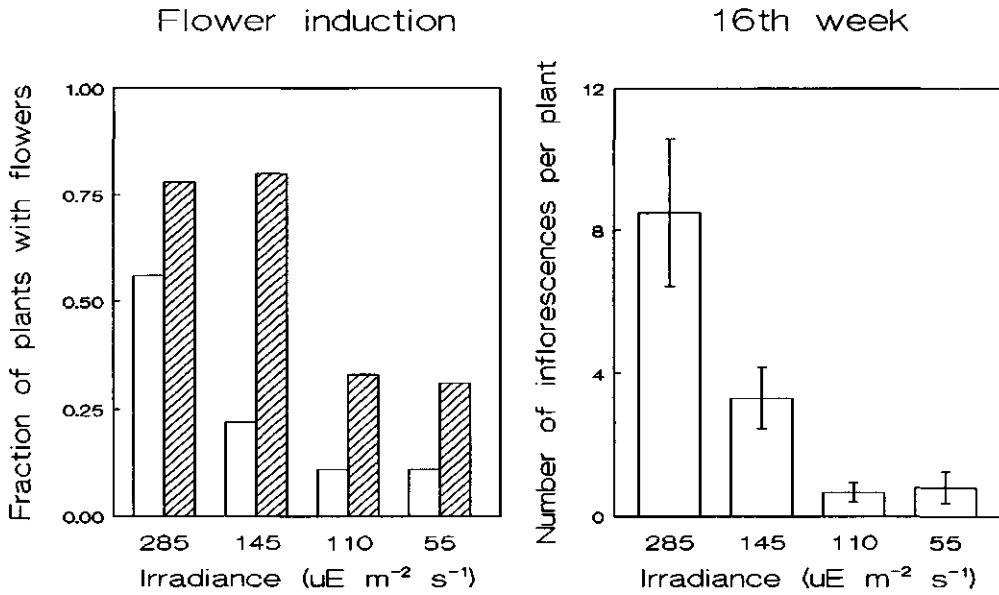


FIG.11: Effect of the irradiance experienced during growth on the initiation of flowering (left) and the flower production (right) of *R. drepanensis*. Left graph: empty bars indicate plants of 11 weeks of age, striped bars indicate plants of 16 weeks of age. Right graph: average  $\pm$  standard error.  $n=18$ .

biomass production was divided by 4 when decreasing the PPFD from LL1 to LL2, and was almost negligible (less than 1 seed per plant) for LL3 and LL4.

### 3.2.4. Chlorophyll concentration

**Experiment 1:** Light period (LP), irradiance (PPFD) and age, and their two- and three-level interactions all resulted in highly significant differences in total chlorophyll and chlorophyll-*a* (Chl-*a*) content (Table 2). Similar results were obtained for the chlorophyll-*b* (Chl-*b*) content, with the exception of the two-level interactions including the factor age. No significant differences were found between treatments in the fraction of chlorophyll-*b*.

As a general trend, both chlorophyll-*a* and -*b* increased with decreasing PPFD, and decreased with increasing age (Fig.12). An important exception was the SS2 treatment, in which the chlorophyll-*a* increased strongly with age together with a marked drop in the chlorophyll-*b*. The effect of the LP is not fully clear, although a trend to higher chlorophyll concentrations with decreasing PAP can be distinguished (but see the multiple comparisons below).

The fraction of chlorophyll-*b* increased with decreasing PPFD in the 11th week measurements, but decreased with decreasing PPFD in the 16th week measurements under short PAP conditions (LS and SS treatments). The effect of age can explain this, as the fraction of chlorophyll-*b* significantly decreased with increasing age in the treatments SS1, SS2 and LS2. Finally, chlorophyll-*b* fraction in the 11th week increased slightly with decreasing PP, and decreased (as described for age) with decreasing PP and PAP (the last only under low PPFD conditions: SS1, SS2 and LS2 treatments) in the 16th week.

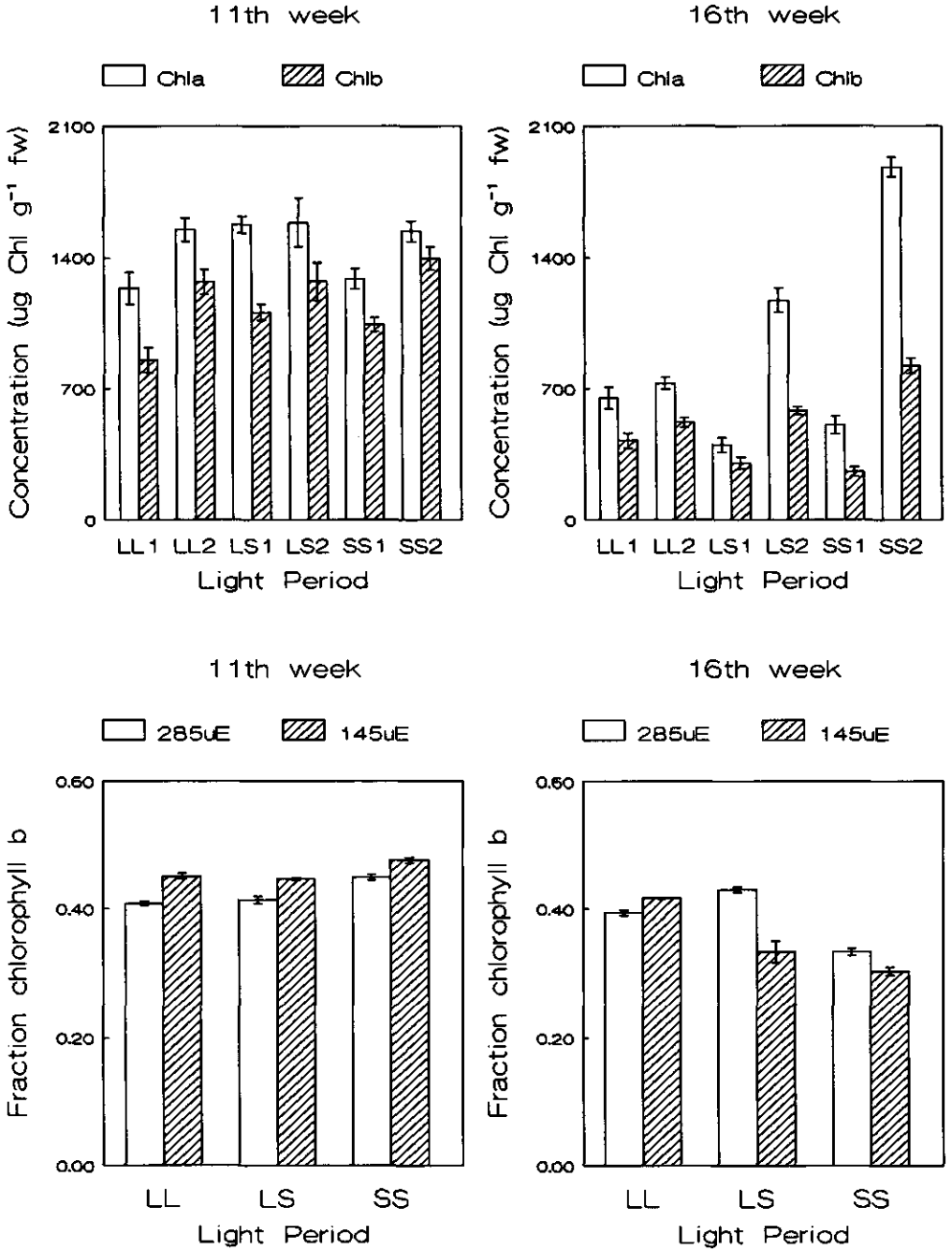


FIG.12: Effect of light period and irradiance on the chlorophyll concentration (above) and the fraction of chlorophyll-*b* (Chl-*b*/Chl.(*a*+*b*), below) in the leaf tissue of the plants after 11 and 16 weeks of growth. See Table 1 for the coding of the treatments. Average  $\pm$  standard error, n=6.

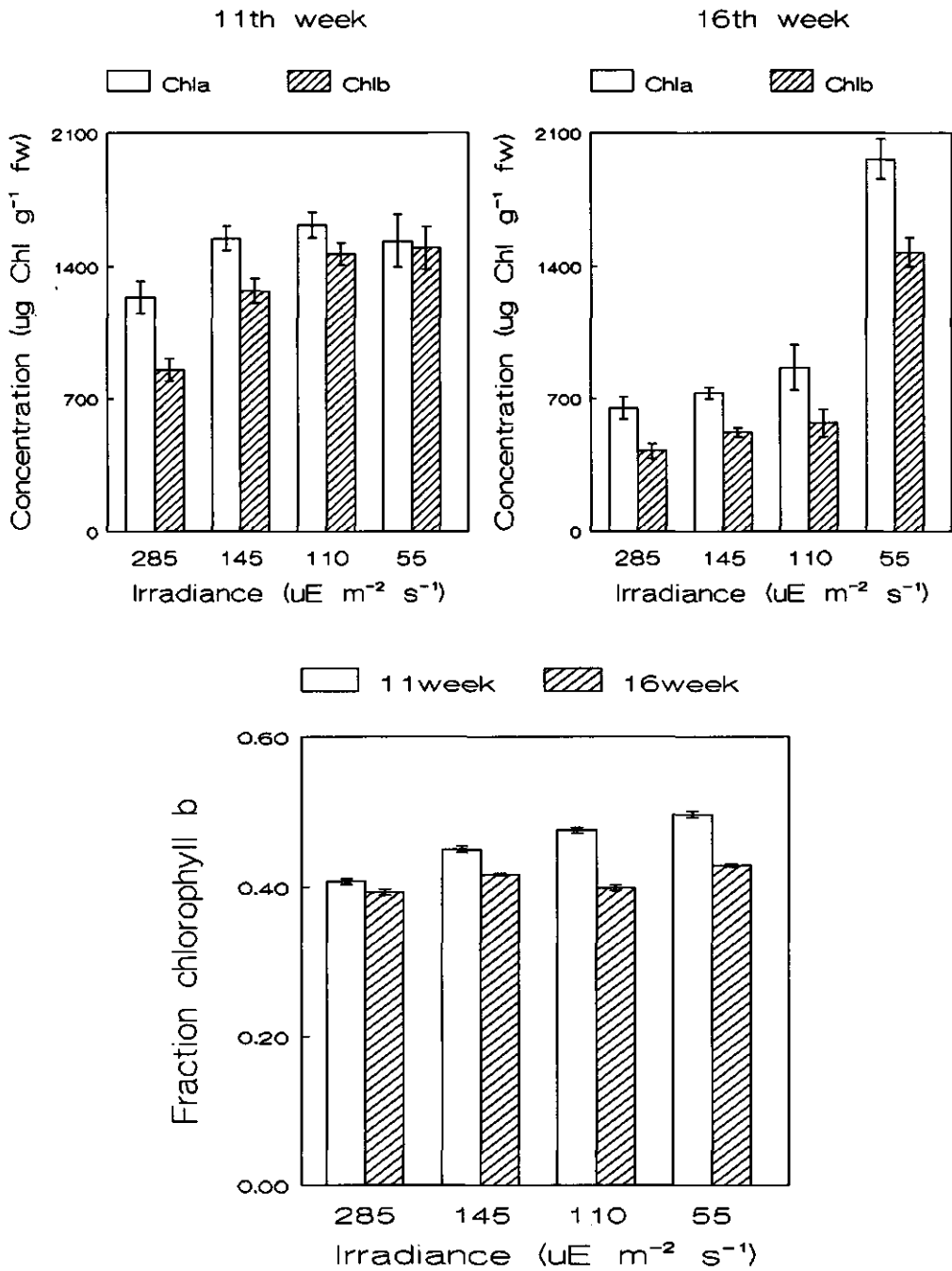


FIG. 13: Chlorophyll concentration and Chl-b/Chl.(a+b) ratio in the leaf tissue of plants grown at four different irradiances, after 11 and 16 weeks of growth. Average  $\pm$  standard error,  $n=6$ .

Thus, changes in chlorophyll concentration with age are quite variable for the different LP-PPFD combinations, which explain the highly significant effect of all two- and three-way interactions.

All comparisons between different ages resulted in significant differences for the Chl-*a* and Chl-*b* concentrations; all but one (SS2) yielded significant results for the total chlorophyll, and all but two (LL1 and LS1) for the fraction of Chl-*b*. In all significant cases but one (SS2), concentration decreased with increasing age, and in all of them Chl-*b* fraction decreased with increasing age.

Multiple comparisons of different PPFDs gave similar results for total chlorophyll, chlorophyll-*a* and chlorophyll-*b* concentrations. Four of the six comparisons between high and low PPFD resulted in significant differences: the two light periods without dim light (LL1 vs. LL2, and SS1 vs. SS2) in the 11th week; and the two short-PAP regimes (LS1 vs. LS2, and SS1 vs. SS2) in the 16th week. In all cases, concentration increased with decreasing PPFD.

LL1 and LS1 showed the only significant difference between different LPs in the 11th week, chlorophyll concentration increasing with decreasing PAP. In the 16th week, all three LPs significantly differed from each other under low PPFD, the concentrations increasing with decreasing PAP and PP.

Eight comparisons yielded significant results for the Chl-*b* fraction. Under long PP, Chl-*b* fraction increased with decreasing PPFD in the 11th week (LL1 vs. LL2, and LS1 vs. LS2), and decreased with decreasing PPFD in the 16th-week comparison (LS1 vs. LS2). SS1 was significantly different from the long PPs (LL1 and LS1) in the 11th week, its Chl-*b* fraction being higher. In the 16th week, all comparisons but one (LS2 vs. SS2) between different LPs resulted in significant differences, the SS1, SS2 and LS2 treatments showing significantly lower Chl-*b* fractions.

*Experiment 2:* Both PPFD and age and their interaction resulted in highly significant effects in the Two-Way ANOVA on total chlorophyll, chlorophyll-*a*, chlorophyll-*b* and Chl-*b* fraction (Table 2).

All age comparisons but the one under LL4 showed significant differences for Chl.(*a+b*), Chl-*a* and Chl-*b*. For the Chl-*b* fraction, all age comparisons but LL1 yielded significant results. Apart from LL4, Chl-*a*, Chl-*b*, Chl.(*a+b*) and Chl-*b*/Chl.(*a+b*) all decreased with increasing age (Fig.13). Indeed, LL4 showed in the 16th week significantly higher Chl-*a*, Chl-*b* and Chl.(*a+b*) concentrations, and also a higher Chl-*b* fraction, than the other three treatments. LL2 showed also a significantly higher 16th-week Chl-*b* fraction when compared with LL1.

In the 11th week, no comparison resulted in significant differences for Chl-*a*. Chl-*b* concentration was significantly lower for the LL1 treatment, and total chlorophyll concentration was significantly lower in LL1 than in LL3 and LL4. All PPFDs differed significantly for the Chl-*b* fraction, which increased with decreasing PPFD.

### 3.2.5. Nutrient tissue content

*Experiment 1:* Light period, PPFD, age and both two-way interactions including PPFD all significantly affected total P content in the plant tissue (Table 2). Tissue phosphorus content was at both ages significantly lower for the LL1 treatment (Fig.14). Tissue phosphorus content significantly decreased with increasing PPFD both under the LL and LS light regimes

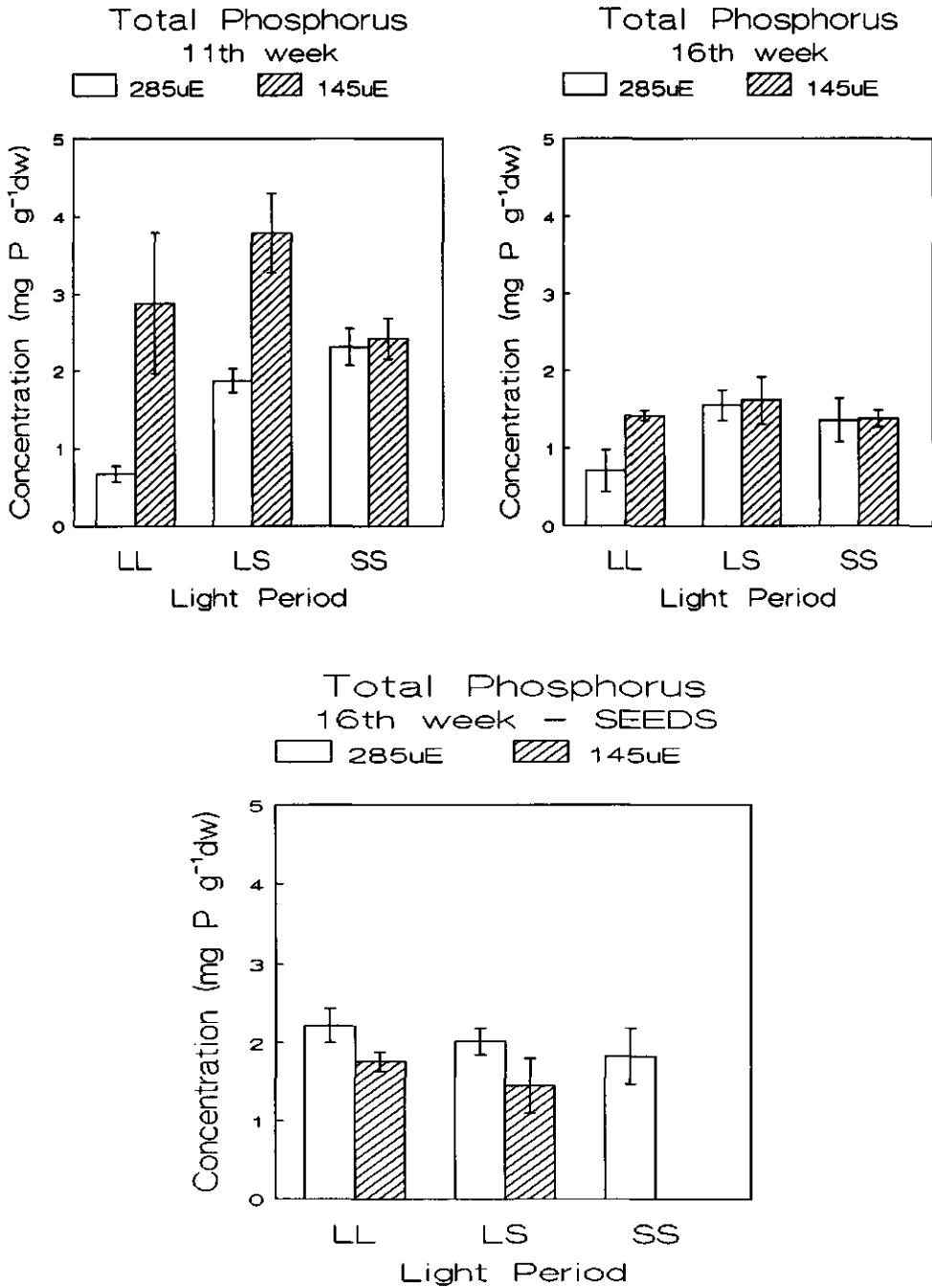


FIG. 14: Total phosphorus concentration in the (pooled above- and belowground) tissue of *R. drepanensis* plants grown under three different light periods two different irradiances, after 11 and 16 weeks of growth. Legend as previous figures. Average  $\pm$  standard error,  $n=5$  to 10.

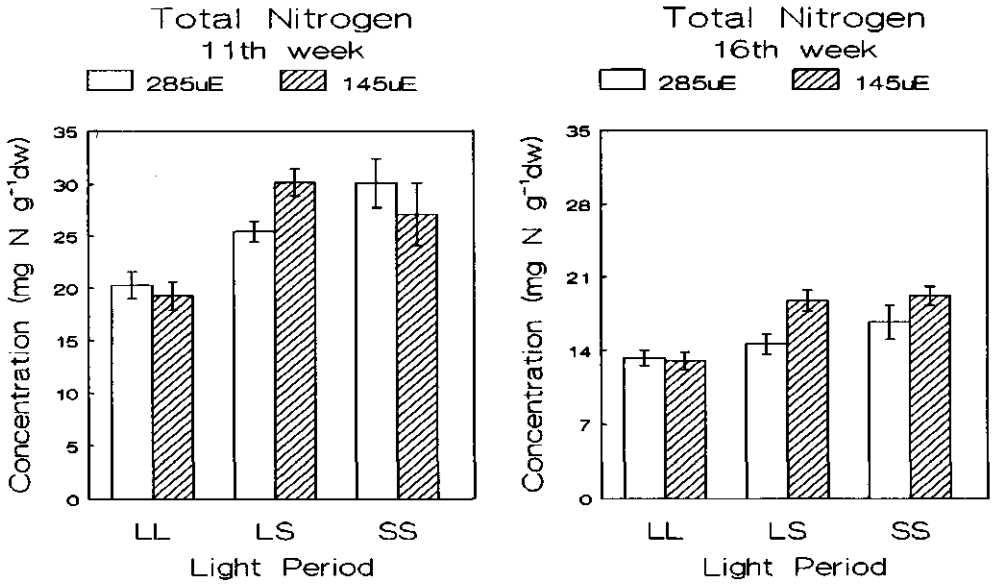


FIG.15: Total nitrogen concentration in the tissue of *R. drepanensis* plants grown under three different light periods and two different irradiances, after 11 and 16 weeks of growth. Average  $\pm$  standard error,  $n=5$  to 10.

(for the LS regime, only in the 11th week), while it significantly decreased with age for the LL2 and LS2 treatments.

Nevertheless, tissue phosphorus content measured in the seeds in the 16th week of the experiment showed high values under all light regimes, all above  $1.3 \text{ mg P g}^{-1} \text{ dw}$  (the critical level reported by Gerloff & Krombholz, 1966). The LL1 treatment had the highest values, above  $2 \text{ mg P g}^{-1} \text{ dw}$ . Although a significant effect of neither light period nor PPFD nor their interaction was detected in a Two-Way ANOVA, tissue phosphorus content tended to decrease with decreasing light period (LL > LS > SS) and decreasing PPFD. Low number of replicates in some of the treatments probably accounted for the high standard errors and thus for the non-significant results in the ANOVA.

Total N concentration in the plant tissue was significantly affected by light period and age (Table 2), while PPFD did not influence it (Fig. 15). Again, LL light regime resulted in the lowest tissue nitrogen contents. No significant differences between LP regimes were found in the 16th week, while in the 11th LL was significantly different from SS, and LL2 of LS2. All age comparisons but those from LL light regimes resulted in significant differences, increasing age resulting in lower tissue nitrogen content.

Unfortunately, sampled seed biomass was not high enough to obtain reliable estimations of their total N tissue content.

**Experiment 2:** Both total N and total P content in the plant tissue were significantly affected by PPFD and plant age ( $p < 0.001$ ; Table 2), while the interactive effect of both factors was not significant. Tissue nutrient content decreased with increasing PPFD (Fig. 16). This resulted in significant differences between LL1 and all other treatments for the total P (at 11



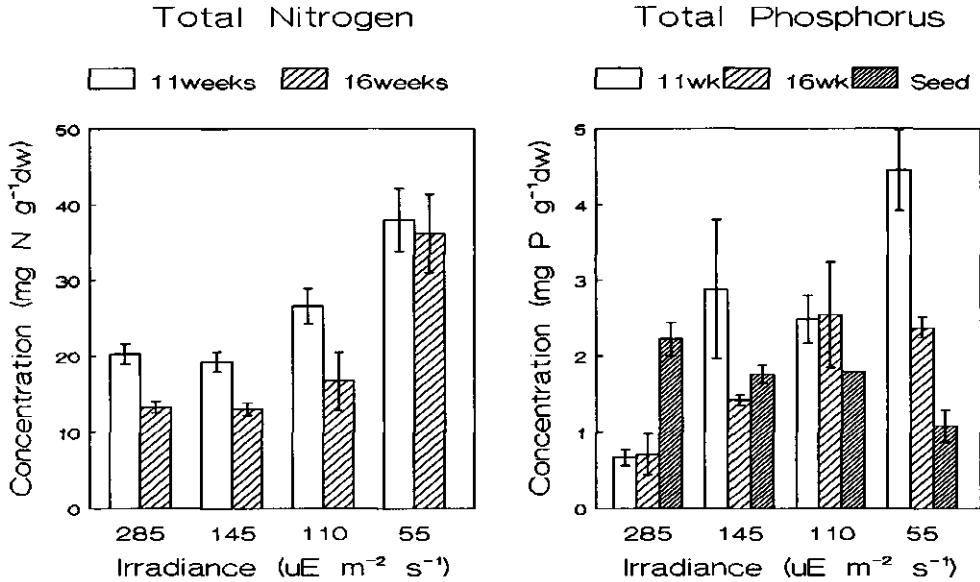


FIG.16: Total nitrogen and total phosphorus concentration in the tissue of plants grown under four different irradiances in the LL treatment, after 11 and 16 weeks of growth. "Seed" refers to the total phosphorus concentration in the seeds of plants of 16 weeks of age. Average  $\pm$  standard error;  $n=5$  to 10, except for the 'seed' values, where  $n=2$  to 15.

and 16 weeks). LL4 significantly differed from all other treatments for the 16th-week total N content. Nutrient tissue content decreased with increasing age, although multiple comparisons only yielded significant results for the tissue phosphorus content of the LL2 and LL4 treatments.

Tissue phosphorus content in the seeds decreased with decreasing PPFd (Fig.16). Nevertheless, effect of light was not significant in the ANOVA ( $F=3.83$ ,  $p>0.05$ ), although lack of replicates for the LL3 treatment obliges to consider the results of this ANOVA with some care (indeed, overall F-value was not significant).

### 3.3. Photosynthesis measurements

#### 3.3.1. Curve fitting

In total, 48 biomass-related and 48 chlorophyll-related light-response curves were obtained. No datapoint had to be rejected as outlier. Each replicate curve fitted well its datapoints (e.g., only in 7 of the 48 biomass-related fits  $r^2$  was below 0.9, and it was always above 0.7), and while some variability was found among replicate curves, it was never as high as reported e.g. in Hootsmans & Vermaat (1994), Van Vierssen & Hootsmans (1994) or Santamaría *et al.* (1994b) (see Fig.17 a-d).

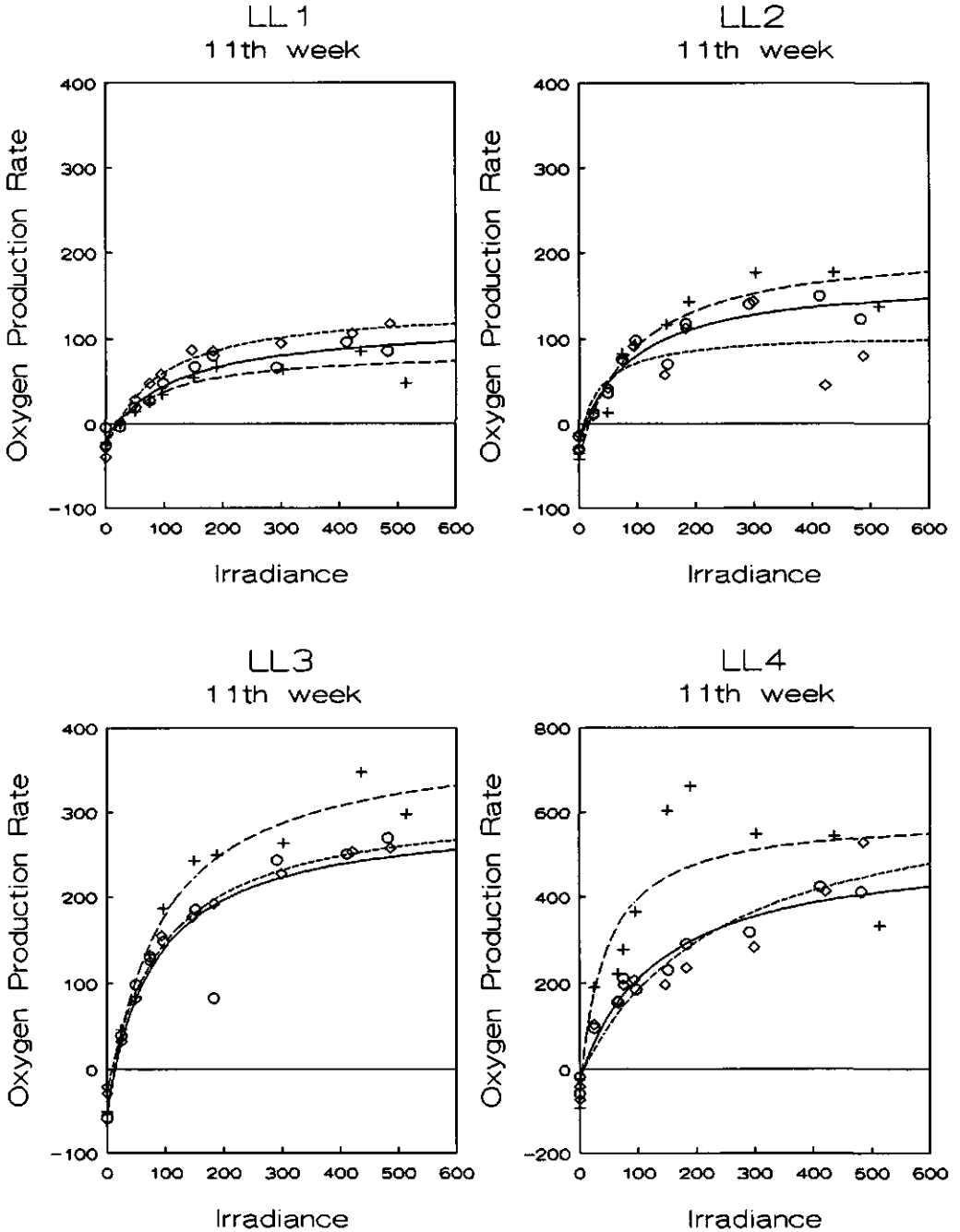


FIG.17a: Effect of the PPFd experienced during growth on the photosynthetic performance of plants of 11 weeks of age, together with the fitted rectangular hyperbola equations. Oxygen Production Rates are in  $\mu\text{g O}_2 \text{ min}^{-1} \text{ g}^{-1} \text{ afdw}$ , Irradiances are in  $\mu\text{E m}^{-2} \text{ s}^{-1}$ . Note the difference in scale for the 'LL4' graph.

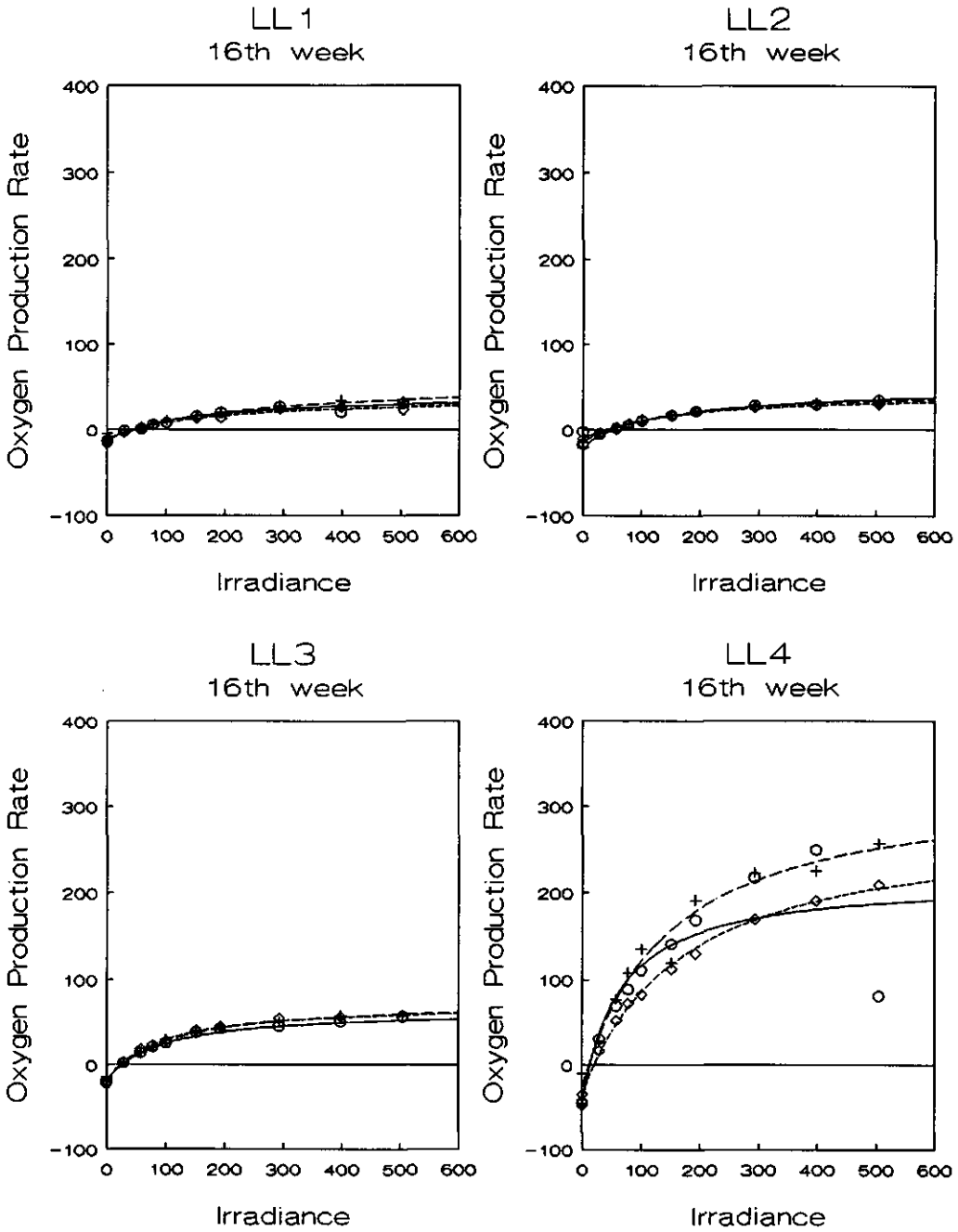


FIG.17b: Effect of the irradiance experienced during growth on the photosynthetic performance of plants of 16 weeks of age, together with the fitted rectangular hyperbola equations. Legend as in fig.17a.

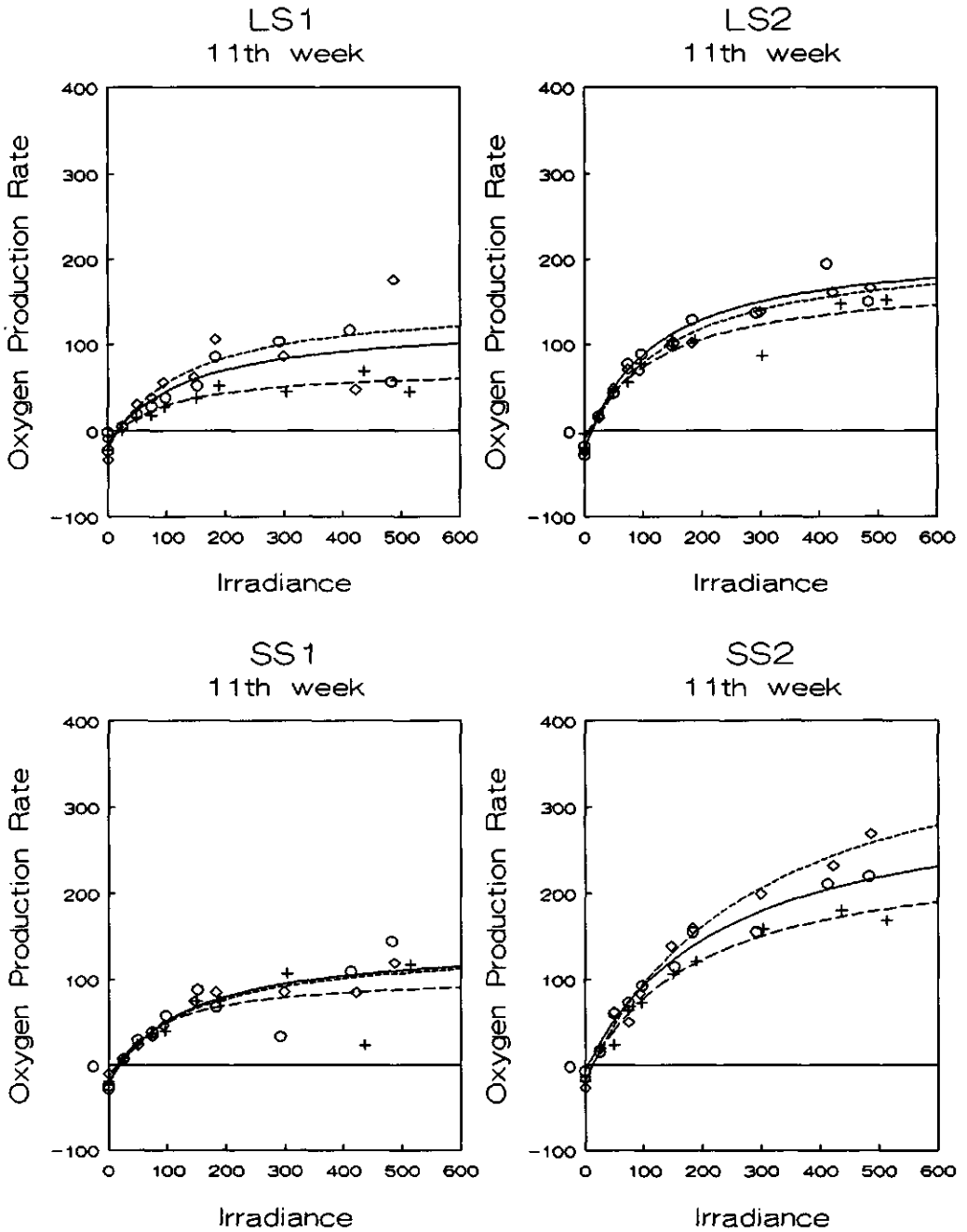


FIG. 17c: Effect of the PP, PAP and irradiance experienced during growth on the photosynthetic performance of plants of 11 weeks of age, together with the fitted rectangular hyperbola equations. Legend as in fig. 17a.

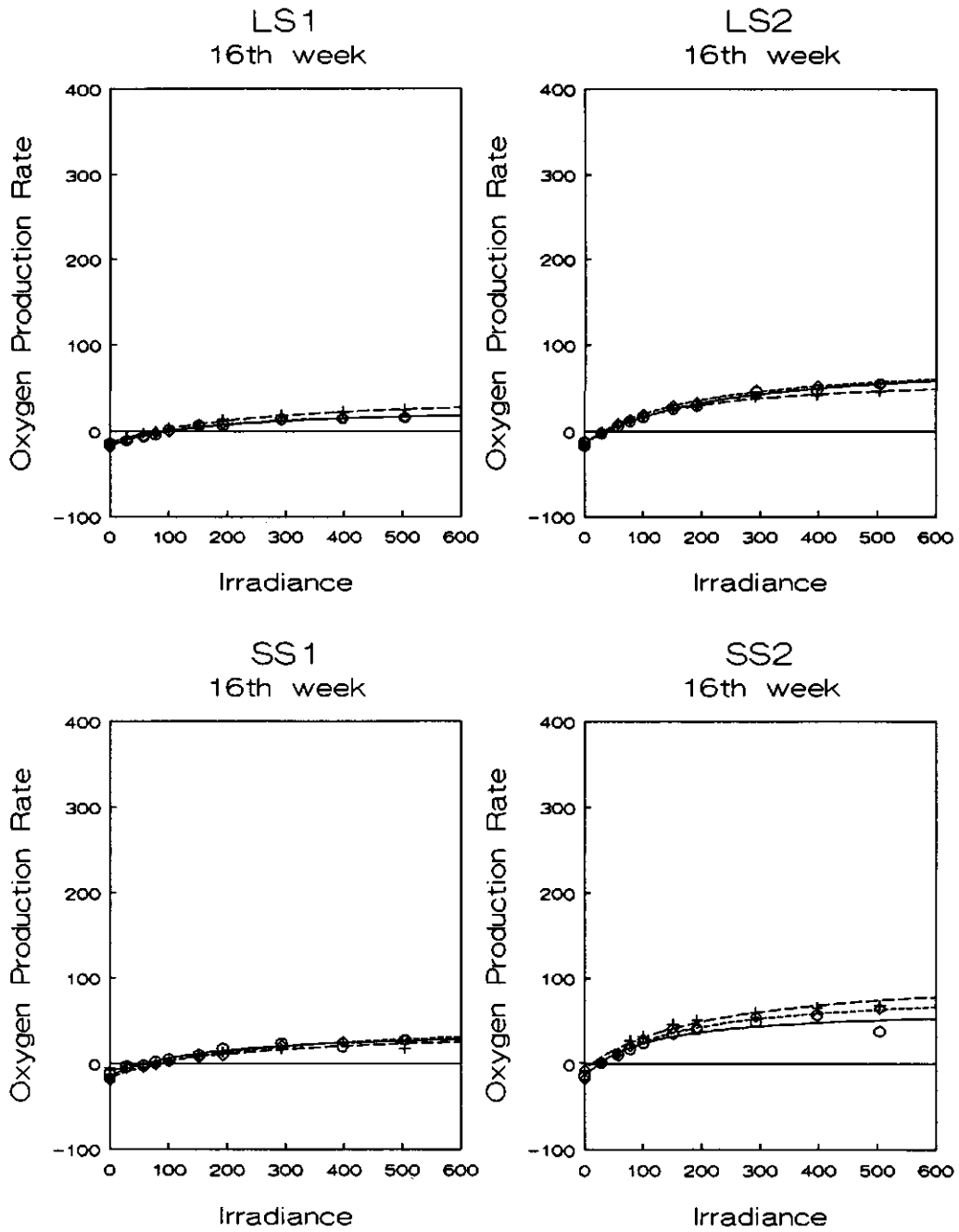


FIG.17d: Effect the PP, PAP and irradiance experienced during growth on the photosynthetic performance of plants of 16 weeks of age, together with the fitted rectangular hyperbola equations. Legend as in fig.17a.

### 3.3.2. Experiment 1

*Biomass-related curves:* LP and age significantly affected all three parameter estimations for  $P_m$ ,  $K_m$  and R, while only  $P_m$  was significantly affected by the PPFD (Table 5). However, when considering the derived parameters light compensation point (LCP), apparent quantum yield ( $\alpha$ ) and actual photosynthesis  $P_{ac}$  (see 'Materials and methods'), all three factors yielded significant results in the ANOVA. Two- and three-way interactions were in general non significant.

$P_m$  increased with decreasing PPFD and decreased with increasing age (Fig.18). Under low-PPFD conditions,  $P_m$  also increased with decreasing PP. Accordingly, all age comparisons resulted in significant differences, PPFD comparisons yielded significant results for the short PAPs (LS1 vs. LS2, and SS 1 vs. SS2) treatments, and LL2 vs. SS2 (11th week) resulted in the only comparison showing significant differences between light periods.

LL2 versus SS2 was also the only comparison which resulted in significant differences for  $K_m$  and R,  $K_m$  increasing and R decreasing with decreasing PP (Fig.18). 2 comparisons between different ages yielded significant results for  $K_m$  (within the LL2 and SS1 treatments), while only one showed significant differences for R (within the LL2 treatment). As a general trend,  $K_m$  increased while R decreased with increasing age.

The apparent quantum yield  $\alpha$  significantly decreased with increasing age in all treatments, and increased significantly with decreasing PPFD under long photoperiods (light periods LL and LS, 11th week; Fig.19). LL2 vs. SS2 was again the only comparison concerning the light period which yielded significant results (11th week),  $\alpha$  decreasing with decreasing PP.

Light compensation point (LCP) significantly increased with increasing age in all treatments, and was only affected by PPFD and LP when considering the 16 weeks old plant material. LCP decreased with decreasing PPFD under short PAP (LS and SS), and decreased also with decreasing PP under low PPFD (LS being intermediate between LL and SS).

$P_{ac}$  significantly decreased with increasing age in all treatments. In spite of a general trend to increasing  $P_{ac}$  values with decreasing PPFD and shorter LP, significant differences were only detected when considering the 16 weeks old plant material.  $P_{ac}$  increased with decreasing PPFD under short PP (LS and SS), decreased with decreasing PAP under high PPFD, and increased with decreasing PAP and decreasing PP under low PPFD.

Summarizing, the combination of a relatively short photoperiod and low PPFD conditions resulted in a higher maximum gross production rate ( $P_m$ ) but a lower apparent quantum yield ( $\alpha$ ), together with a lower dark respiration rate (R). In spite of the increased  $K_m$ , the light compensation point (LCP) tended to decrease. As an overall result, the actual net photosynthesis  $P_{ac}$  increased under such circumstances.

On the other hand, a decrease in the PPFD experienced during growth only affected  $P_m$ , which increased with decreasing PPFD. When concurrent with long photoperiods, decreasing PPFD also resulted in an increased apparent quantum yield ( $\alpha$ ) and a decreased LCP. As a consequence,  $P_{ac}$  increased with decreasing PPFD.

Increased age resulted in a decrease in  $P_m$ ,  $\alpha$  and R and a increase in  $K_m$  and LCP. Thus,  $P_{ac}$  strongly decreased.

*Chlorophyll-related curves:* When relating the PI curves to the chlorophyll concentration in the plants, light period did not significantly affected  $P_m$  any longer, while  $K_m$ , R,  $\alpha$  and LCP still yielded significant results (Table 5). PPFD significantly affected R and LCP, and age maintained its significant influence on every parameter but R.  $P_{ac}$  was now significantly

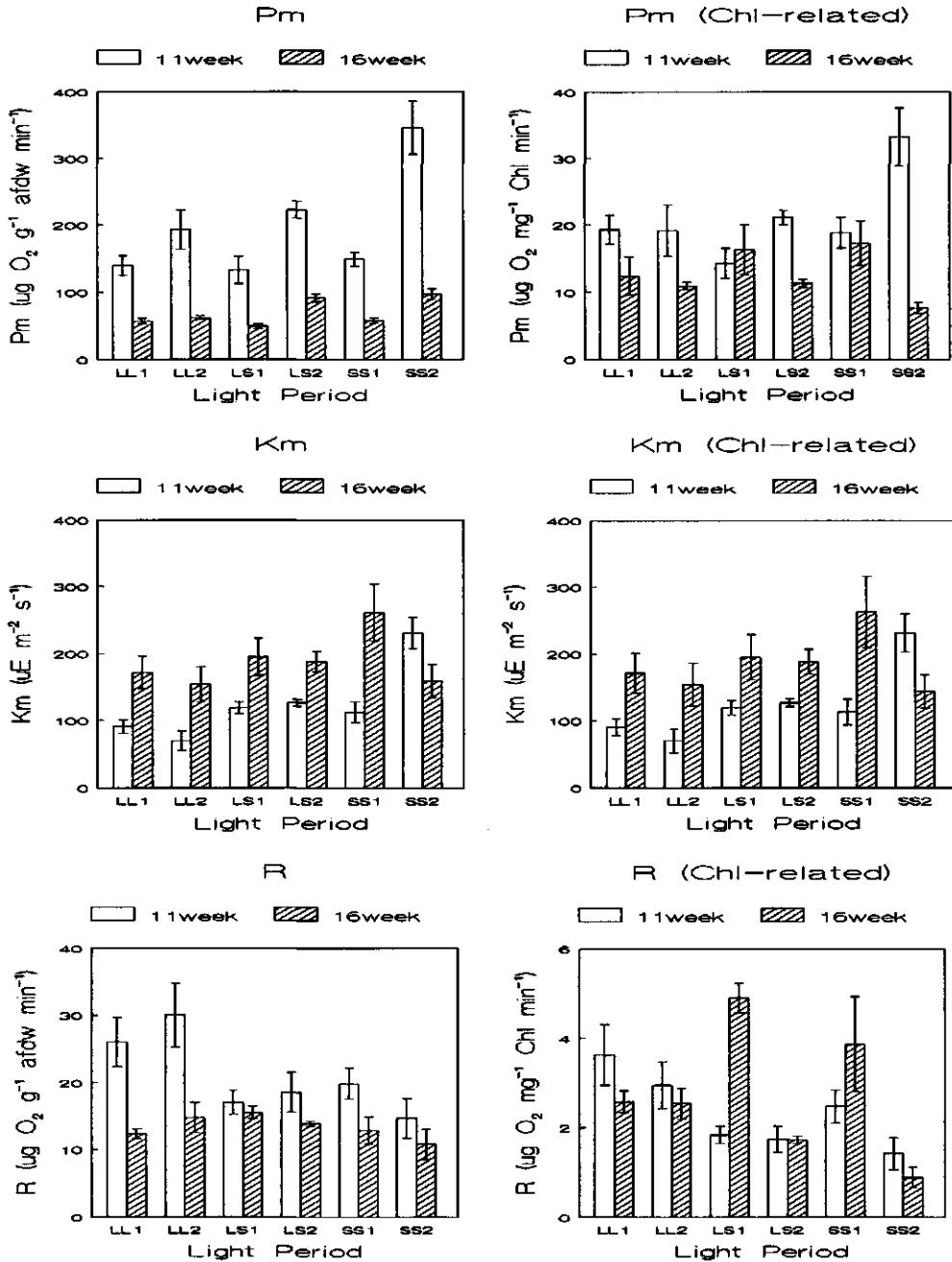


FIG.18: Effect of PP, PAP and age on the different parameter estimates of the fitted rectangular hyperbola equations modelling the photosynthetic performance of *Ruppia drepanensis* plants of 11 and 16 weeks of age. Pm: maximum gross production; Km: half saturation constant; R: dark respiration. Average  $\pm$  standard error, n=3. Rest of the legend as in fig.17a.

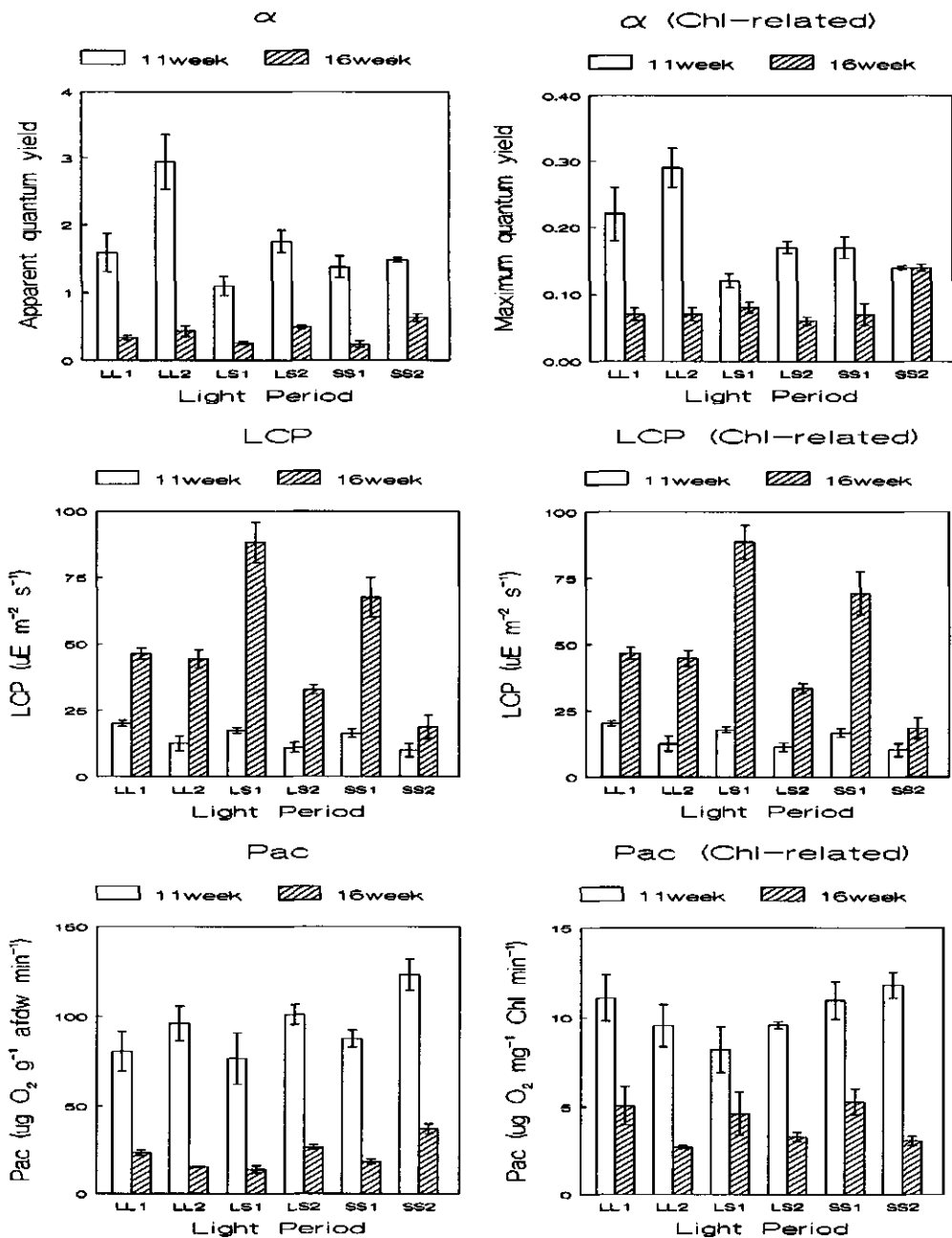


FIG.19: Effect of PP, PAP and age on the different derived parameter estimates of the fitted rectangular hyperbola equations modelling the photosynthetic performance of *Ruppia drepanensis* plants of 11 and 16 weeks of age.  $\alpha$ : maximum quantum yield (in  $\mu\text{g O}_2 \text{g}^{-1} \text{afdw min}^{-1} \mu\text{E}^{-1} \text{m}^2 \text{s}$ ); LCP: light compensation point;  $P_{ac}$ : realised net photosynthesis (see text). Average  $\pm$  standard error,  $n=3$ .



TABLE 5: Results of the ANOVAs for the effect of light period (LP) and irradiance (PPFD) experienced during growth, and age on the parameter estimations of the biomass-related and the chlorophyll-related photosynthetic response of *R. drepanensis* plants to increasing irradiance levels.

	Biomass-related curves					Chlorophyll-related curves						
	$P_m$	$K_m$	R	$\alpha$	LCP	$P_{sc}$	$P_m$	$K_m$	R	$\alpha$	LCP	$P_{sc}$
<i>Experiment 1:</i>												
LP	.008 <sup>***</sup>	.002 <sup>***</sup>	.02 <sup>*</sup>	.03 <sup>*</sup>	.03 <sup>*</sup>	.0021 <sup>***</sup>	.10 <sup>NS</sup>	.003 <sup>***</sup>	.012 <sup>*</sup>	.0001 <sup>***</sup>	.005 <sup>***</sup>	.12 <sup>NS</sup>
PPFD	.0001 <sup>***</sup>	.80 <sup>NS</sup>	.93 <sup>NS</sup>	.0001 <sup>***</sup>	.0001 <sup>***</sup>	.0001 <sup>***</sup>	.59 <sup>NS</sup>	.67 <sup>NS</sup>	.0001 <sup>***</sup>	.38 <sup>NS</sup>	.0001 <sup>***</sup>	.10 <sup>NS</sup>
Age	.0001 <sup>***</sup>	.0001 <sup>***</sup>	.0003 <sup>***</sup>	.0001 <sup>***</sup>	.0001 <sup>***</sup>	.0001 <sup>***</sup>	.0001 <sup>***</sup>	.0002 <sup>***</sup>	.30 <sup>NS</sup>	.0001 <sup>***</sup>	.0001 <sup>***</sup>	.001 <sup>***</sup>
LP*PPFD	.02 <sup>*</sup>	.37 <sup>NS</sup>	.33 <sup>NS</sup>	.69 <sup>NS</sup>	.016 <sup>*</sup>	.0002 <sup>***</sup>	.69 <sup>NS</sup>	.44 <sup>NS</sup>	.014 <sup>*</sup>	.07 <sup>NS</sup>	.0001 <sup>***</sup>	.31 <sup>NS</sup>
PPFD*Age	.28 <sup>NS</sup>	.08 <sup>NS</sup>	.88 <sup>NS</sup>	.58 <sup>NS</sup>	.10 <sup>NS</sup>	.78 <sup>NS</sup>	.0005 <sup>***</sup>	.056 <sup>NS</sup>	.015 <sup>*</sup>	.048 <sup>*</sup>	.0001 <sup>***</sup>	.04 <sup>*</sup>
LP*Age	.56 <sup>NS</sup>	.13 <sup>NS</sup>	.04 <sup>*</sup>	.02 <sup>*</sup>	.26 <sup>NS</sup>	.58 <sup>NS</sup>	.048 <sup>*</sup>	.09 <sup>NS</sup>	.017 <sup>*</sup>	.0002 <sup>***</sup>	.005 <sup>***</sup>	.21 <sup>NS</sup>
L*P*Age	.49 <sup>NS</sup>	.018 <sup>*</sup>	.74 <sup>NS</sup>	.008 <sup>***</sup>	.018 <sup>*</sup>	.003 <sup>***</sup>	.02 <sup>*</sup>	.010 <sup>*</sup>	.053 <sup>NS</sup>	.20 <sup>NS</sup>	.0001 <sup>***</sup>	.64 <sup>NS</sup>
<i>Experiment 2:</i>												
PPFD	.0001 <sup>***</sup>	.65 <sup>NS</sup>	.011 <sup>*</sup>	.0001 <sup>***</sup>	.0001 <sup>***</sup>	.0001 <sup>***</sup>	.0001 <sup>***</sup>	.66 <sup>NS</sup>	.36 <sup>NS</sup>	.23 <sup>NS</sup>	.0001 <sup>***</sup>	.34 <sup>NS</sup>
Age	.0001 <sup>***</sup>	.058 <sup>NS</sup>	.0016 <sup>***</sup>	.0001 <sup>***</sup>	.0001 <sup>***</sup>	.0001 <sup>***</sup>	.0001 <sup>***</sup>	.059 <sup>NS</sup>	.23 <sup>NS</sup>	.013 <sup>*</sup>	.0001 <sup>***</sup>	.001 <sup>***</sup>
PPFD*Age	.0006 <sup>***</sup>	.30 <sup>NS</sup>	.11 <sup>NS</sup>	.0001 <sup>***</sup>	.0001 <sup>***</sup>	.36 <sup>NS</sup>	.44 <sup>NS</sup>	.29 <sup>NS</sup>	.94 <sup>NS</sup>	.77 <sup>NS</sup>	.0001 <sup>***</sup>	.80 <sup>NS</sup>

affected only by age and its interaction with PPFD. About half of the interactions including age had a significant effect, LP\*PPFD being only significant for R and LCP.

Only 3 comparisons resulted in significant differences for  $P_m$ , one concerning each factor.  $P_m$  increased significantly with decreasing photoperiod under low PPFD (LL2 vs. SS2), and with decreasing PPFD under a short PP (SS1 vs. SS2), both for the 11 weeks old plant material (Fig. 18).  $P_m$  significantly decreased with age only in the SS2 treatment.  $K_m$  was only affected by LP, and increased significantly with decreasing PP under low PPFD (LL2 vs. SS2, 11th week). This same comparison resulted in a significant difference for R, which decreased with decreasing PP.  $\alpha$  yielded significant results in 3 comparisons, all with 11 weeks old plant material: it decreased with decreasing PAP under high PPFD (LL1 vs. LS1); and LL2 was significantly higher than LS2 and SS2 (Fig. 19). LCP increased significantly in a sequence LL1 < SS1 < LS1, and decreased significantly with decreasing PP under low PPFD (LS being intermediate between both), all only for the 16 weeks old plant material. But  $P_{sc}$  did not change with light period.

PPFD resulted in significant differences in two of the comparisons concerning R, which decreased significantly with decreasing PPFD under low PAPs (LS and SS; 16th week). One of those comparisons (LS light period) yielded also significant results for LCP, which decreased with decreasing PPFD. Again,  $P_{sc}$  did not significantly change with PPFD.

None of the comparisons concerning the factor age showed significant differences for  $K_m$ . Significant differences were found in only one comparison for R (LS1), in all comparisons but LS1 for  $\alpha$ , and in all comparisons but SS2 for LCP. As a general trend, R and  $\alpha$  decreased with increasing age, LCP increasing with increasing age. Overall consequence was a strong decrease in  $P_{sc}$  with increasing age, significant for all comparisons but LS1.

Summarizing, chlorophyll-related curves showed a much more limited effect of PPFD and LP on the parameter estimates.  $P_m$  was only affected under the most light-limiting conditions (combinations of low PPFD and short PP). The effect of PPFD on  $\alpha$  became

undiscernible, but long PAP proved to increase the maximum quantum yield. Effect of age on  $R$  was only significant for LS1. While old plants showed very high respiration rates under short PAPs at high PPFD (LS1 and SS1),  $R$  remarkably decreased to the lowest values found at short PAPs and low PPFD (LS2 and SS2).

$K_m$  and LCP presented almost identical values for the biomass- and the chlorophyll-related curves.  $P_{ac}$  values, finally, in their chlorophyll-related form are not affected by PPFD and LP, and appear only affected by age.

### 3.3.3. Experiment 2:

*Biomass-related curves:* Both PPFD and age and their interaction significantly affected  $P_m$ ,  $\alpha$  and LCP (Table 5).  $K_m$  was significantly affected by neither factor, while  $R$  and  $P_{ac}$  were affected by both PPFD and age but not by their interaction.

$P_m$  and  $\alpha$  significantly increased with decreasing PPFD and decreased with increasing age, the effect of PPFD being reduced for the 16 weeks old plants (Figs. 20 and 21). LL1 and LL2 were not significantly different in all cases. LCP significantly decreased with decreasing PPFD and increased with increasing age. LL1 and LL2 differed significantly from LL3 and LL4. Respiration tended to increase with decreasing PPFD and to decrease with increasing age, although only one comparison (LL1 vs. LL4, 16th week) yielded significant results.

As a consequence,  $P_{ac}$  increased with decreasing PPFD, and strongly decreased with increasing age. LL3 plants of 11 weeks showed the only significantly higher  $P_{ac}$  when performing multiple comparisons, LL2 and LL4 presenting intermediate values between LL1 and LL3. In the 16th week, LL4 plants are the only ones with a significantly higher  $P_{ac}$ . All age comparisons showed significant differences.

Overall, reduced PPFD resulted in increased maximum rates of net photosynthesis ( $P_m$ ), higher apparent quantum yields ( $\alpha$ ) and lower light compensation points (LCP), although with the 'cost' of higher dark respiration rates ( $R$ ). Plants grown under lower PPFD are able to achieve higher net photosynthetic rates under such lower PPFDs; even for the LL4 treatment (grown at  $55 \mu E m^{-2} s^{-1}$ ),  $P_{ac}$  values are still higher than for LL1.

*Chlorophyll-related curves:* PPFD had a significant effect only on  $P_m$  and on LCP (Table 5). Age affected significantly  $P_m$ ,  $\alpha$ , LCP and  $P_{ac}$ . Interaction was only significant for LCP.

$P_m$  and  $\alpha$  followed the same pattern of variation as for the biomass-related curves, and LCP values were almost identical (Figs. 20 and 21). Much less comparisons yielded significant results for  $P_m$  (only LL1 and LL2 vs. LL4, and no significant differences were found between different ages). No comparison showed significant differences for  $\alpha$ .  $P_{ac}$  was not affected by PPFD, and increasing age induced significant decreases in  $P_{ac}$  only under the two lowest PPFD (LL3 and LL4).

Summarizing, correction for plant chlorophyll content explains all variation in dark respiration rates ( $R$ ) and maximum quantum yield ( $\alpha$ ), while maximum net photosynthesis shows still a significant tendency to increase with decreasing PPFD, and light compensation point seems independent of the chlorophyll content. As a consequence, chlorophyll-related  $P_{ac}$  is not affected by the PPFD experienced during growth, although it still decreases with increasing age.

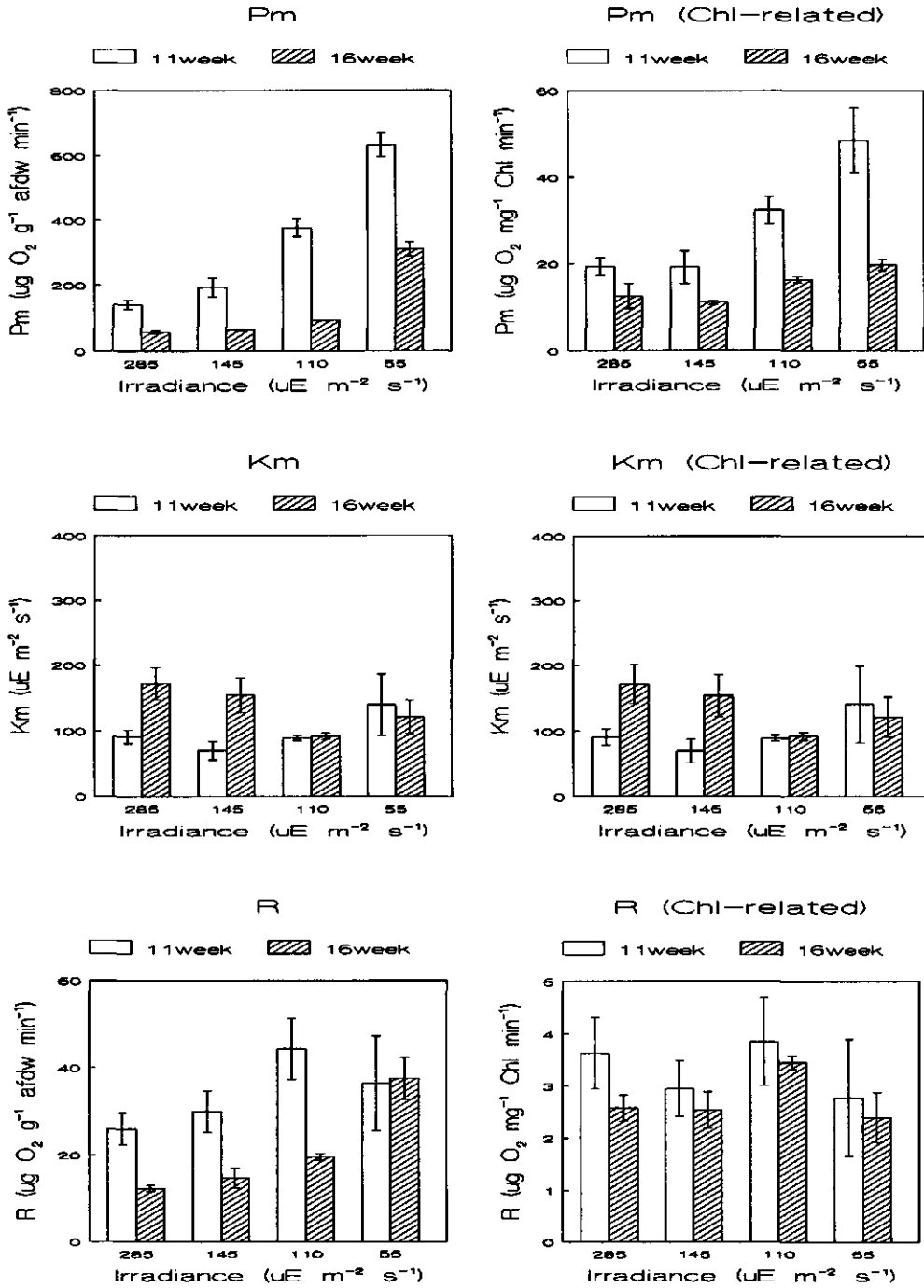


FIG.20: Effect of PPFD and age on the different parameter estimates of the fitted rectangular hyperbola equations modelling the photosynthetic performance of *R. drepanensis* plants of 11 and 16 weeks of age. Legend as in Fig.18.

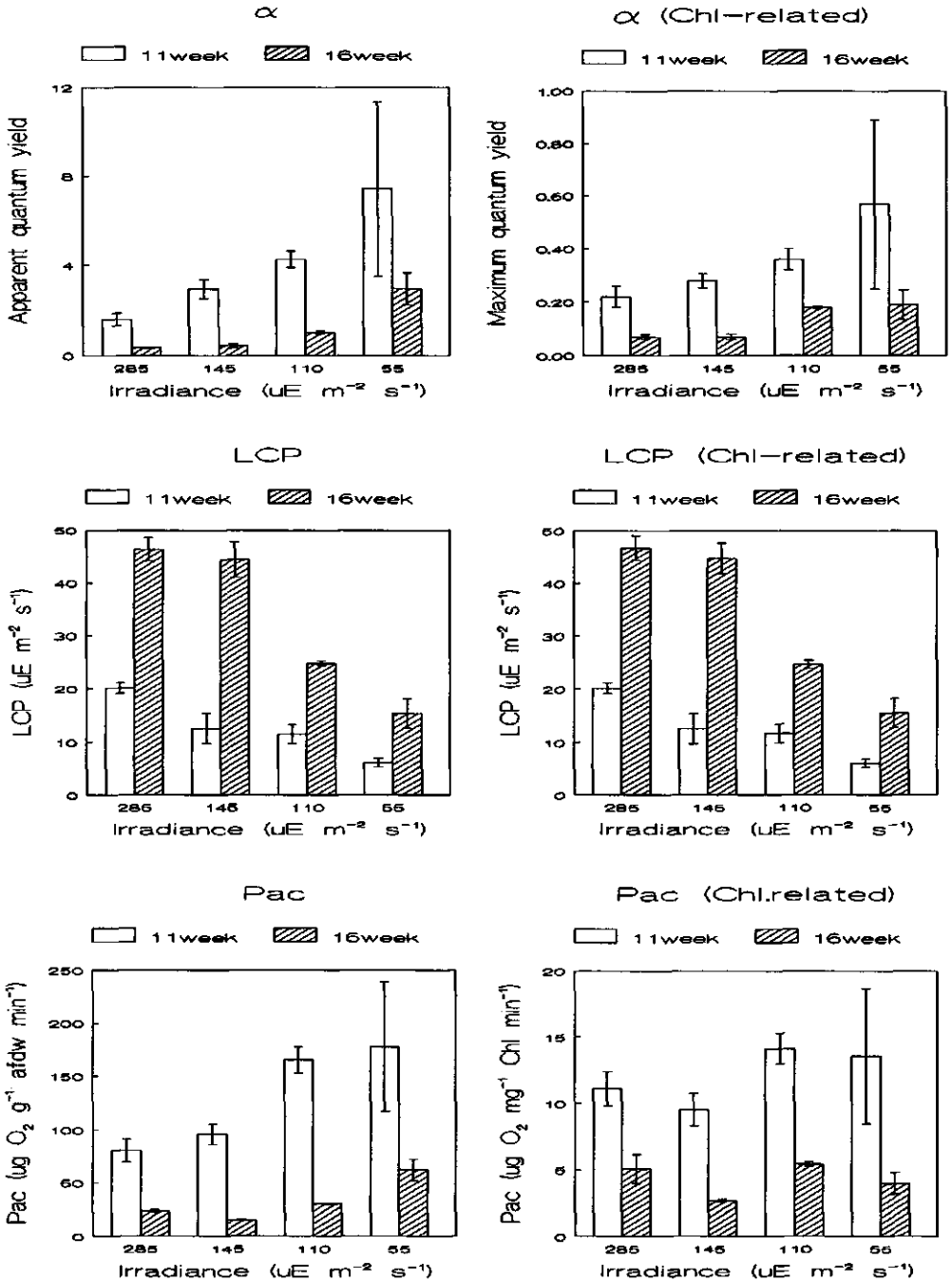


FIG.21: Effect of PPFD and age on different derived parameter estimates of the fitted rectangular hyperbola equations modelling the photosynthetic performance of *Ruppia drepanensis* plants of 11 and 16 weeks of age. Legend as in Fig.19.

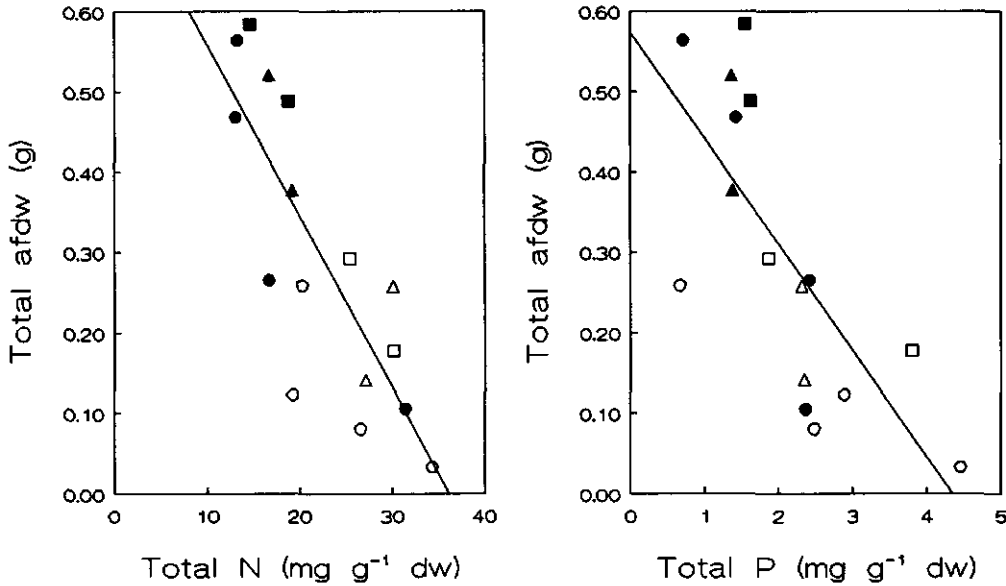


Fig.22: Relation between the total N and total P tissue content and the total biomass (afdw) of *R. drepanensis* plants grown under different light regimes. Circles: LL treatments. Squares: LS treatments. Triangles: SS treatments. Empty symbols: 11th week. Filled symbols: 16th week. Each datapair is the average from 5 to 10 replicates. Lines are linear fits on all datapairs together.

## 4. DISCUSSION

### 4.1. Nutrient availability and plant nutrient content

Orthophosphate concentration in the water column was always very low, and nitrate concentration decreased strongly with time until reaching very low values (0.1 to 0.2 mg N l<sup>-1</sup>) after 7 to 9 weeks (except for LL4). Although the sediment porewater was expected to be the major source of nutrients, the shape of the fits describing the decline in nitrate concentration in the water column (Fig.1) is just the opposite of the curves describing plant biomass increase, suggesting a strong correlation between water column nitrate depletion and plant biomass increase. This may explain the increase in dissolved nitrate concentration with decreasing irradiance in experiment 2. More striking is the lower dissolved nitrate concentration found under long-day conditions, which cannot be correlated with differences in plant growth. It is also remarkable that plants from long-day treatment showed the lowest nitrogen and phosphorus content.

Lower tissue nutrient contents may be attributed to a restricted nutrient availability. However, all treatments were started under the same conditions (identical sediment mixture, and tap water containing approx. 14 mg l<sup>-1</sup> nitrate and 0.01 mg l<sup>-1</sup> orthophosphate). Indeed, low nitrate values in the water column are not enough to conclude a restricted N availability for the plants: differences in above- and belowground nutrient uptake between different treatments could also explain the differences in both water column nitrate concentration and total N plant tissue content.

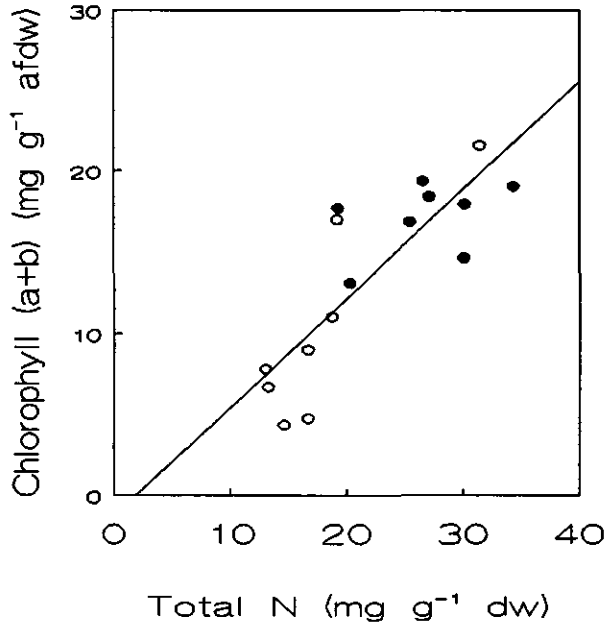


FIG. 23: Relation between leaf chlorophyll-(a + b) content and plant total N content. Each datapair is the average from six replicates. Filled circles: 11th week. Empty circles: 16th week.

Both total nitrogen and total phosphorus tissue content were lower for the long-day treatments, which also showed slightly lower growth rates than the dim and the short-day treatments. Indeed, nitrogen and phosphorus tissue contents from LL1 and LL2 (20 mg N g<sup>-1</sup> dw and 0.67 mg P g<sup>-1</sup> dw) were under the threshold separating nutrient limitation and luxury uptake regions as reported by Thursby (1984) for *R. maritima* (25-30 mg N g<sup>-1</sup> dw and 2.5-3.0 mg P g<sup>-1</sup> dw). Nevertheless, values reported by Gerloff & Krombholz (1966) from 13 different species of submerged macrophytes were much lower (13 mg N g<sup>-1</sup> dw and 1.3 mg P g<sup>-1</sup> dw), total P content from LL1 treatment being still in the region where nutrient limitation is to be expected. While Thursby (1984) based his conclusions on the effect of steady nutrient concentrations (weekly replenished) on 'logarithmic plant growth', Gerloff & Krombholz (1966) measured plant growth as biomass increase once the nutrient in the medium had been depleted. Conditions from Gerloff & Krombholz (1966) being more similar to our experimental conditions, we will stick to their values and reject a possible nitrogen limitation of plant growth.

As may be observed in Fig. 22, total N and total P concentration decreased with increasing plant biomass. Thus, a 'dilution' effect seem to be on the basis of differences in nutrient tissue concentration, similarly to reported by Hootsmans & Vermaat (1994) and Barko & Smart (1981). Moreover, LL1 values do not show any clear decrease of biomass coupled with decreased nutrient content. Still, nutrient limitation may not have resulted in a lower biomass yield, but in the impossibility to achieve higher biomass values than LS1 and SS1 while growing under higher daily irradiances and having higher rates of net photosynthesis (see below). LL1 plants would then be expected to have higher exponential

growth rates, but a lower asymptotic maximum value. This was not the case, neither in experiment 1 nor in experiment 2 (Figs.5a,b). Thus, although photosynthesis and growth of plants from the long-day treatments may have been affected by a relatively low nutrient content relative to the results of Gerloff & Kromholz (1966), we could not find any further evidence of nutrient limitation.

The relation between chlorophyll and nutrient content was analyzed by means of a linear regression. The regression showed a highly significant effect of nitrogen ( $r^2=0.82$ , Fig.23), the resulting equation being  $\text{Chl.}(a+b) \text{ (mg g}^{-1} \text{ afdw)} = -1.23 + 0.67 * \text{nitrogen (mg N g}^{-1} \text{ dw)}$ . The slope of the equation was much higher than in Hootsmans & Vermaat (1994) for *P. pectinatus* (slope was 0.152,  $r^2=0.68$ ), suggesting a relatively higher investment in chlorophyll production in *R. drepanensis*. As LL1 and LL2 datapairs (Fig.23) fitted relatively well in the overall trend, the low chlorophyll content of such plants may probably explain their low nitrogen content. On the other hand, nutrient reallocation into the seeds (as found for phosphorus content, see Figs. 14 and 16) probably accounted for the strong decline in chlorophyll content with increasing age.

#### 4.2. Influence of light acclimation on photosynthetic performance

Light acclimation effect on plant photosynthesis will be based on the concept of 'sun' and 'shade' plant strategies as described from terrestrial plants. First, a brief review on these strategies is presented. Second, we will discuss whether plant acclimation may be fully explained on the basis of the received diel radiation. An alternative hypothesis is that the effect of PPFD and LP have an interactive, non-additive component.

Comparison with reviewed data from other *Ruppia* species will then be used to characterise light acclimation in *R. drepanensis* and *R. maritima* and relate this with their life-cycle. Further comparison with *P. pectinatus* will illustrate the alternative role of morphological and photosynthetic acclimation. The latter, together with the species' life-cycle, may provide the framework to understanding differences in photosynthetic response between different submerged macrophyte species and populations. When not specifically mentioned, discussion on the photosynthetic performance of *R. drepanensis* will be based on our biomass-related PI curves.

##### 4.2.1. Photosynthetic light acclimation in sun and shade plants

Plants occupying sunny habitats (sun plants) are generally capable of higher photosynthetic rates at high irradiances than plants restricted to shaded locations (shade plants). Shade plants show lower rates of dark respiration, lower light compensation points (as low as 0.5 to 2  $\mu\text{E m}^{-2} \text{ s}^{-1}$ ; Boardman, 1977) and, thus, higher net photosynthesis rates at low irradiances. However, light saturation is reached much earlier in shade plants (at 60-100  $\mu\text{E m}^{-2} \text{ s}^{-1}$ , versus 800 up to 2000  $\mu\text{E m}^{-2} \text{ s}^{-1}$  in sun plants). Furthermore, sustained exposure to irradiances in excess of that required to saturate photosynthesis (supersaturation) may lead to a time-dependent inactivation of photosynthesis (photoinhibition).

Light response characteristics of a given plant may be strongly modified by the light regime experienced in the immediate past period. Growth of sun plants in the shade results in photosynthetic light responses tending towards those of obligate shade plants. Both light-saturated photosynthesis and dark respiration rates then show a strong decline with decreasing

light regime, and the light compensation point decreases accordingly. However, there is strong evidence that obligate shade plants have a low potential for photosynthetic light acclimation, largely as a consequence of their inherently low ability to increase their capacity for effective utilization of high irradiances for photosynthesis. Growth of shadow plants under high light regimes does not result in an increased capacity for light-saturated photosynthesis, and may cause a reduction in the quantum yield of photosynthesis at rate-limiting irradiances. In some cases, growth at moderately high irradiances resulted in a decrease in the capacity for light saturated photosynthesis, frequently accompanied by chlorophyll bleaching and other adverse effects (Björkman, 1981).

The limited ability of shade species to increase their photosynthetic rate when grown at high irradiance appears to be due to a failure to increase their level of RuDP carboxylase. The consequent inability to utilize higher irradiances would cause the photochemical traps of the photosystems to be closed for a higher proportion of the time, thus increasing the probability of photoinactivation (Boardman, 1977).

Adaptation to growth at low irradiances appears to be a question of the economical use of available light energy. Shade plants invest a greater proportion of its synthetic capacity in the synthesis and maintenance of the light-harvesting machinery than do sun plants. There are marked decreases in the levels of soluble proteins, including RuDP carboxylase, and the constituents of the electron transport chain. High levels of these latter compounds would be of little use to the plants in their low-light environment, and their synthesis and maintenance would require an increased expenditure of energy. Although the adaptability within a given genotype can be considerable, adaptation for high photosynthetic efficiency under one extreme of irradiance apparently precludes high efficiency at the other extreme (Boardman, 1977).

Aquatic plants living in shallow waters and amphibious plants receive the same full photosynthetically active spectrum of the sun's radiation as terrestrial plants, their responses to light being equivalent to those discussed above (Jeffrey, 1981). Because of shading associated with light absorption by the water column, especially under turbid conditions, submerged macrophytes have generally been considered typical 'shade' plants (Jeffrey, 1981). On the other hand, those macrophytes occurring in shallow or intertidal ecosystems may be expected to show adaptations typical for sun plants (Spence & Chrystal, 1970). This pattern could be further modified by the seasonal timing of the life-cycle and the distribution of the different species or populations, winter-annual and/or temperate plants being more likely to show shade-plant characteristics, and summer annuals and/or tropical and subtropical plants being more likely to show sun-plant characteristics.

The response of *R. drepanensis* to different irradiances experienced during growth (experiment 2) showed all the typical features described above for shade plants. Increased PPFD during growth did not result in increased photosynthetic rates at saturating irradiances. Moreover, both quantum yield at low irradiances (estimated by  $\alpha$ ) and maximum gross production ( $P_m$ ) decreased with increasing growth PPFD. Although dark respiration increased slightly with decreasing PPFD, LCP was lower under low PPFD conditions. The overall result was that net photosynthesis of the plants at the same PPFD they experienced during growth ( $P_n$ ) increased with decreasing PPFD.



#### 4.2.2. The role of PPFD and LP in affecting photosynthetic light acclimation

Most of the experiments dealing with light acclimation of sun and shade species refer to changes in the instantaneous PPFD. Nevertheless, daily irradiance is sometimes reported instead of PPFD (i.e. in Boardman, 1981, Fig.3.2), suggesting that PPFD and light period may have an additive effect in affecting plant photosynthetic response. However, it seems clear that LP and PPFD did not have a purely additive effect of *R. drepanensis* photosynthesis. Although a decrease in the daily PAR ( $\Sigma$ PAR) experienced during growth resulted in a linear decrease in LCP (Fig.24; linear fit:  $r^2=0.93$ ,  $p<0.001$ ), and  $P_m$  decreased exponentially with increasing  $\Sigma$ PAR (exponential fit:  $r^2=0.82$ ,  $p<0.001$ ), other parameters such as  $\alpha$  and the rate of dark respiration did not follow any overall relation. While a decrease in  $\Sigma$ PAR due to decreasing PPFD under long-day conditions (LL) resulted in an exponential increase in  $\alpha$  ( $r^2=0.96$ ,  $p<0.01$ ), an equivalent decrease in  $\Sigma$ PAR achieved by decreasing PP and PAP under the two highest PPFD had little effect on  $\alpha$ : linear equations fitted separately to the long- and short-PAP datapoints (see Fig.24) were significantly different ( $F=8.60$ ,  $p<0.05$ ). Moreover,  $\alpha$  was significantly higher for LL2 than SS2, while  $\Sigma$ PAR was lower in the last treatment.

Similarly, separate linear equations fitted to the long- and short-PAP datapairs for the relationship dark respiration- $\Sigma$ PAR (Fig.24) were also significantly different ( $F=12.82$ ,  $p<0.05$ ). While  $R$  tended to increase with decreasing  $\Sigma$ PAR under long-day conditions, it decreased with decreased  $\Sigma$ PAR caused by a shorter PAP.

Variation in light-saturated photosynthetic rate  $P_m$  is generally attributed to other factors than those affecting maximum quantum yield  $\alpha$  (Björkman, 1981). But when negatively affected in shade plants exposed to high light conditions, both  $P_m$  and  $\alpha$  are supposed to decrease due to the negative effect of supersaturating PPFD. Decreasing leaf chlorophyll content, often reported to accompany this process, was also found at increasing PPFD and PP in our experiment.

The reason why variation in  $\alpha$  under short PAP conditions does not follow the general pattern derived from the experienced  $\Sigma$ PAR may lay in the fact that PAP does not only affect  $\Sigma$ PAR, but also the ratio between 'day' net production and 'night' dark respiration. Investment of energy associated to the maintenance of an efficient light harvesting system may not be sustainable any more when a long night period makes the expenses caused by dark respiration bigger. Restricting respiration losses may then be a need which negatively affects the build-up or the maintenance of a high  $\alpha$ . It is noteworthy that, while under long-day conditions dark respiration increased with decreasing PPFD but remained unaffected once related to chlorophyll content (experiment 2; see Fig.20), it decreased with decreasing PAP both expressed on a biomass and on a chlorophyll basis (experiment 1; see Fig.18).

As an overall result of both processes, the  $P_m/R$  ratio steadily increased with decreasing  $\Sigma$ PAR. Although the SS2 treatment is the only one in which  $K_m$  is higher than the PPFD experienced during growth, indicating a somehow inferior balance between the carbon metabolism rate and the light harvesting process, reduced respiration expenses resulted in higher  $P_m/R$  ratios for the short PAP treatments than those expected on a  $\Sigma$ PAR basis. Concluding, decreasing PAP coupled with decreasing PPFD have shown to affect several photosynthetic parameters synergically, the effect of a restricted daily irradiance ( $\Sigma$ PAR) combined with longer 'respiratory' periods reducing the plant's possibilities for shade acclimation. In spite of the restricted rate of dark respiration, plant net production under such circumstances is lower than expected on a PPFD or a  $\Sigma$ PAR basis.

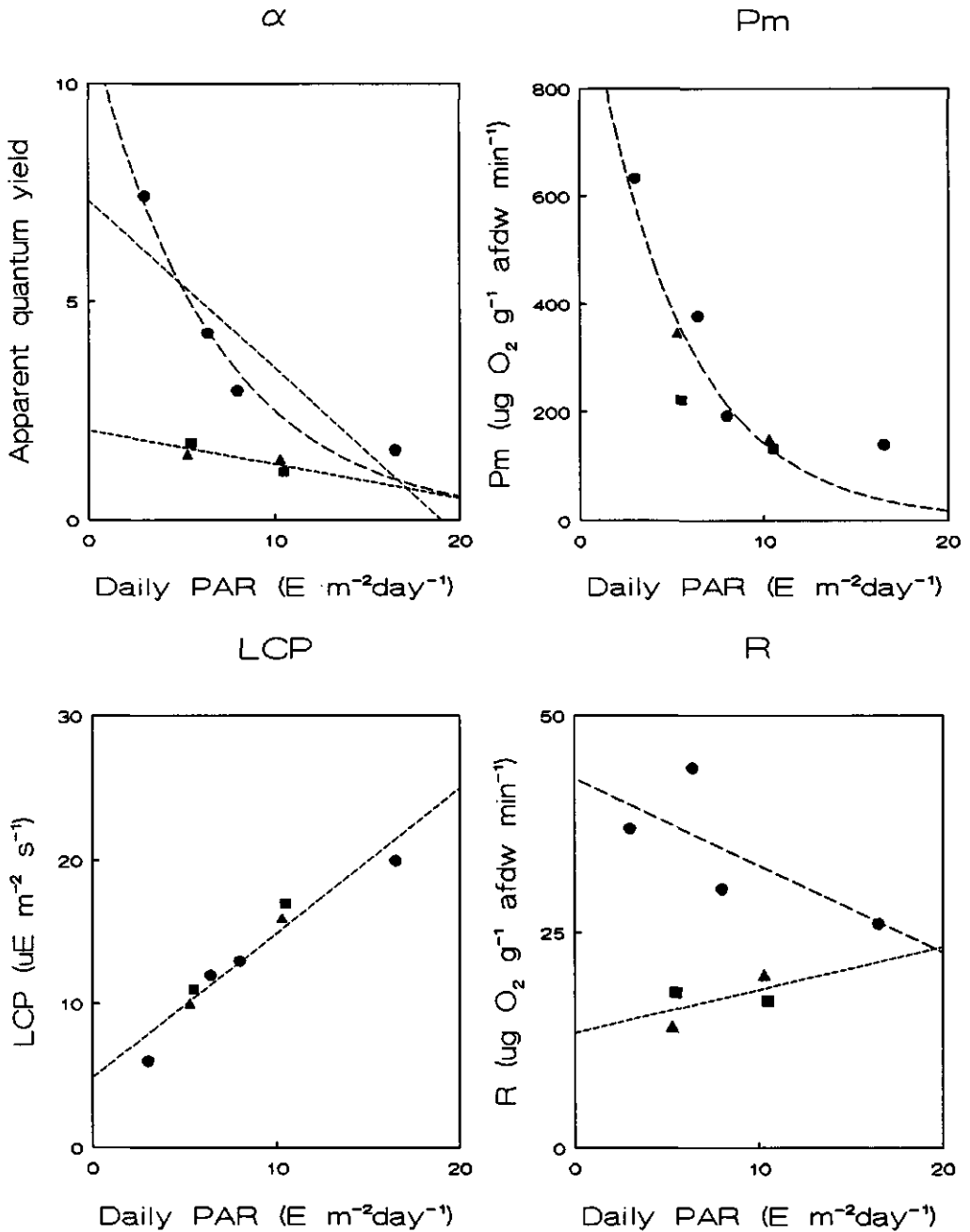


FIG.24: Relation between different photosynthetic parameters and the daily irradiance experienced during growth. Best fitting equations (linear for LCP, exponential for  $\alpha$  and  $P_m$ ) are also indicated. Separate linear fits for the long- and short-PAP datapairs are shown for  $\alpha$  and R. Circles: LL treatments. Squares: LS treatments. Triangles: SS treatments. All values from plants of 11 weeks of age. Each datapoint is the average from 3 replicate values.

## 4.2.3. Comparison with literature data: influence of life-cycle on light acclimation capacity

Shade plants are supposed to show a relative inability to adapt to high irradiance regimes as a consequence of their strong adaptation to low irradiance habitats. A comparison between the photosynthetic characteristics of *R. drepanensis* and the values reported in literature for other brackish water species of *Ruppia* (Table 6) and for other temperate freshwater macrophytes occurring in shallow water bodies (Table 4.17 from Hootsmans & Vermaat, 1994) may allow us to discern whether a shade adapted character is a general feature of submerged macrophytes, or whether it reflects specific adaptations to local conditions (mainly water transparency and growth season). When not specifically mentioned, we will restrict ourselves to measurements performed at temperatures close to 20 °C.

The range of  $P_m$  values reported for all three *Ruppia* species grown under field conditions (92-133 for *R. maritima*, 42-144 for *R. cirrhosa*, 45-178 for *R. drepanensis*, all in  $\mu\text{g O}_2 \text{g}^{-1} \text{afdw min}^{-1}$ ) is similar to what we found for *R. drepanensis* under high PPFD conditions. Low  $P_m$  values are in general explained by a higher age, as they always appear in older plant material. Although Twilley *et al.* (1985) and Beer *et al.* (1991) reported higher net production rates (about 200  $\mu\text{g O}_2 \text{g}^{-1} \text{afdw min}^{-1}$ ), similar to what we found for *R. drepanensis* grown at 145  $\mu\text{E m}^{-2} \text{s}^{-1}$ , none of the reported maximum photosynthetic rates approach the values obtained under our lowest irradiance regimes (347-661  $\mu\text{g O}_2 \text{g}^{-1} \text{afdw min}^{-1}$ ).

Koch & Dawes (1991) reported  $P_m$  values for *R. maritima* plants cultivated under different photoperiods (6, 12 and 18 h, PP=PAP). PPFD was comparable to our LL3 treatment (90  $\mu\text{E m}^{-2} \text{s}^{-2}$ ). Although maximum  $P_m$  values were found at intermediate PP (12 h, 3.9  $\text{E m}^{-2} \text{day}^{-1}$ ), the effect of PP was quite limited. Reported  $P_m$  values (104-207  $\mu\text{g O}_2 \text{g}^{-1} \text{afdw min}^{-1}$ ) were much lower than our findings for *R. drepanensis* grown under a similar PPFD (347 for LL3, 6.4  $\text{E m}^{-2} \text{day}^{-2}$ ), and not remarkably higher than the values shown by *R. maritima* plants collected from field under much higher daily irradiances (126-133  $\mu\text{g O}_2 \text{g}^{-1} \text{afdw min}^{-1}$ ,  $\Sigma\text{PAR}$  approx. 55  $\text{E m}^{-2} \text{day}^{-1}$ ; Wetzel & Penhale, 1983).

$P_m$  values are believed to be limited by the enzymatic activity of the carbon metabolism, mainly RuDP, and by the rate of carbon uptake. Enhancing carbon uptake by lowering the pH from 8.5 to 7 increased the rate of net photosynthesis similarly in *R. maritima* (from 198 to 276  $\mu\text{g O}_2 \text{g}^{-1} \text{afdw min}^{-1}$ ; Beer *et al.*, 1991) and *R. cirrhosa* (from 144 to 259  $\mu\text{g O}_2 \text{g}^{-1} \text{afdw min}^{-1}$ ; Peñuelas & Menéndez, 1990). Peñuelas & Menéndez (1990) concluded that *R. cirrhosa* has the capability to utilize bicarbonate as carbon source. As Beer *et al.* (1991) pointed out, efficient use of bicarbonate may assure high supply rates of  $C_i$ , and it is possible that RuDP activity then limits photosynthetic production. Santamaría *et al.* (in press) reported very low bicarbonate concentrations and high pH values at mid spring in several wetlands where *R. drepanensis* occurs. Although this species may efficiently use bicarbonate as a carbon source for photosynthesis,  $P_m$  was severely reduced by limited carbon availability under such circumstances, and increased significantly after the addition of bicarbonate to the medium.

Maximum quantum yield varied similarly to  $P_m$  for different *Ruppia* species. Values reported for *R. maritima* were all lower than our lowest finding for *R. drepanensis*, but similar to the values reported in the field for the latter species. Older *R. drepanensis* plants showed lower  $\alpha$  values. As  $\alpha$  and  $P_m$  values from 'young' *R. drepanensis* plants from the field were comparable to our findings under LL1 conditions, while the PPFD they experienced during growth was much higher, we may conclude that they would not decrease

TABLE 6: Comparison of various photosynthesis parameters. Salinity (Total Dissolved Solids) is indicated by  $g\ l^{-1}$ .  $\mu E$ :  $\mu E\ m^{-2}\ s^{-1}$ . wk: age of the plants in weeks. Lf: leaf fragments. Sf: shoot fragments, sometimes including rhizome and root fragments. Ip: intact plants. PP in hours, EPAR in  $E\ m^{-2}\ day^{-1}$ , PPFD in  $\mu E\ m^{-2}\ s^{-1}$ .  $P_m$  and  $R$  in  $\mu g\ O_2\ g^{-1}\ afdw\ min^{-1}$ ,  $\alpha$  in  $\mu g\ O_2\ g^{-1}\ afdw\ min^{-1}\ (\mu E\ m^{-2}\ s^{-1})^{-1}$ ,  $K_m$  and LCP in  $\mu E\ m^{-2}\ s^{-1}$ . Most PI curves from references 1, 5 and 9 showed photoinhibition. Values from present study only from the 11 weeks old material.

Conversion factors used:  $afdw=90\%dw=16\%fw$  (leaves or aboveground biomass; own data),  $PQ=1$  (Hootsmans & Vermaat, 1994).

Species: *Rmar*=*R. maritima*, *Rcirr*=*R. cirrhosa*, *Rdrep*=*R. drepanensis*, *Rpoly*=*R. polycarpa*.

References: <sup>1</sup>Koch & Dawes, 1991; <sup>2</sup>Evans *et al.*, 1986; <sup>3</sup>Wetzel & Penhale, 1983; <sup>4</sup>Twilley *et al.*, 1985; <sup>5</sup>Williams & McRoy, 1982; <sup>6</sup>Beer *et al.*, 1991; <sup>7</sup>Peñuelas & Menéndez, 1990; <sup>8</sup>García *et al.*, 1991; <sup>9</sup>Santamaría *et al.*, in press; <sup>10</sup>Santamaría *et al.*, 1994; <sup>11</sup>Present study; <sup>12</sup>Vollebergh & Congdon, 1986.

Species (reference)	Growth conditions	Photosynthesis conditions	PP (EPAR)	NP <sub>max</sub> (PPFD)	P <sub>m</sub>	$\alpha$	K <sub>m</sub> (=L)	R	LCP (=L)		
<i>Rmar</i> <sup>1</sup>	20 $g\ l^{-1}$ , 22°C, 90 $\mu E$ , 9wk Florida population	Lf, O <sub>2</sub> , 24°C, 0-1200 $\mu E$	6 (1.9)	115 (660)	135	2.22	-	5.6	55		
			12 (3.9)	117 (670)	207	2.56	-	71.6	64		
			18 (5.8)	86 (400)	104	0.67	-	8.3	61		
	Florida, 80 $\mu E$ , 14°C	id., 22°C	12 (3.5)	210 (400)	272	1.32	-	44.6	35		
		id., 22°C	12 (3.5)	119 (1030)	158	0.32	-	24.5	88		
		id., 30°C	12 (3.5)	51 (400)	62	0.16	-	29.6	69		
	New Carolina population	6 (1.9)	62 (400 to 1200)	133	0.67	-	6.2	90			
		12 (3.9)	115 (400)	175	1.81	-	48.6	64			
		18 (5.8)	116 (400)	129	0.63	-	13.2	56			
	New Carol, 80 $\mu E$ , 14°C	id., 22°C	12 (3.5)	230 (400)	256	5.77	-	12.5	14		
		id., 22°C	12 (3.5)	206 (680)	237	2.93	-	14.9	27		
		id., 30°C	12 (3.5)	64 (370)	98	2.26	-	24.0	37		
<i>Rmar</i> <sup>2</sup>	Field, 4May, 19°C	Lf, O <sub>2</sub> , 0-520 $\mu E$ ; 19°C	-	-	100	1.89	53	-	-		
	id., 23May, 23°C	id., 23°C	-	-	151	2.71	56	-	-		
	id., 24March, 8°C	id., 18°C	-	-	92	-	-	-	-		
	id., 8April, 12°C	id., 22°C	-	-	136	-	-	-	-		
	id., 24April, 14°C	id., 24°C	-	-	119	-	-	-	-		
<i>Rmar</i> <sup>3</sup>	Field, May '80, 17°C	Lf, 14°C, 17°C	14-15 (55)	-	133	0.38	284	-	-		
	id., Sept '79, 22°C	id., 22°C	14-15 (25)	-	126	0.13	561	-	-		
<i>Rmar</i> <sup>4</sup>	id., May '80, 21°C	O <sub>2</sub> comm. prod. in field	14-15 (55)	-	123	-	-	-	287		
	Exp. ponds, 11 $g\ l^{-1}$	Sf, O <sub>2</sub> , exp. pond, 14:00h	-	188 (575)	-	-	-	-	-		
<i>Rmar</i> <sup>5</sup>	id.	Sf, 14°C, exp. pond	-	197 (1150)	-	-	-	-	-		
	Field, Aug, 30°C, 34 $g\ l^{-1}$	Sf, 14°C, 0-100% sunlight, 30°C	-	154 to 258 (sat.)	-	-	-	-	-		
<i>Rmar</i> <sup>6</sup>	Field, Aug '94	Lf, DIC, 18°C	-	-	-	-	-	-	-		
		2mMC <sub>6</sub> , pH8	-	198 (350)	-	-	-	-	-		
		2mMC <sub>6</sub> , pH7	-	276 (350)	-	-	-	-	-		
<i>Rcirr</i> <sup>7</sup>	Field, winter & spring	Lf, O <sub>2</sub> , 20°C	-	-	-	-	-	-	-		
		3mMC <sub>6</sub> , pH8.5	-	103 (700)	-	-	-	-	-		
		6mMC <sub>6</sub> , pH8.5	-	144 (700)	-	-	-	-	-		
		6mMC <sub>6</sub> , pH7	-	259 (700)	-	-	-	-	-		
		28.3.89, 3mMC <sub>6</sub> , pH8	-	144 (700)	-	-	-	-	-		
		17.4.89, 3mMC <sub>6</sub> , pH8	-	42 (700)	-	-	-	-	-		
<i>Rdrep</i> <sup>8</sup>	Field, April, 7 $g\ l^{-1}$	Lf, O <sub>2</sub> , 25°C, 0-2394 $\mu E$	-	-	45	0.08	236	18	86		
	Field, March '92	Ip, O <sub>2</sub> , 22°C, 0-1800 $\mu E$	-	124 (1365)	178	1.88	95	65	55		
<i>Rdrep</i> <sup>9</sup>	id., May '92	id.	-	63 (1820)	83	0.40	210	27	101		
	20°C, 240 $\mu E$ , 7wk	Ip, O <sub>2</sub> , 20°C, 0-435 $\mu E$	16 (13.8)	142 (410)	219	0.82	268	23	29		
<i>Rdrep</i> <sup>10</sup>	id., 30°C	id., 30°C	16 (13.8)	150 (445)	277	0.75	368	54	97		
	20°C, 285 $\mu E$ , 11wk	Ip, O <sub>2</sub> , 20°C, 0-500 $\mu E$	16 (16.5)	117 (485)	140	1.59	91	26	20		
<i>Rdrep</i> <sup>11</sup>	id.	id.	16 (10.5)	175 (485)	133	1.10	119	17	17		
		id.	8 (10.3)	144 (480)	150	1.38	113	20	16		
		id., 145 $\mu E$	id.	16 (8.0)	178 (435)	193	2.94	70	30	13	
		id.	id.	16 (5.5)	194 (410)	222	1.76	127	18	11	
		id.	id.	8 (5.3)	268 (485)	346	1.49	231	14	10	
		id., 110 $\mu E$	id.	16 (6.4)	347 (435)	377	4.26	89	44	12	
		id., 55 $\mu E$	id.	16 (3.0)	661 (190)	634	7.42	139	37	6	
		<i>Rpoly</i> <sup>12</sup>	59-88 $\mu E$ , 20°C, 0 $g\ l^{-1}$	Sf, O <sub>2</sub> , 18-19°C, 0 $g\ l^{-1}$	10 (2.6)	152 (1150)	-	-	-	11	-
			id., 17 $g\ l^{-1}$	id., 17 $g\ l^{-1}$	10 (2.6)	75 (1150)	-	-	-	9	-
			id., 35 $g\ l^{-1}$	id., 35 $g\ l^{-1}$	10 (2.6)	91 (1150)	-	-	-	16	-

further when increasing the growth-PPFD above our experimental values. A trend to higher  $\alpha$  values at PP=12 h can be found again in Koch & Dawes' (1991) results, and Wetzel & Penhale (1983) reported remarkably low  $\alpha$  values for *R. maritima*, which may be related to the high light regime experienced by the plants ( $55 \text{ E m}^{-2} \text{ day}^{-1}$ ).

Dark respiration values reported by Koch & Dawes (1991) for *R. maritima* are within the range of our findings except for their intermediate photoperiod (12 h) treatment, which showed much higher R values. Respiration of *R. drepanensis* was higher under field conditions, but it strongly decreased with age (Santamaría *et al.*, in press). Respiration values reported for *R. polycarpa* (Vollebergh & Congdon, 1986) are the lowest of the table, although net photosynthesis at saturating irradiances is comparable to other reviewed values.

All LCPs reported for *R. maritima*, and those reported for *R. drepanensis* in the field are at least twice the values we report here for *R. drepanensis*. Under our lowest irradiance regime (LL4), LCP is only  $6 \mu\text{E m}^{-2} \text{ s}^{-1}$ , even lower than our dim PPFD. As we mentioned above, such LCP low values are considered typical for shade-adapted plants.

$P_m/R$  ratios calculated by Koch & Dawes (1991) show an interesting pattern:  $P_m/R$  was minimal at PP=12 h, the treatment with higher  $P_m$  and  $\alpha$  values, and maximal at PP=6 h, mainly due to differences in dark respiration rates. Our above-presented hypothesis (reduction in dark respiration under short PAPs) may also apply remarkably well to this case. Finally,  $P_m/R$  values reported for *R. drepanensis* grown under field conditions are lower than our results.

Overall, *R. maritima* does not seem to vary its photosynthetic rate at saturating irradiances when grown under different light regimes. Although information available is far from being unequivocal,  $P_m$  did not increase when *R. maritima* plants grew under increasing irradiances.

If we exclude the 6 h PP from Koch & Dawes (1991), which is a quite unrealistic photoperiod (much shorter than may be expected to occur under field conditions), photosynthetic performance of *R. maritima* is worse under long-day (PP 18 h) than under short-day conditions (PP 12 h):  $P_m$  and  $\alpha$  decreased with increasing PP. But, as dark respiration also decreased, LCP is similar under both photoperiods.

Williams & McRoy (1981) considered *R. maritima* to be a typical colonizing species, which they related to a higher maximum quantum yield, but a higher sensitivity to high irradiance and a more frequent photoinhibition. According to them, plasticity among the different populations of this species did not result in different  $P_m$  or  $\alpha$  values.

All reviewed data seem to indicate that *R. maritima* is a shade-adapted plant. Williams & McRoy's (1981) considerations indicate how the ecological role played by this species may be determined by its strategy concerning photosynthesis. Still, *R. maritima* may not be considered as an obligate shade plant, as high irradiances do not seem to result in a long-term reduction of its photosynthetic performance. This results in a considerable ecological plasticity, fitting in nicely with its cosmopolitan distribution.

Considering the above-presented results, *R. drepanensis* seems on first sight to be a obligate shade (sun intolerant) species. But photosynthetic performance reported from field, where much higher irradiances are to be expected, are not worse than those from our LL1 treatment, and none of them is inferior to the rates reported from *R. maritima*. Both species having common behaviour at moderately high irradiances, a considerably high plasticity allows *R. drepanensis* to adapt to lower light regimes. This fits well with its winter annual life-cycle and the high turbidity caused by wind-induced sediment resuspension in early spring in the shallow lakes where it occurs.

Comparison with *P. pectinatus*, a cosmopolitan species able to inhabit shallow lakes

with very low water transparency, stresses the remarkable capacity of *R. drepanensis* to adapt its photosynthetic performance to low irradiance regimes. Hootsmans & Vermaat (1994) grew *P. pectinatus* plants from Lake Veluwe (The Netherlands) at four irradiances (200, 150, 100 and 50  $\mu\text{E m}^{-2} \text{s}^{-1}$ ) and measured their photosynthetic performance after 30, 70 and 120 days of age. Irradiances and other culture conditions (clay:sand sediment, 17-20 °C temperature, 16 h photoperiod, PP=PAP) were equivalent to our LL1 to LL4 treatments. Similar to what we found for *R. drepanensis*, they reported a strong negative effect of ageing on plant photosynthesis. Contrary to us, they found the photosynthetic performance of the plants to be reduced under low light regimes. They also found increasing chlorophyll concentrations with decreasing irradiance, but 'not enough to keep photosynthetic rates unaffected'. Indeed, plants from the low irradiance levels had lower  $P_m$ ,  $P_m/R$  and  $K_m$ ; gross and net production at 200  $\mu\text{E m}^{-2} \text{s}^{-1}$  also decreased, while  $\alpha$ , LCP and  $R$  were not affected. A field experiment creating different shading levels in lake Veluwe (100%, 74%, 55% and 27% daylight conditions) gave similar results, except for  $K_m$ . All these results suggest a sun-tolerant plant strategy, with light-saturated photosynthesis and saturating irradiance increasing with increasing light regime during growth, and light-limited photosynthesis remaining unaffected at decreasing light regimes. Their results are clearly opposite to our findings for *R. drepanensis*.

As Hootsmans & Vermaat (1994) already suggest, the solution to the apparent contradiction between the lack of photosynthetic acclimation to low high levels in *P. pectinatus* and its well known ability to maintain considerable biomass levels in highly eutrophicated and turbid shallow lakes 'might be found in morphological adaptations like elongation and the availability of carbohydrate reserves in the tuber'. In contrast, *Ruppia* species, lacking a high morphological plasticity and growing from seeds, were compelled to adapt to low light levels by modifying their photosynthetic performance.

Moreover, life-cycle characteristics have played an extra role in forcing the described adaptations to low light regimes. The strict winter annual character of *R. drepanensis* explains its extreme adaptation to shade conditions inside the genus *Ruppia*. *P. pectinatus*' summer annual character in Lake Veluwe, together with its ability to survive low-light winter conditions using tuber reserves, has resulted in a relative sun-tolerant character. This may have, in our opinion, important consequences when considering different populations of cosmopolitan plants species grown under very different climatic conditions. Indeed, a good example has been recently provided to us by a population of *P. pectinatus* from Bahía Blanca (Argentina), which in a laboratory experiment comparable to ours showed decreasing  $P_m$  values with increasing light regime (Hootsmans, unpublished data), suggesting a strategy comparable to what we found here for *R. drepanensis* under similar underwater light conditions.

Still,  $P_m$  values reported for *P. pectinatus* both from the experiments of Hootsmans & Vermaat (1994) and from the bibliographical review they present were lower (higher value under light saturation is 120  $\mu\text{g O}_2 \text{g}^{-1} \text{afdw min}^{-1}$ ) than our findings for *R. drepanensis*, although in a range comparable to our LL1 treatment. Dark respiration (9 to 44  $\mu\text{g O}_2 \text{g}^{-1} \text{afdw min}^{-1}$ ) and half-saturation constant (15 to 249  $\mu\text{E m}^{-2} \text{s}^{-1}$ ) estimates were comparable to our results, whilst apparent quantum yield (0.3-1.4  $\mu\text{g O}_2 \text{g}^{-1} \text{afdw min}^{-1} \mu\text{E}^{-1} \text{m}^2 \text{s}$ ) and light compensation point (19 -100  $\mu\text{E m}^{-2} \text{s}^{-1}$ ) were lower, their higher limit being similar to our LL1 values.

It seems clear from these results that strong photosynthetic acclimation of *R. drepanensis* to low light levels, although resulting in a decreased photosynthetic performance at increasing irradiances, does not result in lower  $P_m$  values than those shown by the 'sun-

adapted' submerged macrophytes. In fact,  $P_m$  is higher for the 'shade' plants under low light conditions than for the 'sun' plants under high light conditions, and comparable for both groups under high light regimes. Indeed, the only species reviewed by Hootsmans & Vermaat (1994) from the group having a low LCP for which  $P_m$  is also shown, *M. salsugineum* (from Orr *et al.*, 1988), has a distinctively high  $P_m$  value ( $294 \mu\text{g O}_2 \text{g}^{-1} \text{afdw min}^{-1}$ ), a high  $\alpha$  ( $6.5 \mu\text{g O}_2 \text{g}^{-1} \text{afdw min}^{-1} \mu\text{E}^{-1} \text{m}^2 \text{s}$ ) and a low  $K_m$  ( $50 \mu\text{E m}^{-2} \text{s}^{-1}$ ), all similar to our LL3 values. Although shade adaptation has been regarded in terrestrial plants as an 'active' increase in light harvesting efficiency having a negative counter effect on the light saturated photosynthetic rate due to the need to restricting respiratory expenses (Boardman, 1977; Björkman, 1981), this is not the case for at least some submerged macrophytes. We do believe that this reasoning is just a consequence of considering light-limited and light-saturated photosynthesis as two independent processes, while in fact they are interconnected through the whole range of intermediate irradiances. Although maintaining the enzymatic machinery allowing for high  $P_m$  rates may seem useless under low irradiances, it is not at all as far as a highly efficient light harvesting system allows the carboxylation process to work as fast as could otherwise be reached under high irradiances. Indeed,  $K_m$  values in our experiment lay under the experimental PPFD in all treatments except SS2 and LL4, indicating photosynthetic performance at sub-saturating irradiances to influence net carbon balance even at growth-PPFDs as low as  $110 \mu\text{E m}^{-2} \text{s}^{-1}$ .

Concluding, photosynthetic adaptation to low light regimes in submerged plants appeared to involve both a higher light harvesting efficiency and higher photosynthetic rates at saturating irradiances. Although all aquatic plants probably show some degree of shade adaptation, different strategies have been found between three species of shallow brackish water macrophytes, *R. drepanensis* and *P. pectinatus* being the extremes of a shadow-sun adaptation gradient with *R. maritima* in between. These differences in strategy may be related to both plant morphology and life-cycle, the latter providing a framework to understand differences in photosynthetic performance between different populations or ecotypes of the same species.

A final word is warranted on the use of PI curves to extrapolate the behaviour and distribution of submerged plant populations in the field, as e.g. in Lipkin *et al.* (1986) or in most of the models computing plant population dynamics. Adaptive plasticity in plant response and the ageing effect are still relatively unstudied, but this and other works provide evidence of strong modifications on photosynthetic performance under different light climates, its pattern varying not only among species but quite possibly among different populations. A further effort in clarifying which kind of response is to be expected in a given plant population would be very rewarding, as some clear trends have already begun to emerge. At the present level of knowledge, precaution is needed when using plant growth models based on PI curves determined under a single plant light history: extrapolation to any other irradiance may lead to erroneous conclusions. As shown here, increasing irradiance may not always result in increasing photosynthetic rates.

#### 4.3. The influence of chlorophyll concentration on photosynthetic light acclimation

Changes in chlorophyll concentration with changing light climate were clearly correlated with changes in photosynthetic performance. Chlorophyll-*a* and -*b* concentration increased with decreasing PPFD and PAP, although as for  $\alpha$  and  $R$ , the effects of LP and PPFD were not just additive. The same hypothesis presented above for the effect of dark period on  $\alpha$  through

the necessity to reduce dark respiration rates may explain the similar leaf chlorophyll content found in plants from the LL2, LS2 and SS2 treatments. On the other hand, acclimation to dim light may probably explain why the plants LS1 showed higher leaf chlorophyll content than those from SS1, and it is thus reflected in higher  $\alpha$  values.

Chlorophyll-related PI curves may help to understand the extent and meaning of the positive correlation between photosynthesis and chlorophyll content. Chlorophyll content successfully explained variation in maximum quantum yield ( $\alpha$ ), dark respiration (R) and  $P_{\infty}$  with decreasing PPFD (experiment 2). Maximum gross production ( $P_m$ ) still increased with decreasing PPFD, although part of its variation was removed when correcting for chlorophyll-*a* content. This is consistent with the view of the light harvesting system as being mainly influenced by chlorophyll content, maximum gross production being further influenced by enzymatic efficiency of carbon fixation. The link between increasing chlorophyll content and increasing dark respiration clearly shows the expenses of maintaining an efficient light-harvesting system.

Nevertheless, biomass-related  $\alpha$  values should show a saturating increase along increasing chlorophyll concentration, especially at the high concentrations found in this experiment, if chlorophyll would be the only factor responsible for such an increase: as discussed in Gabrielsen (1948), a direct application of Beer's law results in maximum  $\alpha$  values at concentrations above 4 to 5 mg Chl.(*a+b*) dm<sup>-2</sup> for data from terrestrial plants. Contrary to this, our  $\alpha$  values increased exponentially with increasing chlorophyll-*a* values. As we did not express chlorophyll concentration on a leaf surface basis, such exponential increase may be due to differences in leaf morphology, although differences in morphology of the photosynthetic organelles may have also played a role. Still, Hootsmans & Vermaat (1994) reported a linear increase in  $\alpha$  with increasing Chl.(*a+b*) expressed on a leaf area basis; which can be explained by the rather low values (1 mg dm<sup>-2</sup>) they report, and Drew (1979) also found a linear relation between Chl.(*a+b*) content and photosynthesis up to 6 mg dm<sup>-2</sup> in leaf fragments of *Phyllospadix torreyi* S. Watson and *Posidonia oceanica* (L.) Delille. It seems clear that increasing total chlorophyll is not the only factor resulting in an enhanced apparent quantum yield, but it covaries with other mechanisms of acclimation.

Although the photosynthetic response to different light periods is more complex, as relative importance of dark respiration for plant daily production is also modified, chlorophyll successfully explained differences in  $P_{\infty}$  between different light regimes. Combinations of low PPFD and short PP resulted in significantly higher  $P_m$ , while  $\alpha$  decreased with decreasing PAP and R decreased with decreasing PP, all on a chlorophyll-related basis. These results fit well with what has been stated above, increases in  $P_m$  reflecting the negative effect of high daily irradiances on the efficiency of enzymatic carbon fixation, and decreases in  $\alpha$  and R with decreasing light period reflecting the correlation between a reduced dark respiration as responding to longer nights and a reduced efficiency of the light harvesting system.

Comparison with chlorophyll concentration reported from other *Ruppia* species (Table 7) and from other submerged macrophytes stresses again the high capability for photosynthetic acclimation found in *R. drepanensis*. Chlorophyll-*a* concentrations were 3 to 6 times higher than reported for *R. maritima* grown under comparable light regimes (Koch & Dawes, 1991). Values reported from the field for *R. maritima* were even lower. Leaf Chl.(*a+b*) content of *R. drepanensis* plants from the Doñana brackish marsh in April were comparable to our present findings, but they decreased to much lower values in May. A similar set of values was also reported by Vermaat & Hootsmans (1994b) for



TABLE 7: Comparison of chlorophyll content and total plant biomass. Chlorophyll concentration in mg g<sup>-1</sup> afdw. Total plant biomass in mg afdw. Rest of the legend and references as in Table 6, except <sup>13</sup>Thursby, 1984 and <sup>14</sup>Santamaría *et al.*, 1994b. Conversion factors used: afdw=90%dw=16%fw (leaves or aboveground biomass), afdw=80%dw (aboveground + belowground total biomass).

Species (refes)	Growth conditions	PP (EPAR)	Chl.(a+b)	Chl-a	Chl-a/Chl-b	Plant biomass	RGR plant biomass(day <sup>-1</sup> )	RGR number of shoots (day <sup>-1</sup> )
<i>Rmar</i> <sup>1</sup>	20gl <sup>-1</sup> ,22°C,90µE,9weeks							
	Florida population	6 (1.9)	-	2.0	-	74.8	.068	-
		12 (3.9)	-	2.3	-	127.2	.077	-
		18 (5.8)	-	1.5	-	165.1	.081	-
	Florida,80µE,14°C	12 (3.5)	-	3.4	-	54.3	.063	-
	id.,22°C	12 (3.5)	-	2.9	-	191.8	.083	-
	id.,30°C	12 (3.5)	-	2.5	-	147.9	.079	-
	New Carolina popul.	6 (1.9)	-	3.0	-	102.5	.073	-
		12 (3.9)	-	2.5	-	229.9	.086	-
		18 (5.8)	-	1.9	-	302.7	.091	-
	NewCarol,80µE,14°C	12 (3.5)	-	5.0	-	95.1	.072	-
	id.,22°C	12 (3.5)	-	4.4	-	349.4	.093	-
	id.,30°C	12 (3.5)	-	3.7	-	276.3	.089	-
	<i>Rmar</i> <sup>2</sup>	Field,4May,19°C	-	-	0.96	2.81	-	-
id.,23May,23°C		-	-	1.26	3.38	-	-	-
<i>Rmar</i> <sup>13</sup>	20°C,450µE,3weeks	16 (25.9)	-	-	-	72.0	.088	.147
	id.,400µE	16 (23.0)	-	-	-	70.8	.088	.151
	id.,350µE	16 (20.2)	-	-	-	48.0	.080	.133
	id.,300µE	16 (17.3)	-	-	-	48.0	.080	.132
	id.,250µE	16 (14.4)	-	-	-	32.0	.072	.113
	id.,200µE	16 (11.5)	-	-	-	33.2	.072	.121
	id.,150µE	16 (8.6)	-	-	-	24.6	.066	.092
	id.,100µE	16 (5.8)	-	-	-	12.9	.033	.068
	id.,50µE	16 (2.9)	-	-	-	9.2	.046	.056
<i>Rdrep</i> <sup>8</sup>	Field,April,7gl <sup>-1</sup>	-	1.52	1.20	3.69	-	-	-
<i>Rdrep</i> <sup>10</sup>	20°C,240µE,7weeks	16 (13.8)	-	-	-	164.8	.104	-
	id.,30°C	16 (13.8)	-	-	-	114.2	.097	-
<i>Rdrep</i> <sup>11</sup>	20°C,285µE,11weeks	16 (16.5)	13.12	7.77	1.45	258.6	.072	.118
	id.	16 (10.5)	16.86	9.89	1.42	291.8	.074	.124
	id.	10 (10.3)	14.65	8.08	1.23	257.4	.072	.112
	id.,145µE	16 (8.0)	17.70	9.72	1.22	122.6	.062	.072
	id.	16 (5.5)	17.95	9.96	1.24	177.3	.067	.085
	id.	10 (5.3)	18.42	9.66	1.10	140.7	.064	.075
	id.,110µE	16 (6.4)	19.37	10.16	1.10	81.0	.057	.074
<i>Rdrep</i> <sup>14</sup>	id.,55µE	16 (3.0)	19.03	9.63	1.02	33.7	.046	.041
	Field,April,20°C;loc.1	-	10.75	8.00	2.84	-	-	-
	id.;loc.2	-	11.19	8.12	2.65	-	-	-
	id.;loc.3	-	16.06	12.00	2.95	-	-	-
	Field,May,25-30°C;loc.1	-	2.14	1.49	2.30	-	-	-
	id.;loc.2	-	2.46	1.81	2.81	-	-	-
	id.;loc.3	-	2.05	1.34	1.88	-	-	-

(Santamaría *et al.*, 1994a). Low values from García *et al.* (1991) are probably related with age, although they attribute them to the effect of high irradiances.

Hootsmans & Vermaat (1991) reported a strong decrease in total chlorophyll between 30 and 70 days of age in *P. pectinatus*. Still, the total chlorophyll leaf content increased with decreasing irradiance during the first two months, their values from 30 days (7-11 mg Chl.(a+b) g<sup>-1</sup> afdw) being about half our values from 77 days. They attributed this to the investment in morphological adaptation, 'low light history levels leading to a strong elongation of the plant and a lower leaf ratio', the lack of a strong increase in chlorophyll content per total aboveground biomass as adaptation to low irradiances, in agreement with Spence & Chrystal (1970) findings for *P. polygonifolius* Pourr. and *P. obtusifolius* Mert. &

*P. pectinatus* plants after 55 days of growth at 50, 100 and 200  $\mu\text{E m}^{-2} \text{s}^{-1}$  (4, 6 and 9 mg Chl.(a+b)  $\text{g}^{-1}$  afdw, respectively). Leaf Chl.(a+b) content reported by Peñuelas *et al.* (1988) in *P. pectinatus* (4.7 mg  $\text{g}^{-1}$  dw) was 1/3 our lowest 77-day value, while Best & Dassen (1987) found values comparable to those from our 285 and 145  $\mu\text{E m}^{-2} \text{s}^{-1}$  treatments in top sections of *Ceratophyllum demersum* (16.5 mg Chl.(a+b)  $\text{g}^{-1}$  afdw).

Increases in total chlorophyll concentration with decreasing PPFD may be interpreted as a positive adaptation to low light regimes, or as due to adverse effect of high irradiances. Although the latter may have probably played a role, high chlorophyll concentrations found in *R. drepanensis* as compared with other submerged macrophytes suggest positive light acclimation as a strong basis to explain increased chlorophyll concentrations found under low PPFD regimes.

Increasing chlorophyll-*b* fraction has been reported as a widespread response to low irradiances in terrestrial plants (as reviewed in Björkman, 1981 and Boardman, 1977), although results are somewhat conflicting in aquatic macrophytes. Hootsmans & Vermaat (1994) found no change in chlorophyll-*b* fraction with light history and age in *P. pectinatus*, as also found by Vermaat & Sand-Jensen (1987) for *Ulva lactuca* L. Best & Dassen (1987) found slightly increasing chlorophyll-*b* fractions with increasing depth in *Ceratophyllum demersum*. In two studies concerning seagrasses, chlorophyll-*b* fraction was affected in some species, increasing with decreasing light levels in Wiginton & McMillan (1979) and decreasing from winter to summer in Jiménez *et al.* (1987), but it remained unaffected in some other species. We found the chlorophyll-*b* fraction to be affected both by light regime and age, increasing significantly with decreasing light regime. It appears as if different species show different capabilities to adapt their chlorophyll-*b* concentration to low light regimes. Björkman's (1981) suggested that a higher proportion of chlorophyll-*b* reflects an adaptation to variations in the light spectrum, especially to shading by the plant canopy, which is possibly of great importance for plants to maintain their photosynthetic performance under phytoplankton and periphyton shading. Again, dependence of *P. pectinatus* on morphological adaptation (i.e., rapid elongation) to overcome such events may help to explain its different response as compared with *R. drepanensis*.

Indeed, the Chl-*b* fraction found in our cultured *R. drepanensis* plants was much higher than those found in field both for *R. drepanensis* and *R. maritima*. Reviewed field data also suggest no age effect of Chl-*b* fraction, consistent with our finding of a significant age effect only at short light periods. Most of the references cited in the paragraph above show Chl-*b*/Chl.(a+b) values lower than the ones we found here, including Hootsmans & Vermaat (1994) (0.15-0.3, in *P. pectinatus*). Only Wiginton & McMillan (1979) (0.3-0.4, in different species of tropical seagrasses) and Vermaat & Sand-Jensen (1987) (0.4, in *U. lactuca*) found values comparable to ours, although still considerably lower than in our low PPFD treatments. Considering the low LCP exhibited by *U. lactuca*, it does not seem unlikely that a higher capability for maintaining high Chl-*b* fractions is linked with strategies of shade adaptation, as reported for terrestrial plants.

#### 4.4. Effect of light climate on plant morphology and growth

Following a brief summary of the most relevant results, *R. drepanensis* morphological response to varying light regimes is discussed. Then, growth rates derived from morphological variables and from biomass, as well as those estimated from net

photosynthesis data, are compared. As our biomass-derived growth rates did not agree with those calculated from net photosynthesis measurements, a discussion on the factors which may explain such differences is also presented. Finally, comparisons between biomass yields found in this experiment and those reviewed from other aquatic and terrestrial plant species are used to characterise the light acclimation response of *R. drepanensis*.

Long-day regimes (LL) resulted in a significant trend to produce less and shorter leaves, plants grown under dim regimes (LS) showing the highest number of leaves and the longest leaf lengths. Although plant biomass was significantly affected by light period, the values were very similar for all three regimes (no individual comparison resulted in significant differences). Plants grown under dim regimes showed the highest biomass yield.

On the other hand, irradiance (PPFD) affected plant morphometry and biomass yield independently of light period (interaction was non-significant in all cases), a 50% reduction in the PPFD resulting in about 50% less shoots, bundles and leaves per plant after 11 weeks of growth, although leaves were 30% longer (experiment 1). Plants grown at  $145 \mu\text{E m}^{-2} \text{s}^{-1}$  showed also a 50% reduction in biomass yield (as compared to plants grown at  $285 \mu\text{E m}^{-2} \text{s}^{-1}$ ) in the 11th week. This difference became less in the 16th week.

Plants from experiment 2 showed a further decrease in total number of bundles, shoots and leaves per plant with decreasing PPFD. Length of the longest leaf also increased with decreasing PPFD, although this trend was broken at the lowest PPFD (LL4).

#### 4.4.1. Plant morphology

Increasing leaf length with shorter PAP and lower PPFD conditions is probably the only mechanism of morphological adaptation that *R. drepanensis* may use in the early stages of its development, when only horizontal basal shoots are produced ('phase of horizontal propagation', Verhoeven, 1979). When low light regimes are caused by high water turbidity, such a response allows to put an increasing proportion of the leaf at the most irradiated places. As reported by Joanen & Glasgow (1965), once plants have reached the water surface, influence of turbidity is almost completely eliminated. Indeed, maximum leaf length achieved values similar to maximum plant length of *P. pectinatus* (maximum asymptotic value from the logistic fit  $K=46$  cm for LL3, versus a  $K$  value of 45 cm reported for *P. pectinatus* grown at 100 and  $50 \mu\text{E m}^{-2} \text{s}^{-1}$  in Vermaat & Hootsmans, 1994b), a species showing vertical shoot growth from its early development. These lengths may be clearly sufficient for very shallow wetlands as those inhabited by *R. drepanensis* (rarely deeper than 50 cm), although increased leaf length is still not as efficient as an increased shoot length because a considerable part of the photosynthetically active tissue remains in zone with low irradiance.

It seems also clear that potential for morphological adaptation has a limit at very low PPFD, in the case of increasing leaf length when reaching  $55 \mu\text{E m}^{-2} \text{s}^{-1}$ . Similarly, plant length did not increase any longer with decreasing PPFD for *P. pectinatus* between 100 and  $50 \mu\text{E m}^{-2} \text{s}^{-1}$  (Vermaat & Hootsmans, 1994b).

Variation in the length of the longest leaf (LGL) after 35 days of growth was well explained by a linear regression on  $\Sigma\text{PAR}$  ( $r^2=0.95$ ; LL4 was taken out). Thus, morphological response to light climate seems to depend more on the total daily irradiance than on the instantaneous PPFD. This confirms the hypothesis formulated by Vermaat & Hootsmans (1994b), who proposed 'stimulation of elongation to be effectuated by the integral diel irradiance received by the plant'.

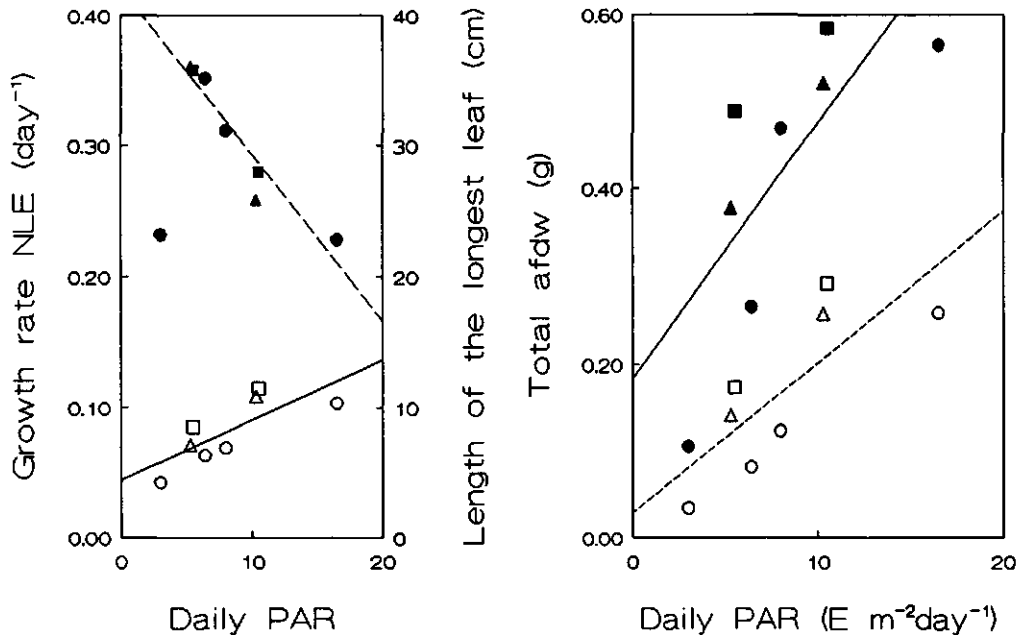


FIG.25: Effect of the daily irradiance (total PAR received per day) on the morphometry (left) and total plant afdw (right) of *Ruppia drepanensis*. Circles: LL treatments. Squares: LS treatments. Triangles: SS treatments. Linear fits are also indicated.

Left graph: Empty symbols: exponential growth rate ( $r$ ) of the variable 'total number of leaves per plant' (NLE). Filled symbols: length of the longest leaf after 35 days of growth (LGL35). Note that the linear fit for LGL35 left out the datapoint corresponding to the LL4 treatment.

Right graph: Empty symbols: 11th week. Filled symbols: 16th week.

After 5 to 7 weeks of growth, plants from all treatments began to produce vertical shoots ('phase of vertical propagation', Verhoeven, 1979). The percentage of leaves allocated in vertical shoots was similar for all treatments in the 11th week, when more than 40 % of the leaves were already allocated in such shoots. In conclusion, a morphological response does not play an important role in the acclimation of *R. drepanensis* to short PAP conditions, and only a limited one as responding to low PPFD conditions.

#### 4.4.2. Plant growth

Total number of leaves (NLE) and bundles (NBU) are directly related to plant primary production, and their pattern of variation closely follows variation in total plant afdw. For all three variables, although relatively good linear fits were found with total daily irradiance ( $\Sigma$ PAR) as independent variable (e.g.  $r^2=0.77$  for NLE), all points from the long-day treatments are situated below the average regression line, while all points coming from the LS and SS treatments are above it (Fig.25). This indicates that, under our present experimental conditions, plant growth did not increase further when increasing the total daily PAR by extending the PAP. In fact, supplying a few extra hours of dim light was the only

TABLE 8: Daily growth rates estimated from biomass yields and from oxygen production/consumption measurements in *R. drepanensis* plants. 'Overall' parameters refer to a calculation assuming the biomass increase to follow a logistic curve. *r*: exponential growth rate ( $\text{day}^{-1}$ ), *K*: maximum asymptotic value (mg). 'Instantaneous' growth rates were obtained by calculating the slope of the tangent to the logistic curve for the specified day. 'Exponential' RGR based on results from this study are averages of the 11th and the 16th week RGR estimates (actual estimates between brackets). RGR estimates from photosynthesis measurements assume a C:afdw ratio of 0.45, similar to Lipkin *et al.*, 1986. All relative growth rates in  $\text{day}^{-1}$ . Rest of the legend and references as in Table 6, except: <sup>15</sup> Lipkin *et al.*, 1986; <sup>16</sup> Vermaat & Sand-Jensen, 1987; *Cdem*=*Ceratophyllum demersum*, *Grac*=*Gracilaria* sp., *Ulac*=*Ulva lactuca*.

Species (refer.)	PPFD (EPAR)	Biomass-based relative growth rate (RGR) estimates:				Exponential	Photosynthesis-based RGR estimates:		
		Overall logistic <i>r</i>	<i>K</i>	Instantaneous logistic 77th day	Instantaneous logistic 112th day		77th day	112th day	Other
<i>Rdrep</i> <sup>11</sup>	285 (16.5)	.097	588	.054	.004	.042 (.072-.012)	.054	.0138	-
	285 (10.5)	.099	604	.050	.003	.047 (.074-.020)	.029	.0007	-
	285 (10.3)	.098	539	.050	.003	.046 (.072-.020)	.030	.0003	-
	145 (8.0)	.096	520	.071	.010	.050 (.062-.038)	.065	.0065	-
	145 (5.5)	.100	515	.064	.006	.048 (.067-.029)	.041	.0078	-
	145 (5.3)	.105	395	.091	.004	.046 (.064-.028)	.052	.0111	-
	110 (6.4)	.098	287	.070	.007	.045 (.057-.034)	.115	.0167	-
	55 (3.0)	.102	112	.071	.006	.039 (.046-.032)	.128	.0350	-
	<i>Cdem</i> <sup>15</sup>	20 (1.1)	-	-	-	.005-.007	-	-	.004-.032
<i>Grac</i> <sup>15</sup>	100 (5.5)	-	-	-	.071-.078	-	-	.008-.079	
<i>Ulac</i> <sup>16</sup>	2 (0.2)	-	-	-	.007	-	-	.010	
	8 (0.7)	-	-	-	.018	-	-	.030	
	50 (4.3)	-	-	-	.085	-	-	.084	

effective way to increase plant primary production under equivalent PPFs.

This seems to be in contradiction with the results from the photosynthesis measurements. Our estimations of the relative growth rate (RGR) based on the PI curves measured in the 11th week show a minimum for the dim treatments, and a maximum for the long-day ones ( $PQ=1$ ; a carbon/afdw ratio of 0.45 was chosen, based on C content values reported in Twilley *et al.*, 1986 and in agreement with Mitchell, 1989). Calculated growth rates for biomass increase show, on the contrary maximum values for the dim treatment, equivalent values for LL1 and SS1, and lower values for LL2 than for SS2 (Tables 6 and 8; exponential RGRs from Table 8 are only presented for comparison purposes, as the calculation method is not the most appropriate).

Plant growth did not follow a purely exponential curve, but a logistic one. Thus, after the inflexion point of the curve had been reached, instantaneous RGR (for our purposes referring to daily growth rate) decreased with time. While overall RGR was determined by biomass increase during the whole experimental period, photosynthesis-estimated RGR may only be compared or used in the small time region around the time for which it was calculated.

Two reasons may explain the continuous change in daily growth rate, even in the absence of supplementary 'limiting factors': first, photosynthetic performance at a given moment is dependent on an overall dynamic acclimation to light conditions, likely to be steadily changing as both the amount of photosynthate and the amount of light perceived by the plant are likely to further modify plant photosynthetic apparatus. Second, age has strong effects on plant photosynthesis (see below; also in Hootsmans & Vermaat, 1994), a process also under the influence of plant productivity as the amount of newly formed, young tissue depends on it. Thus, plant photosynthetic performance at a given moment is a dynamic equilibrium between an optimizing tendency due to growth and light adaptation, and a trend to deterioration due to ageing.

Instantaneous RGR (IRGR) may be calculated from the derivative of the logistic curve for a given time, divided by the biomass value at that moment. Our estimated IRGR for the 77th day, at which photosynthetic performance was measured, follow in part the same trend as the RGR estimated from the PI curves: IRGR is lower for the dim treatment (Table 8). Moreover, our calculated IRGR shows that photosynthesis-calculated RGR overestimate biomass production under low PPFD conditions, and underestimate it under short PAP, both by a factor 2.

Other data reviewed in Table 8 followed the same pattern. Estimations of growth rate of *Ceratophyllum demersum* L. grown at  $20 \mu\text{E m}^{-2} \text{s}^{-1}$  (Lipkin *et al.*, 1986) using  $\text{O}_2$  and DIC methods yielded RGR estimates 2 to 6 times higher than measured from weekly biomass increase assuming exponential growth, while  $^{14}\text{C}$  method yielded RGR estimates equal to or double than those calculated from biomass increase. In the same paper, photosynthetic RGR estimates from *Gracilaria* plants grown at  $100 \mu\text{E m}^{-2} \text{s}^{-1}$  yielded results comparable to measured RGR for the  $\text{O}_2$  and DIC method, and 3 to 7 fold underestimations when using the  $^{14}\text{C}$  method. On the other hand, Vermaat & Sand-Jensen (1987) estimated *Ulva lactuca* RGR from DIC measurements of photosynthesis performance, obtaining estimations equal to actually measured biomass increases for plants grown at  $50 \mu\text{E m}^{-2} \text{s}^{-1}$ , and quite comparable values (about 2/3 of the actual growth rates) for plants grown at 2 and  $8 \mu\text{E m}^{-2} \text{s}^{-1}$ .

Discrepancy between photosynthesis and growth may be due to uncertainties in converting photosynthetic oxygen exchange to cell growth (e.g. variable PQ and RQ ratios; Vermaat & Sand-Jensen, 1987). Indeed, Best & Dassen (1987) found seasonal variation in PQ for *Ceratophyllum demersum* L. to be 0.56-1.76 (average 1.12), quite comparable to the range of variation displayed in Table 8. As concluded by Vermaat & Sand-Jensen (1987), close similarity between RGR values estimated from photosynthesis data and calculated from biomass increase for our LL1 treatment indicate a close balance between the photosynthetic process and growth rate under these precise conditions. Lambers & Rychter (1990) suggested that variation in plant chemical composition is a factor affecting the efficiency with which carbohydrates are converted into biomass. Plant biomass allocation pattern, the cost involved in maintenance processes such as the turnover of proteins and the specific costs for ion uptake are also mentioned as important factors responsible for variation in respiration as dependent on environmental conditions. Differences in plant composition, reflected e.g. in total P and total N content (which increased with decreasing PPFD) and probably associated with the increased investment in pigments, pigment-bound proteins and carbon-fixation enzymes which is to be expected at lower irradiances on the basis of the observed photosynthetic performance, may explain the subsequent overestimation of RGR based on a PQ of 1, as e.g. energetic cost associated to protein synthesis is about 2.5 higher than the one associated to carbohydrate synthesis (Lambers & Rychter, 1990). Associated protein turnover and the necessary increase in nitrogen uptake are also expected to increase the energy expenses, as reflected in the dark respiration rates, which indeed increased with decreasing irradiance.

The effect of LP on plant metabolism is probably more complex. The reduced dark respiration observed under short PAP, such that R did not increase with decreasing PPFD under the LS and SS regimes, reflects in our opinion the savings of the plants as a response to longer dark periods (see Section 4.2.2.). Restricting the protein content associated with the light harvesting system may have been the mechanism used to achieve such a reduction in dark respiration, as the apparent quantum yield was lower than expected while leaf chlorophyll content was higher. Still, total N content was also higher for plants grown under

decreasing light period (LL < LS < SS). In any case, the strategy of plants grown under short light periods seems to be opposite to those grown under low PPFD, the reduced light harvesting efficiency being balanced by reduced metabolic expenses in such a way that overall RGR is comparable or slightly higher than the RGR of plants grown under long-day conditions.

Considering the error associated with all the conversion factors used to estimate the RGR from photosynthesis, our estimates seem reasonably good. Nevertheless, they reflect the risk of directly extrapolating photosynthesis result to predictions of growth dynamics: together with changes in photosynthetic response due to light acclimation and ageing, PQ and RQ coefficients are also likely to change along the acclimation process following changes in protein content and metabolic activity.

#### 4.4.3. Biomass yield: comparison with literature data

Comparison with literature data shows *R. maritima* growth rate and biomass yield to be quite comparable to *R. drepanensis* when both are grown under similar conditions (Table 6). Moreover, the effect of different PPFD regimes under long-day conditions on biomass and total number of shoots of *R. maritima* as reported by Thursby (1984) is very similar to our results, once data are expressed as RGR. He reported both biomass and number of shoots to increase linearly with increasing PPFD in the range 50-450  $\mu\text{E m}^{-2} \text{s}^{-1}$ . After recalculation of his results, we obtained a good linear fit using an exponential RGR based on the total biomass ( $\text{RGR} = 1.03 * 10^{-4} * \text{PPFD} + 0.046$ ,  $r^2=0.96$ ). A similar calculation using our data from the 77th day gave a remarkably similar fit ( $\text{RGR} = 1.05 * 10^{-4} * \text{PPFD} + 0.043$ ,  $r^2=0.92$ ). Thus, in spite of their differences in photosynthetic acclimation, both species seem to show a remarkably similar growth response to decreasing light regimes.

Values reported from Vermaat & Hootsmans (1994b) show an even smaller effect of decreasing PPFD on plant biomass yield and RGR: a decrease in irradiance from 200 to 100 and 50  $\mu\text{E m}^{-2} \text{s}^{-1}$  resulted in a 2-fold decrease in biomass yield after 55 days of growth. Indeed, exponential RGRs calculated from these biomass yield data are almost identical (0.086, 0.091 and 0.097 respectively).

Shade-tolerant species have a higher capacity to maintain its growth rate with decreasing irradiance. Björkman (1981) reviewed RGRs of sun and shade species grown at decreasing daily irradiances ( $\Sigma\text{PAR}=2, 4$  and  $10 \text{ E m}^{-2} \text{ day}^{-1}$ , comparable to 50, 100 and 200  $\mu\text{E m}^{-2} \text{ s}^{-1}$  under a 16 h PP), reporting RGR to be 0.06, 0.09 and  $0.10 \text{ day}^{-1}$  for the shade-tolerant *Impatiens parviflora* Dc., and 0.01, 0.05 and 0.09 for the shade-intolerant *Helianthus annuus* L. In comparison with these results, Vermaat & Hootsmans (1994b) concluded that *P. pectinatus* can be seen as a comparatively shade-tolerant species in terms of maintenance of RGR, and that most aquatic macrophytes may be postulated to be comparatively shade-tolerant when considering the similarity in elongation rates under low irradiances and the relative low irradiances characteristic of the aquatic environments. Still, they point out, plants restricted to open shallow waters may be a possible exception.

*R. drepanensis* and *R. maritima* may be also considered as shade-tolerant species regarding their RGR under low irradiances. But an important difference is that *R. drepanensis*, contrary to all other species referred in Vermaat & Hootsmans (1994b), does not base its strategy for tolerating low irradiances in a strong elongation of the vegetative apparatus, but in an enhanced photosynthetic performance. A similar RGR is attained, but the limited morphological response is expected to restrict *R. drepanensis* to shallower water

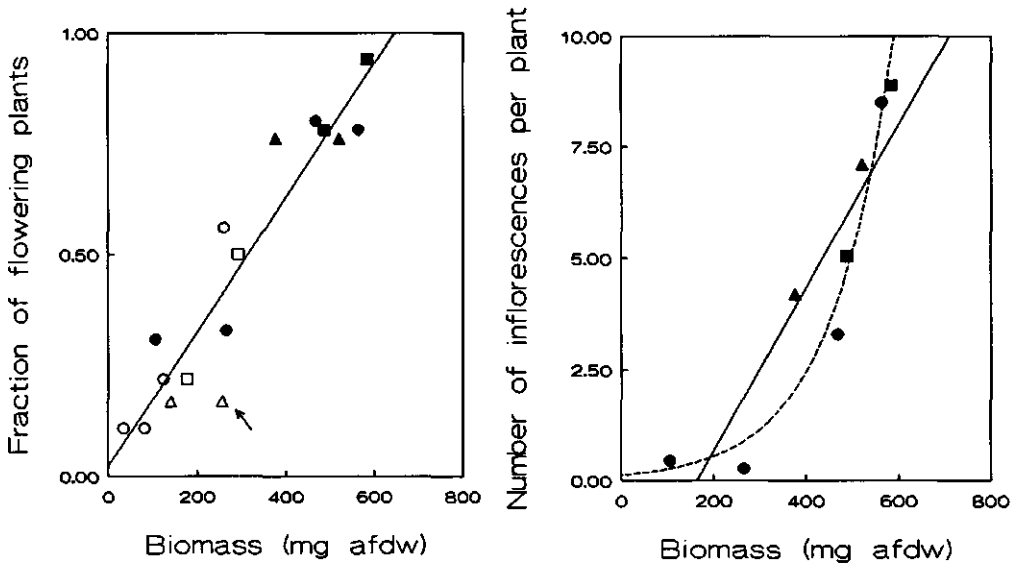


FIG.26: Flower induction (as fraction of plants with flowers) and flower abundance (as number of flowers per plant) versus individual plant biomass. Linear and exponential fits are indicated. Datapoint symbols as in Fig.25 (right graph). Arrow indicates the SS1 treatment.

bodies, and extreme photosynthetic adaptation seems to have resulted in a sun-intolerant species, adequately fitted to a winter-annual life-cycle but probably unable to develop a summer-annual cycle in more permanent wetlands. *R. drepanensis* distribution, limited to shallow, Western Mediterranean temporary wetlands, is clearly correlated with its particular photosynthetic and morphological characteristics.

#### 4.5. Effect of light climate on reproduction

Both short-day conditions and reduced PPFD regimes resulted in a postponed flower initiation and a lower flower production (lower number of inflorescences per plant). Also seed production was strongly affected both by PPFD and by light period, short PAP regimes (LS and SS) yielding less than half the seed biomass as under long-day regimes, and low irradiances resulting in an 80% reduction in seed biomass yield.

Regarding the effect of PP on flower induction, *R. drepanensis* seems to be a quantitative long-day plant, flowering being accelerated by long-days (Salisbury, 1981). This is in agreement with the winter-annual character of *R. drepanensis*, increasing photoperiod inducing flowering and reproduction before the waterbody dries in late spring. Decreased seed yield under short PP seems to be just the result of a postponed flowering, as the average number of inflorescences per plant was not significantly different in the 16th week.

PAP did not significantly affect flower induction, which was almost simultaneous for LL1 and LS1. Flower production not being significantly affected by PAP, we have to propose differences in pollination or in seed growth maintenance to explain differences in



seed yield between LL1 and LS1. Although resource availability, related with both plant biomass and tissue nutrient concentration, can influence seed production (Titus & Hoover, 1990), both effects may be rejected as plants from the LS1 treatment showed higher nutrient tissue content and higher biomass than LL1. The effects of a restricted metabolism as discussed for photosynthesis and growth could be at the basis of the lower seed yield in LS1, as seed yield is comparable to SS1 in spite of the postponed flowering of the latter treatment. Apparently, differences in photosynthate allocation allow plants grown under short PAP to maintain a high growth rate, but they cannot afford other energy expenses, either consisting of building up an efficient light harvesting system or in maintaining the production of a high amount of seeds.

A light period effect was clearly manifested only at the highest irradiance. At low PPFs, flowering is equally postponed under all three light periods. Besides the delay in flowering found for SS1, differences in flower induction and in flower abundance are well explained by plant biomass (Fig.26). Linear regression yielded very good fits for both variables on plant biomass ( $r^2$  was 0.93 and 0.91 respectively), and an exponential equation resulted in an equally good fit for flower abundance ( $r^2$  0.906). These results are in agreement with Titus & Hoover (1991), who found a linear increase of the percentage of flowering plants with increasing biomass in *Vallisneria americana* Michx., 100 % being reached above 2.0 g dry weight. Nevertheless, saturation of flower induction was not clearly reached in our data, nor was 100% induction reached in any treatment. Although Titus & Hoover (1991) reported biomass in a controlled greenhouse experiment to be 'a far stronger correlate of the occurrence of flowering... than for a natural population', they still defended the use of the 'size threshold' concept for field plants, paying attention to differences in nutrient availability as a possible interfering factor.

Comparatively, the size effect seems more important in affecting flowering than the moderate acceleration in flowering due to long photoperiods. Unfortunately, studies concerning flower induction in aquatic plants are really scarce, as noted by Sculthorpe (1967). Dawson (1980) found little influence of photoperiod on flowering of *Ranunculus penicillatus* (Dum.) Bab. var. *calcareus* (Butcher) Cook. Titus & Hoover (1991), reinterpreting also data from Szemeja (1987) and Farmer & Spence (1987) on flowering of *Lobelia dortmanna*, suggest that 'daylength and temperature, if they are at all important, interact with other factors to determine when flowers will appear'.

As expected from the biomass dependence of flower initiation and abundance, and from the resource dependence of seed filling, variability in seed production was also well explained by plant biomass, an exponential fit resulting in  $r^2$  values of 0.952 and 0.873 for seed number and seed biomass respectively (Fig.27, left). Still, plants grown under dim conditions show lower seed production than predicted from this relation (biomass is slightly higher in LS1 than in LL1, while seed production is much lower), showing that PAP may modify the plant biomass effect. Again, the dependence of seed production on biomass did not saturate for our range of datapoints.

When considering all these results, restricted light climate both in the form of a lower PPF or a shorter PAP seems to have a much more dramatic effect on long-term population survival than may be expected on the basis of photosynthesis or vegetative growth alone. While plants grown at very low irradiances showed an enhanced photosynthetic performance, and plants grown under short PAP showed an increased growth and biomass yield, the strong reduction in seed yield may lead to a strong decline of *R. drepanensis* populations following any significant decrease in light climate (Fig.27, right).

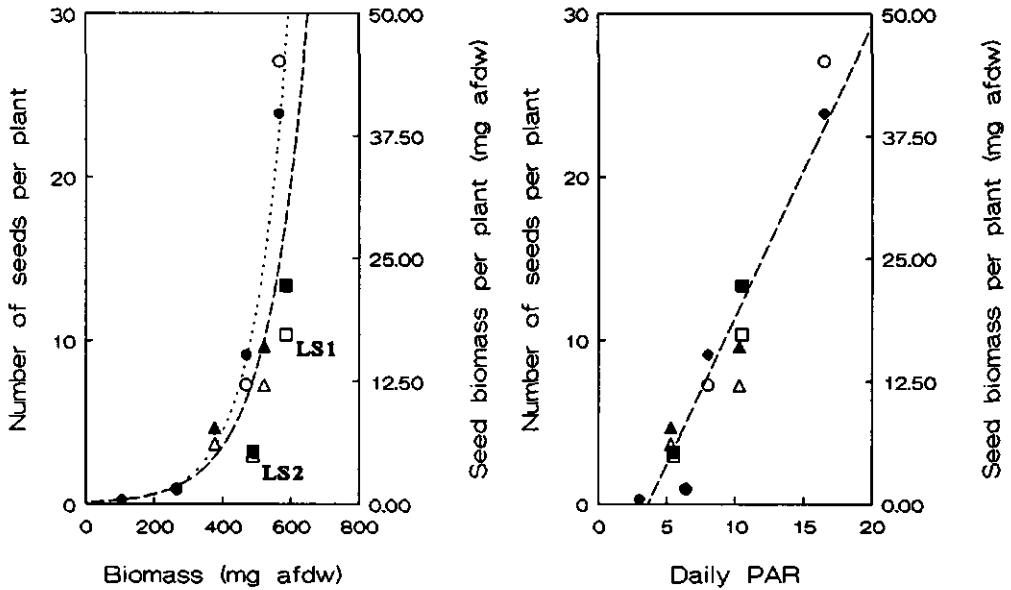


FIG. 27: Seed production versus plant biomass at harvest (left) and versus daily PAR (right). Datapoint symbols as in Fig. 25. Empty symbols: seed biomass. Filled symbols: number of seeds. The broken lines represent exponential (left graph) and linear (right graph) fits on the number of seeds, the dotted lines similar equations fitted only on the 4 long-day datapairs. 'LS1' and 'LS2' indicate the datapairs corresponding to the dim treatment.

#### 4.6. Effect of ageing and reproduction on plant photosynthesis

PI curves measured in the 16th week illustrate the strong combined effect of ageing and reproduction on the photosynthetic performance of *R. drepanensis*. Net production dropped in all curves, the decrease in photosynthesis being more pronounced in the high PPFD treatments.  $P_m$ ,  $\alpha$  and  $P_{ac}$  decreased, and  $K_m$  and LCP increased significantly with increasing age in all treatments (Figs. 18 to 21). Dark respiration (R) decreased also with increasing age, especially under long-day conditions.

Leaf chlorophyll-*a* and -*b* content also showed a strong reduction between the 11th and the 16th week (Figs. 12 and 13). Nevertheless, chlorophyll-*a* content of the LS2 treatment was not affected by age, and chlorophyll-*a* content increased with age in the SS2 and LL4 treatments. Overall, Chl-*b* fraction decreased also with increasing age in all treatments but LS1.

LS2 and SS2 treatments showed the lowest seed production of all light periods tested, and LL4 resulted also in a virtual absence of seeds. Thus, it seems clear that the decline in leaf chlorophyll content is not only a consequence of an increasing age, but much more likely of the expenses of reproduction (mainly seed production). As found in the chlorophyll-related PI curves, both  $P_m$  and  $\alpha$  still decreased with age once the chlorophyll effect was removed, suggesting that other components of the light harvesting system and the enzymes regulating the carbon metabolism were also negatively affected by reproduction and age. High

chlorophyll-related respiration rates found in the 16th week for LS1 and SS1 (the only treatments in which R increased with increasing age), when the plants are likely to have a maximum reproductive demand to maintain the production of most of the new seeds (flower abundance is similar to LL1, but seed biomass is much lower) also support the hypothesis of a direct effect of seed production on a decay of the vegetative apparatus.

All these results are in agreement with Hootsmans & Vermaat (1994), who reported strong decreases in  $P_m$ , net and gross production at  $200 \mu\text{E m}^{-2} \text{s}^{-1}$ , R and  $P_m/R$ , as well as increasing LCP, with increasing age in *P. pectinatus*. Such an effect was especially marked in the first two months of plant growth. They also found chlorophyll content to decrease with increasing age. Contrary to our results,  $K_m$ ,  $\alpha$  and R were all unaffected, although chlorophyll-corrected R also increased with increasing age. They also reviewed equivalent results from Van der Bijl *et al.* (1989), Jana & Choudhuri (1979) and Drew (1979), working with *P. pectinatus*, *Vallisneria spiralis* L. and *Posidonia oceanica* (L.) Delile respectively. Nevertheless, in all the above-mentioned cases the reported decrease in photosynthesis, respiration and chlorophyll content was attributed only to ageing, and only Drew (1979) refers to a leaf senescence process, which he attributed to 'daylength changes rather than ... irradiance or temperature changes'. So it seems that age in itself can induce a quite strong decline in vegetative apparatus of submerged macrophytes, which may bring some doubts on the relative importance of reproduction as a causal factor for senescence.

Comparison with data from Santamaría *et al.* (1994b) may help to discriminate both effects. They measured 3 replicate PI response curves using plants grown as in the present experiment, and under a light regime analogous to our LL1 treatment ( $240 \mu\text{E m}^{-2} \text{s}^{-1}$ , 16 h PP) after 7 weeks of growth. The resulting parameters estimates were compared with our 11th- and 16th-week values from LL1. As flower production and seed formation occurred between the 11th and the 16th week of growth, we may assume that differences between the 7th and the 11th week data are just due to ageing, while further differences between the 11th and the 17th week are due to additional senescence caused by seed production. Moreover, our three age treatments are now equivalent to those presented in Hootsmans & Vermaat (1994), who found effect of age to be mainly evident between the 30th- and the 70th-day measurements.

ANOVA resulted in significant differences for all parameter estimations but R ( $p < 0.01$ , except for  $K_m$  and LCP, where  $p < 0.05$ ). Multiple comparisons yielded similar results for  $P_m$ ,  $P_{sc}$  and  $\alpha$ : 49th- and 77th-day values did not differ significantly, and both were significantly different from the 112th-day one.  $K_m$  yielded no significant differences, while for LCP only the 77th- and the 112th-day values differed significantly. So it seems that an age effect was only strongly manifested after the initiation of reproduction. Nevertheless, two different trends may be appreciated in the values of the parameter estimates (Fig. 28): while  $P_m$  tends to decrease with increasing age,  $\alpha$  shows a maximum at the intermediate age which is also reflected in a corresponding minimum of  $K_m$  and LCP at such an age. Overall,  $P_{sc}$  only decreases in the 'oldest' measurement. Thus, although a progressive decline of  $P_m$  may be postulated to be caused by ageing (as reported by Hootsmans & Vermaat, 1994), it is coupled with increasing  $\alpha$  and decreasing  $K_m$  and LCP, and it seems reasonable to link it with the progressive effect of light acclimation along plant development (indeed,  $P_{sc}$  does not change). Later on, reproduction is followed by a rapid deterioration of both photosynthetic subsystems (light harvesting and carbon metabolism), and  $P_{sc}$  decreases strongly.

It is to be expected from an annual species that it will senesce and die after seed production. Nevertheless, it is always difficult to assess whether the decay of vegetative apparatus is due to ageing, plant exhaustion due to the high energetic cost of seed production,

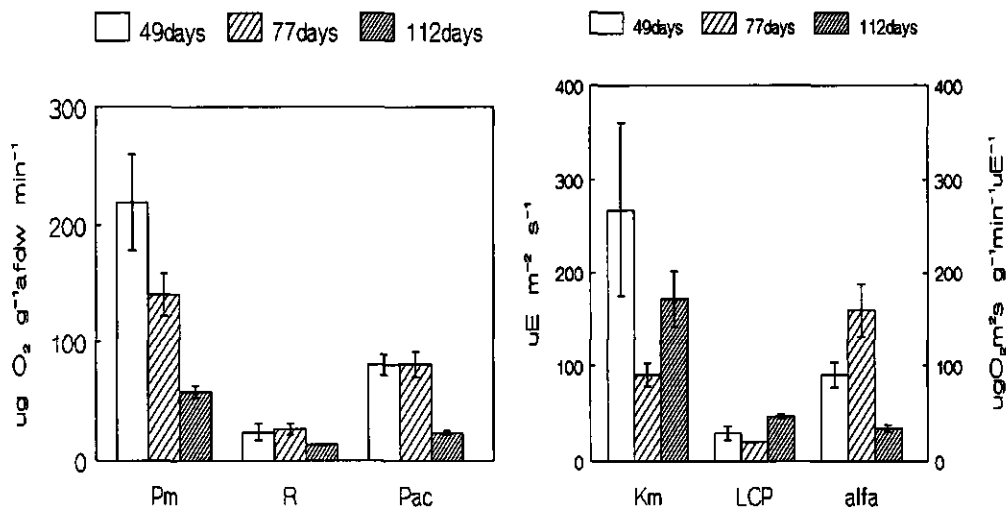


FIG.28: Effect of age on photosynthesis. Meaning and units of the different parameters estimates as previous figures. Data from 49 days of age from Santamaría *et al.*, 1994a.

or to induced senescence. The same photoperiod conditions reported to promote flowering have been frequently reported to promote also senescence, although in some cases senescence stimuli were different, and sometimes the coincidence of factors inducing senescence with flowering and seed production was required (Salisbury, 1981). In our case, at least for the photoperiodic effect, a direct effect of reproduction on senescence seems the most plausible explanation for our data, and we do not find any evidence for supporting a more complicated hypothesis.

Thus, once reproduction has begun, *R. drepanensis* plants probably dedicate most of the energy and nutrients available to fill the seeds, the high demand generated by this process resulting in a rapid deterioration of the vegetative apparatus. Thus, it seems unlikely that an extended inundation period following flowering and seed production would result in a much higher offspring for *R. drepanensis* populations.

## 5. CONCLUSIONS: OVERALL EFFECT OF LIGHT CLIMATE ON THE LIFE-CYCLE OF *Ruppia drepanensis*

*R. drepanensis* plants grown at different combinations of photoperiod, photosynthetic period and instantaneous irradiance showed different responses in their photosynthetic performance, growth dynamics or reproduction. Regarding its photosynthetic performance, *R. drepanensis* can be qualified as a shade-tolerant plant with a relatively high sun-intolerance. Both light harvesting and carbon metabolism improved with decreasing growth irradiances, and apparent net photosynthetic production was thus higher. Plants grown under a shorter photosynthetic period (PAP) showed an improved carbon metabolism, but a less efficient light harvesting system. The overall increase in net photosynthesis with shorter PAP was attributed to reduced respiratory needs.

These results are in apparent contrast with the growth rates estimated from biomass increase and other morphometric variables, which decreased with decreasing irradiance and were maximal for the dim-treatments. We attribute these differences to changes in the PQ as explained by the different photosynthate partitioning and different metabolic expenses associated with the different chemical composition of plants grown under different light regimes. Plants grown under low irradiances are predicted to show a lower PQ, probably due to the high expenses of building and maintaining an efficient light harvesting system. Plants grown under short PAP are predicted to show a higher PQ, their restricted metabolic needs obtained at the cost of a lower light harvesting efficiency.

*R. drepanensis* exhibited a quite limited capacity for morphological adaptation to restricted light regimes. Its capacity for photosynthetic acclimation, however, was considerable. Probably as a response to the strong wind-induced wave action in the shallow lakes where it occurs, development of vertical shoots is a relatively late event in *R. drepanensis* life-cycle, which may explain its limited morphological response.

Induction of flowering was significantly postponed under short PP and low PPFD conditions. Flower abundance was not affected by light period, but it was restricted under low irradiances. Nevertheless, short photosynthetic periods resulted in a strong reduction of seed production, which was also lower at lower PPFD and virtually null at irradiances equal or lower than  $110 \mu\text{E m}^{-2} \text{s}^{-1}$ . Thus, offspring appeared to be strongly restricted under any conditions lowering light climate below an optimum found under long-day conditions and moderately high irradiances.

Importance of the life-cycle as an overall framework to interpret all these results should be stressed. *R. drepanensis* is a winter-annual species, early production of propagules being of great importance to survive desiccation of the wetlands in late spring. The limited capacity for morphological acclimation (through e.g. elongation) fits well with the shallowness of the temporary habitats where it occurs, but it may be also related with the limited amount of reserves available in the seeds to sustain early seedling development. The high capacity of photosynthetic acclimation is associated to a sun-intolerant character, but while shade acclimation is strongly needed if turbidity is further restricting winter low daily irradiances, effective photosynthesis under high irradiances is not expected to be really necessary as they are normally accompanied by plant reproduction and subsequent wetland desiccation. Finally, its quantitative long-day character assures stimulation of the reproduction under increasingly longer daylengths, whilst postponement of reproduction until such late moment is necessary regarding the metabolic costs associated with a short PAP.

Our care at regarding the use of results on photosynthesis or growth dynamics to extrapolate the future behaviour of plant populations (both their distribution and dynamics) must be stressed here. As we have demonstrated, interpretation of such results without a precise knowledge of the integrated plant response as determined by its life-cycle strategy may yield very conflicting results. Neglecting the differences in PQ caused not only by differences in nutrient availability, but also by the continuous process of plant ageing and light acclimation, may bias the derivation of estimated growth rates. On the other hand, basing population dynamics on vegetative growth data (in other words, assuming reproductive success not to be directly affected by changes in the plant's environment), may result in completely erroneous predictions if temporary habitats or strict annual species are under consideration.

In conclusion, interaction of low irradiances and shorter PAP hypothesized to be associated with an increased water turbidity resulted in a strong reduction of *R. drepanensis* seed production. In spite of the high plasticity shown by *R. drepanensis* in photosynthetic

acclimation to low light regimes, restricted offspring could easily result in a strong decline of its population in a few generations (=years). The highly dynamic character of temporary wetlands may result, as hypothesized, in an extreme sensitivity to disturbance events, such as increased turbidity due to cultural eutrophication. While seed bank reserves may easily overcome elements of stress as a succession of dry years, disturbances as cultural eutrophication have a more permanent character (e.g., nutrients remain in the sediment and are released every year to the water column), thus reducing the possibilities of a re-establishment of submerged macrophyte populations.

## 6. ACKNOWLEDGEMENTS

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## Chapter 3

# The effect of temperature on the growth, photosynthetic performance and reproduction of *Ruppia drepanensis* Tineo

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**ABSTRACT:** Seedlings of *Ruppia drepanensis* Tineo obtained from seeds collected in the Doñana National Park brackish marsh (SW Spain) were grown in two different experiments: the first one studied growth, reproduction and photosynthetic acclimation at 20 and 30 °C, while the second one determined the effect of temperature (14 versus 20 °C) and photoperiod (10 versus 16 h) on the induction of flowering.

Plants showed maximal vegetative growth at 20 °C, with lower growth rates at 14 and 30 °C. A short photoperiod resulted in a lower biomass yield when combined with low temperatures. Reproduction was also optimal at 20 °C: low temperatures (14 °C) inhibited flower induction, while flower development was very poor at 30 °C (no seed was produced). Photoperiod had little effect on flowering, as compared with temperature.

According to its photosynthetic performance, we can define this species as cold-adapted, with a wide temperature optimum performing well at temperatures as low as 10 °C and capable of acclimate to higher temperatures. Photosynthetic plasticity allowed for high net production rates at 10 °C, independently of the temperature experienced during growth. At 30 °C, however, net photosynthetic rates were lower in plants grown at 20 °C than at 30 °C.

Calculation of relative growth rates (RGR) on the basis of photosynthesis data resulted in a severe underestimation of the biomass-based RGR at 30 °C, which discourages the use of the same PQ coefficients at different temperatures.

The overall effect of temperature on *R. drepanensis* metabolism fits well with the environmental conditions prevalent in Mediterranean temporary wetlands. The cold winter period is effectively used for vegetative growth, and mild spring temperatures (about 20 °C) allow abundant flowering and seed production. Photosynthetic acclimation to higher temperatures (30 °C or more) can be useful to prolong the reproductive period of the plants in exceptionally wet years, although these temperatures are normally associated with a fast drying of the wetland.

## 1. INTRODUCTION

This paper deals with the temperature response in the vegetative growth and reproduction of *Ruppia drepanensis* Tineo. It is part of a study assessing the key phases in the life cycle of this species and the tuning to its specific habitat. *R. drepanensis* is a winter-annual inhabiting Western Mediterranean temporary wetlands, and it is also the dominant submerged macrophyte in the brackish marsh area of the Doñana National Park (SW Spain). The general aim of the study is to provide a conceptual model of the life cycle of this species, useful for the development of management strategies.

Mediterranean temporary wetlands annually dry up during late spring and summer. The duration of the inundation period depends strongly on highly variable winter and spring rains. Shallowness of the wetlands and strong seasonal variation result in water temperature ranges between 10 °C (winter) and 35 °C (prior to dry-up). Maximum vegetative growth of the submerged vegetation is generally occurring during the first, colder months, while flowering and seed production is realized during the last, warmer months.

In temporary aquatic habitats, the timely production of sufficient drought resistant seeds is essential for year-to-year survival. Thus, any factor delaying plant development and reproduction may have a dramatic effect on the survivorship of the plant population. Triggering of developmental events along the life-cycle of species inhabiting these wetlands, such as flower induction, have to adjust to the year-to-year variation of the inundation period. In these circumstances, seasonal stimuli which are independent of the climatic variability (for example photoperiod) become less reliable, while other factors such as plant size, temperature or nutrient status may gain importance.

In this study, we experimentally tested the following hypotheses:

- Because of its winter-annual life cycle, growth and photosynthesis of *R. drepanensis* is expected to be optimal at relatively low temperatures (10 to 20 °C), and poorer at high temperatures (30 °C).
- Acclimation to high temperatures (30 °C) might result in enhanced photosynthetic performance at such temperatures. According to Berry & Björkman (1980), it should also result in a poorer photosynthetic performance at low temperatures (10 °C).
- Temperatures below 15 °C inhibit the induction of flowering, while temperatures above 30 °C result in flower deterioration (as suggested by Setchell, 1924; and Verhoeven, 1979).

To support our hypotheses, we will first elaborate on the effect of temperature on angiosperm life-cycles, and launch a proposal for the identification of several temperature acclimation strategies in submerged angiosperms. Explicit incorporation of information on terrestrial angiosperms was considered necessary since many ecophysiological studies have focused on these. Subsequently, our experimental results will be presented.

## 2. GENERAL PATTERNS IN THE TEMPERATURE RESPONSE IN ANGIOSPERMS

Temperature is known to be a major factor determining the distribution and productivity of plants. Plant morphology, especially the root to shoot ratio, is also strongly affected by temperature. Finally, temperature is one of the environmental parameters controlling other developmental events, such as the induction of flowering and the germination of seeds. In the following sections, a brief account is given of the effects of temperature on growth and photosynthesis of aquatic and terrestrial macrophytes. Research in this field is presently much

more developed for terrestrial macrophytes, so we will devote first some attention to them (based mainly on Berry & Björkman, 1980 and Berry & Raison, 1981). Subsequently, information on seagrasses is presented, mainly from Bulthuis (1987), followed by a review of non-marine aquatic macrophytes.

Two important concepts in the following discussion are temperature acclimation and the acclimation capacity of the plants. Berry & Björkman (1980) define acclimation as the 'environmentally induced phenotypic modifications that may be interpreted as being adaptative', and acclimation capacity as 'the ... genetically determined ... ability to acclimate'. As Bradshaw (1965) clearly pointed out, acclimation is just one form of phenotypic plasticity, which he applies strictly to variation which is not directly genetic in origin. Adaptation, in contrast, refers to changes which are strictly genetic in origin. He also specifies that 'the plasticity of a character is an independent property of that character and is under its own specific genetic control'. Hence, the use of the term 'acclimation' will be restricted here to environmentally induced plasticity, while 'acclimation capacity' will be used to refer to the genetic control of such plasticity.

### *2.1. State of the art, with special reference to terrestrial plants*

One of the difficulties in assessing the effect of temperature on the macrophytes lies in the difficulty of extrapolating its primary effects at the molecular level to the integration level of physiological processes or plant growth (Berry & Raison, 1981). In the following paragraphs, we will concentrate as much as possible on the response of the individual plant or plant organ as a whole, descending only to overall physiological processes such as photosynthesis or nutrient uptake. The effect of temperature at the biochemical level will generally be ignored.

The analysis is further complicated by two factors. Firstly, plant tissue temperature may differ significantly from that of the environment (air or water). As a consequence, morphological characteristics which may enable plants to maintain a more favourable temperature may represent an evolutionary alternative to physiological adaptations. Secondly, other factors may co-vary with temperature in a given habitat. Seasonal changes in temperature are linked with changes in day length, solar elevation and precipitation regimes. This obvious covariance precludes the identification of either irradiance or temperature as key environmental factors from field data alone.

#### *2.1.1. Primary productivity*

The temperature response in angiosperm growth generally follows an optimum curve (hereafter referred as the 'temperature response curve'). Studies on plant growth response to contrasting thermal regimes showed that adaptations which permit efficient performance of the plants at one extreme can be detrimental at the opposite extreme. Moreover, differences between species in growth response correspond very well to differences in the short-term response of leaf photosynthesis to temperature. However, this relation is complicated by long-term effects such as photosynthetic acclimation or temperature-induced damage to the photosynthetic system (Berry & Raison, 1981).

The photosynthetic response to temperature interacts strongly with other environmental factors, the most important being irradiance and CO<sub>2</sub> concentration. Hence,

meaningful comparisons of temperature response curves of leaves require that the irradiance dependence of photosynthesis is known. For example, irradiance required to saturate photosynthesis is lower at low temperatures. While maximum temperature dependence is observed under rate-saturating irradiances, the temperature response becomes flatter and broader as irradiance is progressively lowered. When irradiance is low enough to be limiting over the entire temperature range, net photosynthesis declines with increasing temperature. This decline is expected since net photosynthesis would decrease with increasing respiratory rates associated with increasing temperatures (Berry & Björkman, 1980).

In general, studies on respiratory activity indicate that differences in the temperature optimum and tolerance range of respiration of plants acclimated to contrasting thermal regimes parallel differences seen for photosynthesis. Nevertheless, respiration is considerably more conservative than the photosynthesis of the same plant. As a general rule, the relative increase in respiratory activity between 10 and 30 °C is about the same for all plants (Berry & Raison, 1981).

An important concept here is the division of respiration into a portion associated with growth (and directly linked to photosynthesis) and another portion linked to maintenance of the life functions of the plant. Berry & Björkman (1981) hypothesized that a significant portion of total plant respiration may be proportional to new growth and independent of temperature (excluding the temperature dependence of growth itself), while temperature-dependent losses would be restricted to that portion of respiration associated with maintenance. According to them, the apparent temperature dependence of respiration might thus lead to significant overestimates of the impact of respiration on the net carbon balance.

Physiological acclimation may permit a plant to grow over a wider range of thermal regimes. Differences in acclimation capacity were found to underlay the seasonal pattern of physiological activity in summer-active and winter-annual species. More importantly, while plant clones originating from hot localities have been generally found to be able to shift their photosynthetic response towards lower temperature optima when grown at lower temperatures, the clones originating from colder localities were adversely affected by growth at higher temperatures (Berry & Raison, 1981).

The adaptations leading to an improvement in the photosynthetic performance at one temperature extreme generally result in decreased performance at the opposite extreme. In general, as Berry & Björkman (1980) concluded, 'plants native to habitats characterized by great variations in temperature over their growth season do not necessarily possess a broader temperature range of adequate photosynthetic performance or tolerance ..., but they tend to possess a higher ... potential for photosynthetic temperature acclimation.' Such temperature acclimation involves changes in several components of the photosynthetic apparatus. Understanding the effect of temperature on the processes that underlie the synthesis of the photosynthetic apparatus is thus needed to complete the picture of the photosynthetic temperature acclimation.

Studies of the temperature dependence of development of the primary organs of germinating seedlings from stored reserves have indicated a strong inhibition of growth processes at both high and low temperature, suggesting that growth is not strongly temperature dependent at intermediate temperatures. The strong response of protein synthesis to high and low temperature may be related to the effects of temperature on growth and chloroplast development. Biosynthetic processes being most likely autocatalytic, the temperature dependence on a sustained basis may however be much stronger than suggested by the temperature effects on the instantaneous rate over a short time interval (Berry & Raison, 1981).

Biomass partitioning is also likely to have a significant influence on plants' response to temperature. In general, low root temperatures and low nutrient availability both result in higher root to shoot ratios. Root/shoot ratio increases also at supra-optimal temperatures. Strong interaction with soil nutrient status suggests that changes in allocation might be interpreted as compensatory responses required to maintain a balance between shoot and root functions as temperature is changed (Berry & Raison, 1981).

### 2.1.2. Temperature coefficients

Temperature coefficients are used to describe the variation of different process rates as a function of temperature. These coefficients are normally derived from the analysis of the rates of isolated chemical reactions. Caution is obviously needed when applying them to physiological rates, which integrate a multitude of different reactions. Nevertheless, they may provide objective criteria useful in the analysis of biological phenomena (Berry & Raison, 1981).

Different formulations of such temperature effects on process rates are in use. The Arrhenius theory states that the rate of a (single) reaction changes exponentially with the inverse of the absolute temperature, according to the equation

$$k = A e^{-\frac{E_a}{RT}} \quad (1)$$

where  $k$  is the reaction rate,  $A$  is an integration constant,  $E_a$  is the activation energy of the reaction,  $R$  is the universal gas constant ( $8.314 \text{ J mol}^{-1} \text{ K}^{-1}$ ), and  $T$  is the absolute temperature (in K).

The  $Q_{10}$  concept has been widely used in physiological works. It does not imply any mechanistic explanation, and it is defined as the ratio of the rate at one temperature to that at a temperature  $10^\circ \text{C}$  lower. The  $Q_{10}$  function assumes a power function to describe the relation to temperature (in  $^\circ \text{C}$ ), so the rate at any temperature can be calculated from the rate at a known temperature (assuming  $Q_{10}$  to remain constant) according to the expression:

$$k_2 = k_1 Q_{10}^{\left(\frac{T_2 - T_1}{10}\right)} \quad (2)$$

where  $k_1$  and  $k_2$  are the physiological rates, and  $T$  the temperatures (in  $^\circ \text{C}$ ). Most biochemical and chemical reactions have a  $Q_{10}$  near 2.

Arrhenius plots often show abrupt changes in the value of the  $E_a$  and  $Q_{10}$ , indicating significant changes in the factors controlling the rate of the process (Berry & Raison, 1981). Obviously, both  $E_a$  and  $Q_{10}$  estimates should not be used outside the temperature range from which they were calculated.

Most physiological processes reach a maximum rate at an optimum temperature. The decline in temperature dependence near the optimum and the negative temperature dependence beyond it are not explained by the Arrhenius or the  $Q_{10}$  function. Johnson *et al.* (1974) proposed a model using temperature-dependent proportions of active and inactive conformations of an essential enzyme (catalyzing a rate-limiting reaction). The rate of the reaction as a function of temperature is described as the product of the Arrhenius reaction and the concentration of the active conformers of the enzyme, according to the expression:

$$k = A \frac{1}{1 + \kappa} e^{\frac{-E_a}{RT}} \quad (3)$$

where  $\kappa$  is the equilibrium constant of the reaction responsible for the enzyme conformational change.  $\kappa$  depends itself on the absolute temperature according to the expression:

$$\kappa = e^{\frac{-(\Delta H - T\Delta S)}{RT}} \quad (4)$$

where  $\Delta H$  and  $\Delta S$  are the increase in enthalpy and entropy involved in the enzyme's conformational change, respectively.

The importance of a thermal inactivation equilibrium of the enzymes as a controlling factor in physiological processes was first demonstrated with bacterial luminescence, and afterwards has been applied to a wide spectrum of physiological phenomena (Johnson *et al.*, 1974). It is considered to be a mechanism of widespread importance, applicable to any reaction showing a quantitative reversibility of the diminished rate when returning to the optimum temperature after brief exposure to higher temperatures. After long exposures, a non-reversible destruction of the system may also take place.

### 2.1.3. The induction of flowering

In plant reproduction, two major events are strongly temperature-dependent: the initiation of flowering, and the relative success of seed germination (through the influence of temperature on seed imbibition and the initiation of germination). In the following we will concentrate on the effect of temperature on flowering, and particularly on its induction.

Most studies dealing with flower induction have concentrated on the effect of photoperiod. Such photoperiodic response has been extensively reported to be modified by temperature (Evans, 1969), although the critical day or critical night length remained relatively resistant to changing temperatures (Salisbury, 1981). In particular, flowering of many species is promoted by a brief to prolonged exposure to temperatures close to the freezing point (i.e. these plants can be 'vernalized'). Most commonly, vernalization does not result in day-neutral (DN) flowering requirements, but is still followed by a qualitative or quantitative response to photoperiod (Salisbury, 1981).

It is common for the photoperiodic response itself to change with temperature. While all combinations are virtually possible, probably the most common combination is a DN response at one temperature with a quantitative photoperiod response at another (Salisbury, 1981). It is important to note that temperature was generally treated as a factor *modifying* the photoperiod response of the flowering plants, never as a primary factor affecting flower induction with independence of the photoperiod.

## 2.2. The light versus temperature polemic in seagrass field studies

Aquatic habitats are generally well-buffered against extreme diurnal and seasonal fluctuations in temperature. Nevertheless, strong differences in thermal regime can be expected both among different geographical regions, and among seasons in temperate climates. Habitat type, mainly regarding the volume of the waterbody and the position of the plant community



within it, introduces a further factor of differentiation. Plants from coastal, intertidal areas and from shallow waterbodies are usually exposed to contrasting temperature ranges along their life-cycle.

Temperature effects on seagrass growth and photosynthesis have received relatively high attention. Nevertheless, as stressed by Bulthuis (1987), the relative importance of temperature for the growth of seagrasses has been a major point of controversy. Since Setchell (1929) suggested temperature as the major factor controlling seasonal differences in growth rate, numerous workers have claimed to provide either collaborating or conflicting evidence. Most of these studies have relied on correlations (or the lack thereof) of environmental field data with seasonal growth patterns. Indeed, Bulthuis (1987) concluded that the role of temperature in controlling the seasonal growth of temperate seagrasses was unresolved, and stressed the need for other approaches besides measuring *in situ* growth rates (including studies of the temperature response of photosynthesis, growth in laboratory cultures under controlled conditions and *in situ* field experiments).

In our opinion, the polemic between light and temperature as the predominant factors determining seagrasses seasonal growth is particularly unfortunate. It probably originates from a narrow application of the limiting factor theory. This theory argues that, in a process being regulated by the simultaneous effect of various factors, the factor resulting in the lowest rate will determine the velocity of the whole process. But diurnal and seasonal fluctuations of irradiance and temperature jeopardize the application of the limiting factor concept to the regulation of photosynthesis and growth, since in most temperate aquatic habitats it seems unlikely that only one of both factors will be limiting during the whole day or season. Furthermore, the evidence presented above for their interactive effect on photosynthesis almost completely excludes the identification of one of these factors as 'major limiting factor'.

Most of the works concluding in favour of a temperature effect on growth and photosynthesis (see Bulthuis, 1987) or taking into consideration the influence of temperature on photosynthesis (Biebl & McRoy, 1971; Drew, 1979; Kerr & Strother, 1985; Leuschner & Rees, 1993) just ignored the effect of temperature acclimation. Although some of them described seasonal changes in the temperature response (Drew, 1978; Evans *et al.*, 1986; Pérez & Romero, 1992), and Pirc (1986) concluded a significant influence of age on the photosynthetic response to a 5 °C increase in temperature, the role of temperature acclimation in modifying the temperature-response curve to our knowledge has only been separately addressed by Zimmerman *et al.* (1989).

Zimmerman *et al.* (1989) found that *Zostera marina* L. plants grown at 20 °C showed a higher temperature optimum for light-saturated photosynthesis, and decreased photosynthetic and respiratory rates when compared at the same incubation temperature, than those grown at 10 °C. They describe these findings as being 'consistent with the generality that improved performance at high temperatures results in decreased performance at low temperatures and vice versa', and conclude that 'the potential for thermal acclimation suggest that seasonal fluctuations in ambient temperature may not affect metabolism and growth of eelgrass nearly as much as had been extrapolated from studies of short-term thermal effects'.

In his review on the temperature effect on seagrass growth and photosynthesis, Bulthuis (1987) concluded the following:

- (1) The apparent quantum yield (the initial slope of the PI curve,  $\alpha$ ) does not change with temperature 'within the normal physiological limits'. But as the high temperature limit is approached (in a reported range of 35 to 45 °C),  $\alpha$  is reduced.

- (2) Irradiance-saturated photosynthesis was maximal at 25-35 °C, and dropped at temperatures above this optimum. Nevertheless, lower optimum temperatures had been reported for at least one temperate species (*Z. marina*, 22 °C).
- (3) Optimum temperature decreased as much as from 30 to 5 °C as irradiance is reduced to near the light compensation point.
- (4) The rate of dark respiration increased with increasing temperature, although not following a general exponential relationship;
- (5) The light compensation point increased with increasing temperature.

Other works have considered the temperature effect on seagrass photosynthesis after Bulthuis's (1987) review (Kerr & Strother, 1985; Marsh *et al.*, 1986; Pirc, 1986; Zimmerman *et al.*, 1989; Pérez & Romero, 1992; Leuschner & Rees, 1993). Among them, data published by Kerr & Strother (1985), Pérez & Romero (1992) and Leuschner & Rees (1993) were in agreement with the above conclusions ( $T_{opt}=30-32$  °C for the irradiance-saturated photosynthesis and dark respiration of *Cymodocea nodosa* (Ucria) Aschers. and *Zostera muelleri* Irmisch ex Aschers., and  $T_{opt}=21$  °C for the irradiance-saturated photosynthesis of *Z. marina*; see also Table 1). On the contrary, both Marsh *et al.* (1986) and Zimmerman *et al.* (1989) found irradiance-saturated photosynthesis of *Z. marina* to be optimal at 25-30 °C.

Finally, Bulthuis (1987) hypothesized that, as found for terrestrial plants, 'phenotypic plasticity in photosynthetic response to temperature' (referring here to photosynthetic acclimation) is to be expected for temperate seagrasses that experience contrasting thermal conditions in winter and summer. Unfortunately, he could find no evidence supporting or rejecting this hypothesis.

In conclusion, temperature is one of the important variables affecting growth of seagrasses at both high and low irradiance levels. In the view of the present experimental evidence, ignoring a temperature effect seems a serious simplification, even under strongly limiting irradiances.

### 2.3. The effect of temperature on growth and photosynthesis of submerged non-marine macrophytes

Because of the interactive effect of temperature and light as outlined above, field studies are excluded from this review and stress will be on temperature effects established under controlled conditions. First, different morphological and ecophysiological subjects will be treated separately, integration is sought in the concluding section where a series of hypotheses is launched.

#### 2.3.1. Morphology, growth and productivity

Barko *et al.* (1982) investigated the effect of irradiance (100, 600 and 1500  $\mu\text{E m}^{-2} \text{s}^{-1}$  midday irradiance) and temperature (12, 16, 20, 24, 28 and 32 °C) on the growth and morphology of the macrophytes *Elodea canadensis* Michx., *Potamogeton nodosus* Poirlet and *Vallisneria americana* Michx. grown in greenhouse cultures. They found irradiance and temperature to influence both biomass and morphology in an interactive way. As a general trend, they concluded that all three species showed increased shoot growth at increased temperatures up

to at least 28 °C, although at the lowest irradiance (100  $\mu\text{E m}^{-2} \text{s}^{-1}$  midday irradiance) the growth optimum occurred at lower temperatures (24 °C). Root biomass response to temperature differed strongly between species. Nevertheless, root to shoot ratio tended to decrease with increased temperature in all three species. Finally, shoot length tended to increase with increased temperature up to 24 to 28 °C in all three species.

In a previous study, Barko & Smart (1981) found *Egeria densa*<sup>1</sup> and *Hydrilla verticillata*<sup>1</sup> to show contrasting growth responses to temperature (16, 20, 24, 28 and 32 °C) after 6 weeks of growth, the interactive effect of irradiance and temperature being of minor importance. While *E. densa* shoot biomass was not affected by temperature up to a sudden drop at 32 °C, *H. verticillata* shoot biomass increased strongly with temperature up to 32 °C. These data gain interest when contrasted with the measurements of net photosynthesis (at saturating irradiance, 515  $\mu\text{E m}^{-2} \text{s}^{-1}$ ) and respiration made by Barko & Smart (1981) one week after the biomass determinations. Their estimations of the daily production on the basis of such data (applying a 14 h photoperiod) decreased with temperature for *E. densa*, and showed a relatively sharp maximum at 24 °C for *H. verticillata*. In conclusion, there is not a very good agreement between the response of growth and metabolic rates (net photosynthesis and respiration) to temperature, the growth response showing a lower degree of variation with temperatures.

Division of respiration into two independent, growth- and maintenance-related portions was proposed by Berry & Raison (1981) to explain the unbalanced impact of respiration on net carbon balance. Although this explanation is in agreement with the results reviewed here, we do not find it fully satisfactory. In our opinion, any measured increase in respiration results in losses in the carbon balance, independently of the physiological processes in which the energy yielded by the respiratory process is invested. Furthermore, photosynthate and growth partitioning are affected by temperature too, which may also explain the observed differences between growth and photosynthetic carbon balance.

Similar results were obtained by Koch & Dawes (1991) while investigating the ecotypic differentiation between two populations of *Ruppia maritima* L. (from Florida and North Carolina, respectively). They grew three clonal replicates from each population at 14, 22 and 30 °C, and estimated their growth and photosynthetic performance after two months. Leaf, root and total biomass were all significantly lower at 14 °C for the North Carolina population, while only leaf biomass was lower at 14 °C for the Florida population. Net photosynthesis was significantly lower for the plants cultured at 30 °C for both populations. Again, there seems to be an important disagreement between the growth and the photosynthetic response to temperature. We conclude that some caution is needed when translating photosynthesis measurements into growth rates.

Finally, Vermaat & Hootsmans (1994b) reported temperature (13, 15 and 22 °C) and irradiance (50, 100 and 200  $\mu\text{E m}^{-2} \text{s}^{-1}$ ) to affect the growth (biomass yield and morphometry) of *Potamogeton pectinatus* L. interactively and nonlinearly. Both increasing temperature and increasing irradiance had a positive effect on plant size, resulting in a higher number of leaves and shoots per plant, and a higher biomass yield after 55 days of growth. No significant differences were found between different irradiances for the plants grown at low temperatures: inhibition of growth at low temperatures thus seems to outweigh any concurrent effect of irradiance, as reported also by Barko *et al.* (1982; see above) and

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<sup>1</sup> Authorities name is only added to a species name if it was indicated in the original paper.

Tobiessen & Snow (1984; using *Potamogeton crispus* L.).

Application of the  $Q_{10}$  concept to the relative growth rates of the logistic fits for several morphometric parameters rarely resulted in significant fits (only 4 of 60 fits had  $p < 0.10$ ; *P. pectinatus*, Vermaat & Hootsmans, 1994b). Thus, the rate at which the various morphometric characteristics were formed or grew was not influenced by temperature in a simple, loglinear way. It is interesting to note that the dark treatments, in which growth rates clearly depended on respiratory rates (as growth is then exclusively sustained by tuber biomass in *P. pectinatus*) had a clear optimum at 15 °C. Thus, the limitations of the  $Q_{10}$  concept are obvious even when applied to relatively simple conditions (no interaction with light nor with photosynthate partitioning).

### 2.3.2. Chlorophyll concentration

Spencer & Ksander (1990) cultivated *Potamogeton gramineus* plants sprouted from winter buds collected from a Californian irrigation channel at 25 combinations of temperature (10, 15, 25, 30 and 35 °C) and irradiance (8, 15, 22, 58 and 189  $\mu\text{E m}^{-2} \text{s}^{-1}$ ) during three weeks. They found chlorophyll to decrease sharply with increasing irradiance and to increase with increasing temperature, while anthocyanin (a colour pigment) content decreased with increasing temperature and increased slightly with increasing irradiance. The relationships between both temperature and irradiance and the pigment levels were not linear.

Similar results were reported by Barko & Filbin (1983), who found chlorophyll content in *E. canadensis* and *V. americana* to increase with increasing temperatures when grown under various irradiances, while *P. nodosus* did not show such a relationship. On the contrary, Koch & Dawes (1991) reported a (non-significant) trend to decreased chlorophyll concentrations after growing *R. maritima* plants at increased temperatures. Vermaat & Hootsmans (1994b) also found chlorophyll concentration to decrease with increasing temperature (13, 15 and 22 °C) in *P. pectinatus*. As biomass yield increased with increasing temperature, they proposed a 'dilution' effect to account for the decreased chlorophyll concentration.

### 2.3.3. Photosynthesis

In general, two types of methodologies have been used: while some authors measured the photosynthetic performance of plants grown or collected at a certain temperature over a range of experimental temperatures (Titus & Adams, 1979; Pokorny & Ondok, 1980; Wedge & Burris, 1982; Orr *et al.*, 1988; Madsen & Adams, 1989, although they allowed for a two-weeks acclimation; Leuschner & Rees, 1993), other authors grew the plants at several different temperatures and then measured their photosynthetic performance at the same temperature at which the plants were grown (Barko & Smart, 1981; Koch & Dawes, 1991). While the former procedure gives information on the intrinsic temperature plasticity of the plants, the latter simulates more closely the field conditions.

In none of the papers we studied has the influence of acclimation been addressed separately. We did not find any reference in which the influence of the growth temperature on the temperature response (i.e., the acclimation potential) of a submerged macrophyte was studied.

We have derived a set of hypotheses from the literature on photosynthesis we

TABLE 1: Set of proposed hypotheses for the effect of temperature on the photosynthesis of submerged non-marine macrophytes.  $P_m$ ,  $K_m$  and  $R$  are parameter estimations from the rectangular hyperbola fit of PI curves.  $\alpha$  is calculated from  $P_m$  and  $K_m$  ( $\alpha = P_m/K_m$ ; Hootsmans & Vermaat, 1994).

1. *Photosynthesis at saturating irradiance*

- 1a. The maximal temperature dependence of photosynthesis occurs at saturating irradiance. Both the maximum gross production ( $P_m$ ) and the net photosynthesis at saturating irradiance ( $NP_m$ ) show an optimum response curve, increasing exponentially from low temperatures and decreasing sharply above the optimum. The Arrhenius equation as modified by Johnson *et al.* (1974) is proposed to model such a curve (see equations 3 and 4, Section 2.1.2.).
- 1b. Different species are expected to show different temperature optima and tolerance ranges, meaningfully coupled to distribution range and life cycle.

2. *Photosynthesis at low irradiance*

- 2a. Both the half saturation constant ( $K_m$ ) and the light compensation point (LCP) increase with increasing temperatures.
- 2b. Apparent quantum yield ( $\alpha$ ) does not vary with temperature 'within the physiological limits'. Only at very high and very low temperatures, it may decrease strongly due to permanent damage of the photosynthetic system.

3. *Dark respiration*

- 3a. As for  $P_m$ , dark respiration ( $R$ ) is expected to increase exponentially with temperature and then follow an optimum curve, with a sharp decrease at temperatures above a certain optimum.

reviewed above. This set is formulated in Table 1. We attempted to apply explicit mathematical formulations as much as possible since this generally facilitates rigorous testing of hypotheses. In the case of photosynthesis we used the rectangular hyperbola, since it is one of the most frequently used equations and has been shown to fit PI curves satisfactorily (Hootsmans & Vermaat, 1994). The formula of the rectangular hyperbola is as follows:

$$P = \frac{P_m * I}{K_m + I} - R \quad (5)$$

where  $P$  is the dependent variable net oxygen production,  $I$  is the independent variable irradiance,  $P_m$  is the maximum rate of gross production,  $K_m$  is the half-saturation constant, and  $R$  is the dark respiration.

The shape hypothesised for the temperature dependence curve of irradiance-saturated net photosynthesis ( $NP_m$ ) can clearly be observed in Titus & Adams (1979) for both *Myriophyllum spicatum* L. and *V. americana* (although modelled with a purely empirical equation). As also hypothesized in Table 1, both species show different tolerance ranges.

Figure 1 and Table 2 summarize maximum gross production ( $P_m$ ) and  $NP_m$  values calculated from literature data. Most curves clearly show the expected asymmetric optimum temperature dependence (for example, *P. pectinatus* from Madsen & Adams, 1989), centred at different temperature optima (from 14 to 30 °C). Two species showed optima above 30 °C (*Myriophyllum salsgineum* A.E. Orchard and *Spirodela punctata* (G.F.W. Meyer) Thompson, from Orr *et al.*, 1988 and Wedge & Burris, 1982, respectively), with no available data for the declining right part of the curve. In two cases (the Florida population of *R. maritima* and *Batrachium aquatile*, from Koch & Dawes, 1991 and Pokorny & Ondok, 1980, respectively), the temperature optima were at the lowest end of the range (14 °C), and

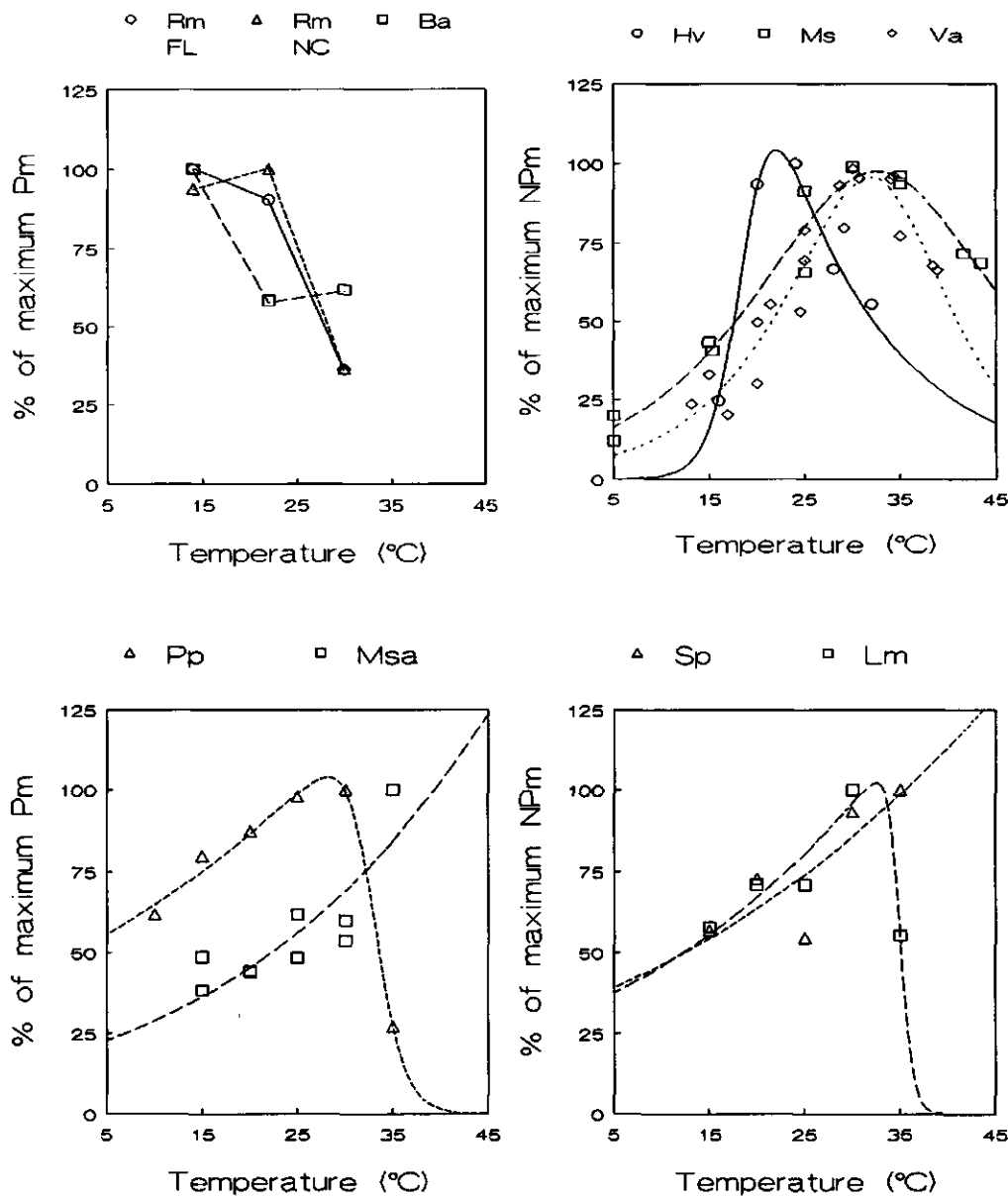


FIG.1: Effect of temperature on the irradiance-saturated photosynthesis ( $P_m$ : gross photosynthesis;  $NP_m$ : net photosynthesis) estimated from the rectangular hyperbola fits of PI curves from bibliographic data. Lines represent the fits of the temperature response curve as proposed by Johnson *et al.* (1974; equations (3) and (4) in the text). Y-axis: percentage of the maximum  $P_m$ - or  $NP_m$ -value measured.

Ba: *Batrachium aquatile*, Pokorny & Ondok (1980); Rm: *Ruppia maritima*, Koch & Dawes (1991); Hv: *Hydrilla verticillata*, Barko & Smart (1981); Va: *Vallisneria americana*, Ms: *Myriophyllum spicatum*, both from Titus & Adams (1979); Pp: *Potamogeton pectinatus*, Madsen & Adams (1989); Msa: *Myriophyllum salsugineum*, Orr *et al.* (1988); Lm: *Lemna minor*, Sp: *Spirodela punctata*, both from Wedge & Burris (1982).

TABLE 2: Parameter estimations of the temperature response curve of the maximum gross production (net production at saturating light intensity in the species marked with an \*) in different aquatic macrophytes. Headings are coefficients of the formula proposed by Johnson *et al.* (1974; see formulas 3 and 4, Section 2.1.2.). Datasets with less than 4 datapairs were left out. In all cases,  $r^2 > 0.96$ . Zm: *Zostera marina*, Leuschner & Rees (1993); Hv: *Hydrilla verticillata*, Ed: *Egeria densa*, both from Barko & Smart (1981); Pp: *Potamogeton pectinatus*, Madsen & Adams (1989); Msa: *Myriophyllum salsugineum*, Orr *et al.* (1988); Ms: *Myriophyllum spicatum*, Va: *Vallisneria americana*, both from Titus & Adams (1979); Sp: *Spirodela punctata*, Lm: *Lemna minor*, both from Wedge & Burris (1982).

	A	$E_s/R$	$\Delta S_i/R$	$\Delta H_i/R$	Fitted $T_{opt}$	n	p
Seagrasses:							
Zm	$e^{38}$	9867	313.6	93336	22.6	10	<.001
Submerged macrophytes:							
Hv*	$e^{160}$	45264	182.0	53171	22.0	5	NS
Ed*	$e^{34}$	8789	123.0	36726	22.8	5	NS
Pp	$e^{13}$	2400	201.1	61544	28.2	6	<.001
Msa	$e^{16}$	3732	—	—	(35)	9	<.001
Ms*	$e^{29}$	7357	52.8	16178	32.3	12	<.001
Va*	$e^{36}$	9551	81.6	25037	32.0	18	<.001
Floating macrophytes:							
Sp*	$e^{13}$	2642	—	—	(35)	5	<.01
Lm*	$e^{15}$	3159	472.6	145551	32.5	5	NS

thus no increase with temperature was detected.

In all cases with at least 6 datapairs available, significant fits were obtained, and  $r^2$  was always above 0.97. Clear differences can be detected among species regarding their sensitivity to suboptimal and to supra-optimal temperatures, as expressed by  $E_s/R$  (high values reflecting a sharp increase in  $P_m$  with temperature) and by  $\Delta H/R$  (high values meaning a sharp drop in  $P_m$  at temperatures above  $T_{opt}$ ) respectively.

In conclusion, although photosynthesis at saturating irradiance appeared to increase exponentially with temperature in almost all cases, it decreased sharply (as a general rule) at temperatures above a certain optimum. Temperature optima for saturated photosynthesis varied for the different species, in a range from 14 to higher than 35 °C. Therefore, no general trend may be derived for all aquatic macrophytes (contrary to the findings of Bulthuis, 1987, for seagrasses and a more limited dataset).

It is of interest to relate temperature optima with life-cycle strategies and/or distribution patterns for the different species reviewed. *B. aquatile* from Czechoslovakia, which showed the lowest temperature optimum, germinated before flooding occurred, and grew during early winter (Pokorny & Ondok, 1980): its low temperature optimum fits well with its winter-annual character. Temperature optima between 20 and 25 °C were found for *R. maritima* obtained from Florida and New Carolina (SE USA; Koch & Dawes, 1991), and for *E. densa* and *H. verticillata* obtained from Florida (Barko *et al.*, 1982). *P. pectinatus* collected from Wisconsin (USA) had its optimum temperature at 30 °C (Madsen & Adams, 1989), while higher optima were found for *Spirodela punctata* and *Lemna minor* L. (Wedge & Burris, 1982) obtained from Pennsylvania (USA), *M. salsugineum* from Victoria

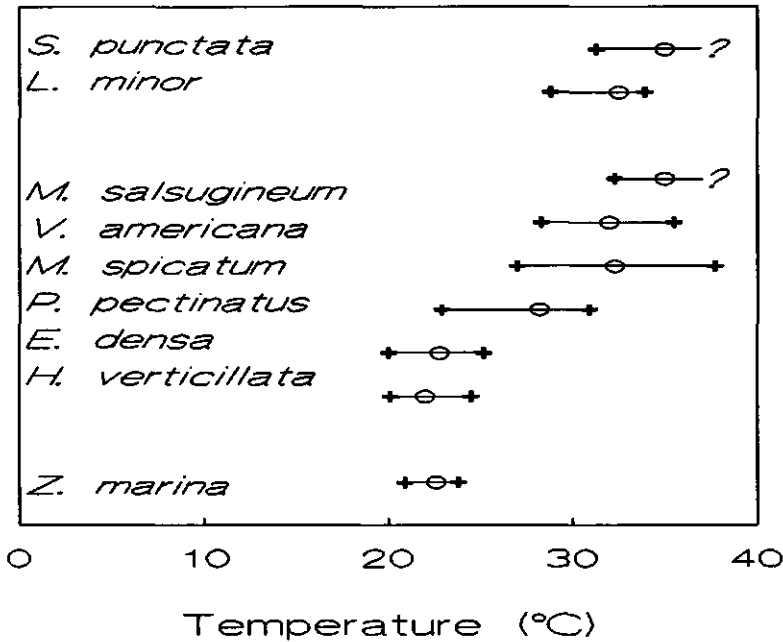


FIG.2: Temperature optima and 90% optimum ranges for the irradiance-saturated photosynthesis of several aquatic macrophytes. Data obtained from Fig.1, and from Leuschner & Rees (1993) for *Z. marina*.

(Australia; Orr *et al.*, 1988), and *V. americana* and *M. spicatum* from Wisconsin (Titus & Adams, 1979; see also Fig.2).

Apparently, a shift to higher temperature optima occurred when moving to colder climates, as indicated by the positive correlation between optimum temperature for  $P_m$  and latitude (Fig.3, left). This apparent contradiction is probably explained by the switch from winter- to summer-annual life-cycles of the species in our database along the transition from subtropical to temperate climates. This may be appreciated in a plot of the climatic temperature (annual average and range of monthly averages) versus the  $P_m$  temperature optimum (Fig.3, right): we hypothesise the datapoints above the line  $y=x$  to have a winter annual strategy (as optimum temperature is realised in winter, the lower section of the bar range in the plot), and those below the line to have a summer-annual strategy. The winter- or summer-annual character should be stronger with increased distance from the  $y=x$  line: *M. salsugineum*, *R. maritima* from North Carolina and *B. aquatile* may thus be expected to have an intermediate strategy (with optimal temperatures realised in spring and autumn).

Although the relationships discussed above left out both the influence of acclimation and the photosynthetic behaviour at low irradiance levels, the emerging patterns are of doubtless importance. Once the life-cycle strategy was incorporated in the latitudinal effect on the climatic temperature, a relatively clear picture has been obtained. Basing us on Fig.3, we expect our study object, the winter-annual *R. drepanensis*, to have low temperature optima for saturated photosynthesis. This hypothesis will be examined in the second part of this paper.

Similar to the irradiance-saturated photosynthesis, dark respiration increased



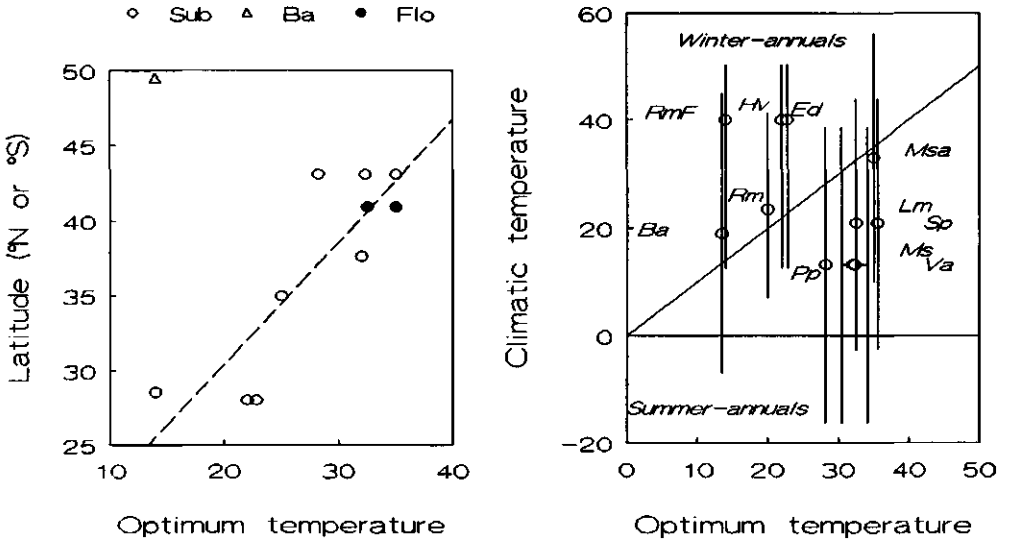


FIG. 3: Left: Relationship between the temperature optima for  $P_m$  and latitude for several aquatic macrophytes. Sub: submerged macrophytes. Ba: *Batrachium aquatile*. Flo: floating macrophytes. Line represents a fitted linear equation ( $y=0.81x+14.11$ ;  $r^2=0.86$ ).

Right: Relationship between climatic temperature and optimum temperature for irradiance-saturated photosynthesis (bars represent the range of average air temperatures between the coldest and the hottest months of the year, dots the annual average). The line where climatic temperature coincides with the optimum temperature ( $y=x$ ) is also indicated.

Ba: *Batrachium aquatile*, Pokorny & Ondok (1980); RmF: *Ruppia maritima* from Florida, Rm: *R. maritima* from North Carolina, both from Koch & Dawes (1991); Hv: *Hydrilla verticillata*, Ed: *Egeria densa*, both from Barko & Smart (1981); Va: *Vallisneria americana*, Ms: *Myriophyllum spicatum*, both from Titus & Adams (1979); Pp: *Potamogeton pectinatus*, Madsen & Adams (1989); Msa: *Myriophyllum salsugineum*, Orr *et al.* (1988); Lm: *Lemna minor*, Sp: *Spirodela punctata*, both from Wedge & Burris (1982).

exponentially with temperature, and in two cases it decreased at temperatures above a certain optimum (Fig. 4). Table 3 shows the parameter estimations and statistics of the fitted curves. Unfortunately, only two datasets with more than 5 observations were available, but in both cases the fits were highly significant, and  $r^2$  values were above 0.99. Ranges of  $E_a/R$ ,  $\Delta H/R$  and  $\Delta S/R$  estimations were similar for R and for  $P_m$ .

In conclusion, optimal temperatures for dark respiration are similar or higher than the optimum for saturating photosynthesis. While for certain species the response curve of dark respiration followed quite closely that of  $P_m$  (as for *E. densa*, *P. pectinatus* and *M. salsugineum*), this was not so for other species: R increased with temperature up to 30 °C for *H. verticillata* and the New Carolina population of *R. maritima*, which both had optimum  $P_m$ -temperature at 20-24 °C.

Parameter estimations from our fits of macrophyte literature data and several examples of parameter values estimated for the temperature response of different biological rates provided by Johnson *et al.* (1974) are given in Table 4. Ranges are quite comparable, with a trend to lower  $E_a$  values (about half) for the metabolism of the aquatic macrophytes and higher  $\Delta S_1$  values for the  $P_m$  of the aquatic macrophytes (double for the submerged

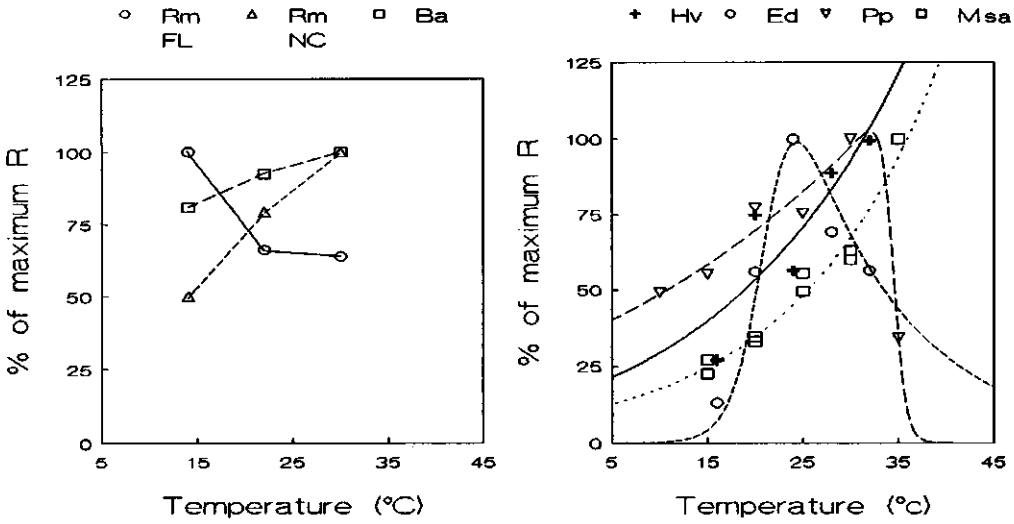


FIG.4: Effect of temperature on the dark respiration (R) estimated from the rectangular hyperbola fits of PI curves from bibliographic data. In the right graph, temperature response fits are shown (see text, equations (3) and (4)). Y-axis: percentage of the maximum R-value measured.

Rm: *Ruppia maritima* from Florida (FL) and North Carolina (NC), Koch & Dawes (1991); Ba: *Batrachium aquatile*, Pokorny & Ondok (1980); Hv: *Hydrilla verticillata*, Ed: *Egeria densa*, both from Barko & Smart (1981); Pp: *Potamogeton pectinatus*, Madsen & Adams (1989); Ms: *Myriophyllum salsugineum*, Orr *et al.* (1988).

macrophytes, four times higher for the floating macrophytes).

The existence of a significant effect of temperature on  $K_m$  and  $\alpha$  was tested by analyzing the significance of linear and exponential fits (only datasets with at least 3 datapoints were used). Exponential fits were used to prevent concluding a non-significant effect of temperature due to non-linearity.

Though less data are available than for  $P_m$ , it appears that  $K_m$  does not generally increase with increasing temperature (Fig.5). While this was so for *M. salsugineum*,  $K_m$  was maximal at intermediate temperatures for *R. maritima*, and decreased with increasing

TABLE 3: Parameter estimations of the temperature response curve of the dark respiration in different aquatic macrophytes. Optimum temperature is between brackets when it refers to the highest temperature at which respiration was measured. In all cases,  $r^2 > 0.96$ . Code of the species and data source as in Table 2.

A	$E_a/R$	$\Delta S_1/R$	$\Delta H_1/R$	Fitted $T_{opt}$	n	p (F-test)	
<b>Submerged macrophytes:</b>							
Hv	$e^{21}$	4938	---	---	(32)	5	<.01
Ed	$e^{160}$	45662	184.0	54216	24.4	5	NS
Pp	$e^{14}$	2990	500.9	154004	32.1	6	<.001
Ms	$e^{24}$	6092	---	---	(35)	9	<.001

TABLE 4: Parameter estimations of the temperature response curve of the maximum photosynthesis and respiration in different aquatic macrophytes, as compared with the parameter estimations for the temperature response of several biological processes. Temperature response of all bacterial processes reviewed from Johnson *et al.* (1984).  $E_a$  and  $\Delta H_1$  in Joules,  $\Delta S_1$  in e.u.

	A	$E_a$	$\Delta S_1$	$\Delta H_1$
Submerged macrophytes				
$P_m$	$e^{13}-e^{160}$	19954-376325	439-1672	134504-511677
R	$e^{14}-e^{160}$	12519-191187	184- 500	227002-644815
Floating macrophytes				
$P_m$	$e^{13}-e^{15}$	21966- 26264	3929	1210111
Luminiscence <i>Vibrio</i>				
	$e^{24}-e^{48}$	62805-125610	164- 329	209350-418700
Luminiscence <i>Achromobacter</i>				
	$e^{56}$	142358	266	334960
Oxygen consumption <i>Rhizobium</i>				
	$e^{21}$	56106	306	401952
Meth. blue reduction <i>Rhizobium</i>				
	$e^{21}$	56943	400	527562
Multiplication rate <i>Escherichia</i>				
	$e^{23}$	628050	476	62805
Range bacterial metabolism				
	$e_{21}-e_{48}$	56106-628050	164- 476	62805-527562

temperature for *P. pectinatus*. Linear and exponential regression were significant both for *M. salsugineum* and *P. pectinatus* (Table 5).

Similar results were found for  $\alpha$  (Fig.6). Although no temperature-dependence could be defined for *R. maritima*, a relatively clear trend to decreasing  $\alpha$  with increasing temperatures was found for *B. aquatile* and *M. salsugineum*. On the contrary,  $\alpha$  increased with temperature up to a breaking point at 30 °C for *P. pectinatus*. Exponential regression was significant for *M. salsugineum* and *P. pectinatus*, and linear regression was significant for *P. pectinatus* once removing the 35 °C datapoint.

In conclusion, the few data available suggest that  $K_m$  and  $\alpha$  are also temperature dependent, the shape of the response curve being very different for different species. With  $K_m$ , LCP and  $\alpha$  being parameters strongly dependent on the interaction between irradiance and temperature, we conclude that a more thorough knowledge of the mechanisms that explain this interaction is needed to adequately model their temperature dependence.

#### 2.3.4. Seasonal acclimation

To our knowledge, up till now no paper has examined the effect of acclimation on the photosynthetic temperature response of any aquatic macrophyte. As the modification of the temperature response curve due to acclimation may hamper seasonal extrapolation, a common practice in modelling exercises, the magnitude of acclimation capacity of aquatic macrophytes is of significant interest.

The reviewed evidence from terrestrial macrophytes suggests that acclimation to contrasting temperatures does not result in wider temperature response curves, but in a shift in such curves (Berry & Björkman, 1980; see above). If this is also the case for aquatic macrophytes, the photosynthetic temperature response curve of plants acclimated to different temperatures should not differ in width from each other, while the temperature response

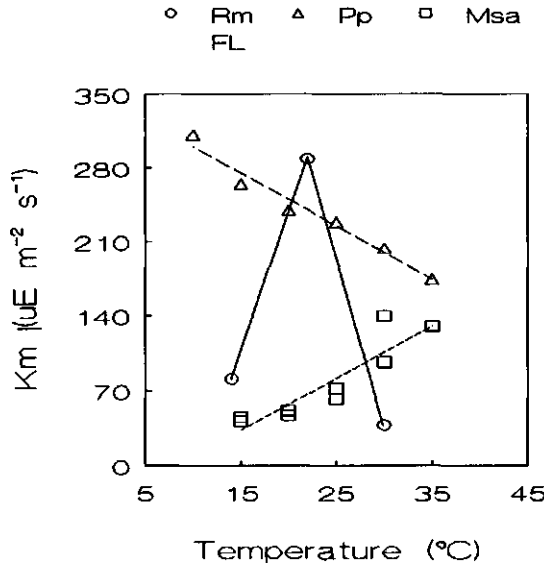


FIG.5: Effect of temperature on the half saturation constant ( $K_m$ ) estimated from the rectangular hyperbola fits of PI curves from bibliographic data. Species coding as in Fig.4. Linear fits are also shown for *P. pectinatus* and *M. salsugineum*. *R. maritima* from North Carolina (Koch & Dawes, 1991) is not shown here, as it followed the same trend as the Florida population.

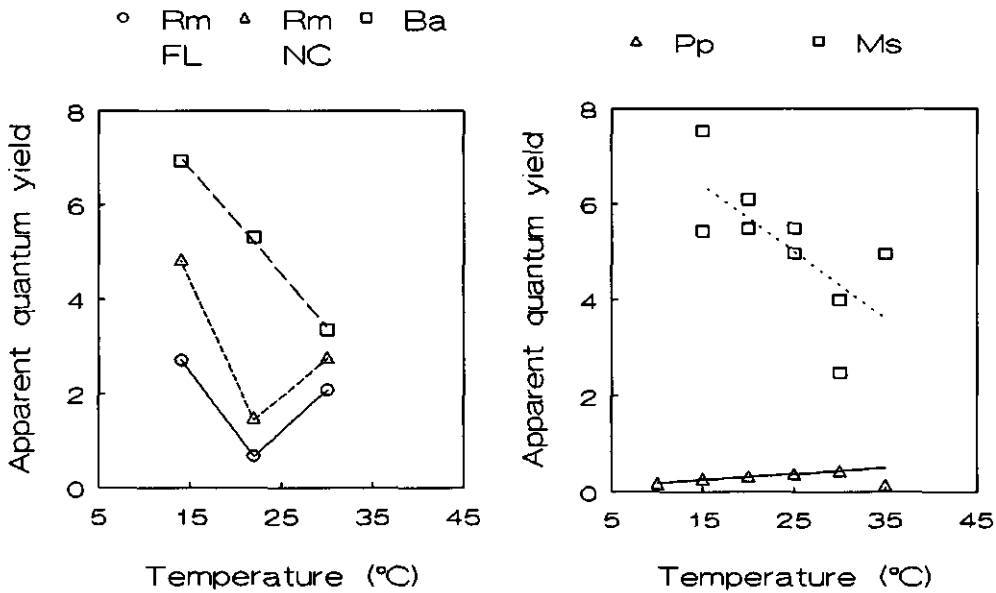


FIG.6: Effect of temperature on the apparent quantum yield ( $\alpha$ , in  $\mu\text{g O}_2 \text{ g}^{-1} \text{ afdw min}^{-1} \mu\text{E}^{-1} \text{ m}^2 \text{ s}$ ) estimated from the rectangular hyperbola fits of PI curves from bibliographic data. Coding of the data as in Fig.4. Linear fits are also shown for *B. aquatile*, *P. pectinatus* and *M. salsugineum*.

TABLE 5: Results of the linear and exponential regression of the half saturation constant ( $K_m$ ) and apparent quantum yield ( $\alpha$ ) of the PI curve of two submerged macrophytes modelled using a rectangular hyperbola. F-test for significance of the regression fit is also provided. Highest temperature datapoint (37°C) was excluded from the dataset for the linear regression for *P. pectinatus* (Pp).

	n	Linear regression ( $Y=a+bX$ ):				Exponential regression ( $Y=a * e^{bX}$ ):			
		a	b	r <sup>2</sup>	p	a	b	r <sup>2</sup>	p
$K_m$									
Ms	9	-39.1	4.83	0.82	<.001	15.6	0.063	0.97	<.001
Pp	6	350.5	-5.05	0.97	<.01	378.3	-0.021	0.999	<.001
$\alpha$									
Ms	9	8.5	-0.14	0.48	NS	9.9	-0.028	0.97	<.001
Pp	5	0.06	0.013	0.99	<.01	0.25	0.006	0.88	<.05

curve measured on progressively acclimated plants (such as measured in the field along the different seasons) should be wider than any temperature response curve assessed at one, single moment. This hypothesis will be tested in the second part of this paper using our own experimental data, while its implications for the seasonal 'temperature-response' curve will be briefly examined here.

Seasonal and 'instantaneous' data for the temperature dependence of photosynthesis and respiration taken from Maberly (1985) and Evans *et al.* (1986) are shown in Figs. 7 and 8. Contrary to what is expected, the 'instantaneous' temperature response curve for  $P_m$  is wider than the seasonal temperature response curve in both graphs. Although the influence of light acclimation and plant age cannot be excluded, a damage to the photosynthetic system caused by prolonged exposure to supra-optimal temperatures, (as mentioned by Berry & Raison, 1981) might also explain this figure. It is thus reasonable to keep in mind that, concurrent with the improvement in photosynthetic performance after a prolonged exposure to supra-optimal temperatures due to acclimation, the occurrence of long-term, non-reversible damage to the photosystem might result in poorer photosynthetic rates.

While a similar figure is shown for  $K_m$  in Fig. 7, no clear trend may be concluded for the dark respiration. In conclusion, none of both graphs shown here support the existence of a significant widening of the temperature response curve due to acclimation along the seasons.

### 2.3.5. Summary

We intended to discern general patterns in the temperature response of submerged non-marine macrophytes. Data from 10 different species were reviewed from literature, and showed the characteristic optimum curve hypothesized for the temperature response of irradiance-saturated photosynthesis ( $P_m$ ) and dark respiration ( $R$ ). Moreover, the model proposed by Johnson *et al.* (1974) successfully fitted all large datasets, showing that reversible thermal inactivation is a mechanistic description worthwhile to apply to these metabolic rates.

On the contrary, general trends could not be observed for the apparent quantum yield

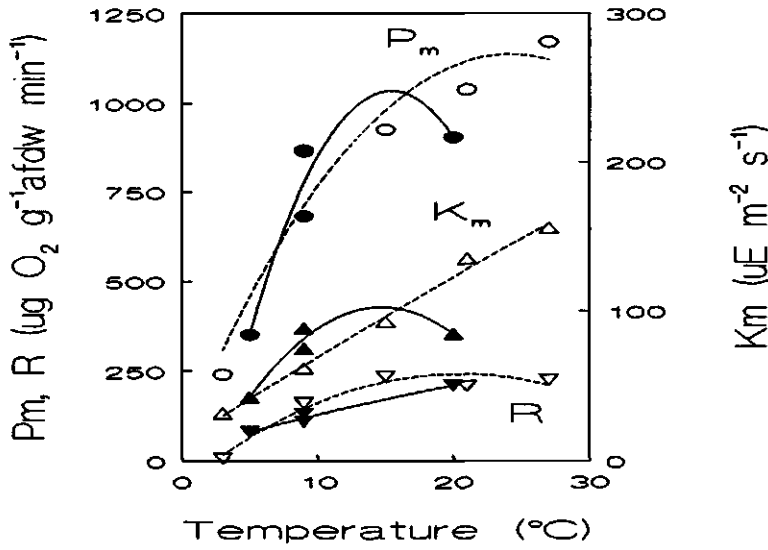


FIG.7: Effect of temperature acclimation on maximum rate of gross photosynthesis ( $P_m$ , circles), half saturation constant ( $K_m$ , triangles) and dark respiration ( $R$ , inverted triangles) estimated from the rectangular hyperbola fits of PI curves in *Fontinalis antipiretica* Hedw. Data from Maberly (1985). Open symbols: photosynthesis measured at different temperatures from plants collected in November. Filled symbols: photosynthesis measured from plants collected in November, March, May and August, at the actual temperature of the lake at the moment of collection.

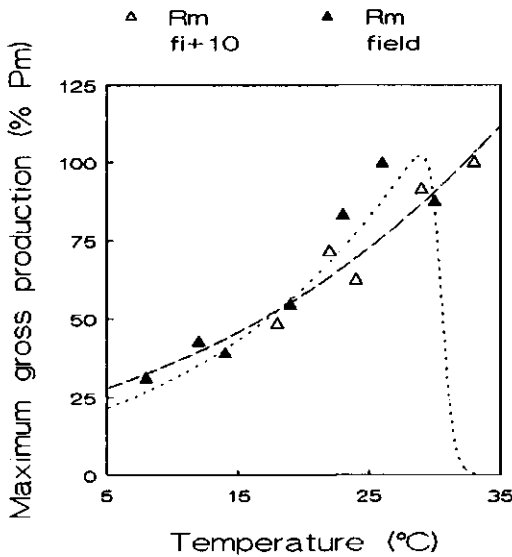


Fig.8: Effect of temperature acclimation on the maximum rate of gross photosynthesis ( $P_m$ ) estimated from the rectangular hyperbola fits of PI curves in *Ruppia maritima* L. Data from Evans *et al.* (1986). Lines represent the fits of the temperature response curve as proposed by Johnson *et al.* (1974). 'field': photosynthesis measured at the same temperature as in the moment of collection in the field. 'fi+10': photosynthesis measured at 10 °C above the field temperature. Y-axis: percentage of the maximum  $P_m$ -value measured.

( $\alpha$ ), light compensation point (LCP) and half saturation constant ( $K_m$ ). As these parameters depend strongly on the interaction with irradiance, the present simplified models are not capable of explaining adequately their temperature dependence.

We also formulated a hypothesis for a formal coupling of the temperature effect on photosynthesis with the species life-cycle. This hypothesis was based on a comparison between the optimal temperature for saturated photosynthesis and the 'climatic' temperature range in the locality of origin of the different species reviewed. The shift from winter- to summer-annual life cycles from subtropical to temperate latitudes was found to agree well with the positive correlation between latitude and optimal temperature for  $P_m$ .

Finally, the effect of acclimation on seasonal extrapolation of parameter estimates was briefly examined. Contrary to what we expected, none of both datasets reviewed from literature supported the existence of a significant widening of the temperature response curve due to acclimation. If anything, the temperature response curve was narrowed along the spring-summer period. We concluded that, concurrent to the improvement of photosynthetic performance due to acclimation, the occurrence of long-term damage of the photosystem might result in poorer photosynthetic rates when plants are exposed to gradually increasing temperatures.

### 3. PHOTOSYNTHETIC ACCLIMATION AND FLOWER INDUCTION IN *Ruppia drepanensis*

#### 3.1. Materials and methods

##### 3.1.1. Experiment 1: Acclimation to high temperatures

###### 3.1.1.1. Plant material cultivation

Seeds attached to plant material were collected in an old channel of the Salinas de San Isidoro (Doñana National Park, SW Spain), at the end of the growing season (June 1991). A mixed population of *R. drepanensis* and *Athenia orientalis* (Tzvelev) García-Murillo & Talavera occurs in this locality. Seeds and wet plant material were stored in plastic bags at 4 °C, until October 1991.

The seeds were germinated in tap water, in the dark and at room temperature (20 °C). After three days, germinated seedlings were selected and transferred to containers with brackish water (0.5 g l<sup>-1</sup> artificial sea salt), where they stayed for one week under low irradiance conditions (10 to 20  $\mu\text{E m}^{-2} \text{s}^{-1}$ ) and at room temperature. Previous experience had shown that this treatment resulted in the best survival percentage. After one week the seedlings, of between 3 and 5 cm size, were randomly distributed over the treatments and subsequently planted.

Two levels of temperature (20 $\pm$ 0.5 and 30 $\pm$ 0.5 °C) were used, each consisting of two aquaria (60 x 40 x 40 cm<sup>3</sup>) containing 39 plastic cups (150 cm<sup>3</sup>) filled with a mixture of sand and clay (3:1 by weight) and covered by 1 cm of washed sand. One seedling was planted in each separate plastic cup. Space among the cups was also filled with washed sand, and the remaining volume of the aquaria was filled with tap water one week before planting the seedlings.

The four aquaria were placed in one phytotron, having a common light source of 17

fluorescent tube lamps (Philips, colour 84, 36 W) providing an irradiance (PAR) of  $240 \mu\text{E m}^{-2} \text{s}^{-1}$  at 1 cm below the water surface (measured with a LICOR LI-192S sensor). The photoperiod was 16 hours. Demineralised water was used to replenish the aquaria when necessary.

### 3.1.1.2. Characterization of plant material

During the first five weeks of the experiment, the following parameters were recorded in 20 randomly-selected plants from each treatment (10 from each of the two aquaria): number of shoots per plant, number of leaves per plant, length of the longest leaf and number of flowers per plant. The same plants were measured each week.

After four weeks, six plants were harvested from each treatment to determine their biomass (above- and below-ground fractions). Fresh weight and dry weight ( $70^\circ\text{C}$ , 24 h) were recorded, and ash-free dry weight was calculated with a dw/afdw regression from the 7-weeks plant material (see below).

Plants collected after 7 weeks for the photosynthesis measurements were also used for biomass determination (fresh, dry and ash-free dry weights, the last after 3 h ashing at  $520^\circ\text{C}$ ). Subsamples were taken to determine the aboveground to belowground biomass ratio. The same procedure was followed with the remaining 18 plants, which were harvested in the 13th week.

Leaf subsamples from the 7th week were used for chlorophyll-*a* and -*b* determination, according to Winterman & De Mots (1965). Phaeopigments were not measured as they were always non-detectable in previous experiments (Santamaría & Van Vierssen, 1994). A fw/afdw regression line for the aboveground biomass samples from the remaining plant material was used to express chlorophyll concentration per g afdw.

### 3.1.1.3. Photosynthesis measurements

The experimental set-up and procedure for photosynthesis measurements was as described in Santamaría & van Vierssen (1994). Temperature during the incubation was kept at  $\pm 1^\circ\text{C}$  of the desired value. The temperatures selected were 10, 20 and  $30^\circ\text{C}$ . A full PI set of measurements was performed for each temperature, PI determinations for each of the different temperatures being performed on separate days. Irradiances at 1 cm below the water surface were on average 25, 50, 75, 100, 150, 190, 265, 300, 380 and  $435 \mu\text{E m}^{-2} \text{s}^{-1}$  respectively.

### 3.1.2. Experiment 2: effect of low temperatures and seedling vernalization on flower induction

Seeds were collected at the same place and date as in experiment 1, and stored as described above until December 1992. The seeds were germinated likewise, but germinated seedlings stayed only three days in brackish water.

Seedlings of four days of age were transferred to freshwater, and half of them was randomly selected and subjected to vernalization conditions ( $7^\circ\text{C}$ , hereafter referred to as the 'vernalization pre-treatment'), while the other half was kept at  $20^\circ\text{C}$ . Low irradiance



was provided to limit seedling growth during the pre-treatment period. After one week, seedlings were randomly distributed and planted under the different treatments. Each treatment consisted of 19 vernalized and 20 non-vernalized plants, regularly interspersed in each respective aquarium.

Two temperatures (20 and 14 °C) and two photoperiods (16 and 10 h) were combined in three treatments. Treatments were 20 °C, 16 h photoperiod; 14 °C, 16 h photoperiod; and 14 °C, 10 h photoperiod (hereafter referred as '20L', '14L' and '14S' respectively). The set-up of the different treatments (1 aquarium per treatment) was as described above for experiment 1. Irradiance inside the phytotrons was  $250 \mu\text{E m}^{-2} \text{s}^{-1}$ .

The number of plants with flowers was recorded weekly. After 12 weeks, half of the plants (10 vernalized, 9 non-vernalized) were randomly selected and harvested for the determination of biomass, tissue nutrient content and number of flowers per plant. Half of the harvested plants were dried (70°C, 24 h) and stored until further determination of the nutrient tissue content. The remaining plants were divided in belowground parts (rhizomes and roots), basal shoots and vertical shoots, dried (110°C, 24 h) and ashed (520°C, 3h) to calculate the ash-free dry weight (afdwt). An afdwt/dwt regression line fitted using the latter data set was used to calculate the afdwt of the 'nutrient' subsample. Nutrient determination followed Novozamsky *et al.* (1983), and consisted of a digestion of the plant material in a mixture of selenium, hydrogen peroxide, salicylic acid and sulphuric acid, followed by a determination of the total N and total P using a Technicon auto-analyzer.

For the 14L and the 14S treatments, the remaining 20 plants were divided in two groups of 10 plants (5 vernalized, 5 non-vernalized): one group was kept under the same growth conditions, while the other was transferred to a higher temperature (14L -> 20L) or a longer photoperiod (14S -> 14L) respectively. The 20 plants of the 20L treatment were kept under the same conditions. The number of plants with flowers was recorded weekly, and all plants were harvested in the 17th week (5 weeks after the transfer) for determination of the number of flowers per plant, number of seeds per plant, biomass and nutrient tissue content (as described above).

### 3.1.3. Calculations and statistical analysis

Statistical analyses closely followed Santamaría & van Vierssen (in prep.), and were performed with the SAS statistical package (SAS Institute Inc., 1988). Morphometric variables from experiment 1 were fitted to an exponential equation (as a logistic growth model was not needed in the first 5 weeks of growth), using a non-linear iterative technique based on the Marquardt algorithm (Conway *et al.*, 1970). A separate curve was fitted for each of the 20 plants measured in every treatment.

Parameters of the curves (experiment 1) and number of flowers, biomass, leaf chlorophyll content and nutrient tissue content data (experiment 1 and 2) were compared by means of Analysis of Variance and Student-t test. ANOVA tests were done using the General Linear Models (GLM) procedure in SAS, after  $\log_{10}$  or arcsine square root transformation if the residuals were not normally distributed or the residual variances were not homogeneous. Two- and Three-Way ANOVA were followed by multiple comparisons among treatments, using the LSMEANS option in SAS and adjusting the comparisonwise error rates (CER) to maintain an experimentwise error rate (EER) of 0.05.

The number of flowers was zero for all plants in some of the treatments. In such cases, strong heterogeneity of the variances prevented the use of ANOVA and Student-t test.

This problem was tackled by performing an One-Tail Student-t test to falsify the null hypothesis 'the average number of flowers per plant (in the treatments having flowered) is zero'. Significant results in such t-tests were interpreted as indicating significant differences between the examined treatment and the treatment(s) showing no plant with flowers.

The number of flowering plants was compared by means of a chi-square test, adjusting the CER to an EER of 0.05 when several separate  $\chi^2$  were performed within the same experiment. Interaction between two factors was tested using an n-Way Classification  $\chi^2$  test as described in Steel & Torrie (1981).

Photosynthesis data sets relating oxygen concentration to time for a particular irradiance level were checked for measurement errors and lag phases were excluded. Oxygen exchange rates were calculated by linear regression and expressed in  $\mu\text{g O}_2 \text{ g}^{-1} \text{ afdw min}^{-1}$  (biomass-related curves) and in  $\mu\text{g O}_2 \text{ mg}^{-1} \text{ Chl(a+b) min}^{-1}$  (chlorophyll-related curves). A total of 18 biomass-related and 18 chlorophyll-related light-response (PI) curves was obtained from the experiment (3 replicate curves times 3 'experimental' temperatures times 3 'growth' temperatures, 'experimental' temperatures hereafter referring to the temperatures at which photosynthetic rates were measured, and 'growth' temperatures to the temperatures at which the plants were previously grown).

The resulting data set for each treatment replica consisted of the experimental irradiance levels and the corresponding  $\text{O}_2$  exchange rates. A rectangular hyperbola was fitted to each data set using the non-linear (NLIN) procedures of the SAS statistical package (SAS Institute Inc., 1988). The fitted equation was as described above (equation 5, Section 2.3.3.).

The resulting model parameter estimates were used for Two-Way ANOVA and multiple comparisons (as above), together with two derived parameters, the apparent quantum yield ( $\alpha = P_m/K_m$ ; Hootsmans & Vermaat, 1994) and the light compensation point (LCP). Estimated net oxygen production rates at the irradiance at which the plants were grown ( $P_{250}$ ) and at  $500 \mu\text{E m}^{-2} \text{ s}^{-1}$  ( $P_{500}$ ) were also tested.

Biomass and flowering data from experiment 1 and 2 were combined to test an overall temperature effect. Biomass and flowering data from experiment 2 were also combined with data from Santamaría & van Vierssen (in prep.) to assess the existence of an interactive effect of photoperiod and temperature. Number of flowering plants were compared using multiple  $\chi^2$  tests and a n-Way Classification  $\chi^2$  test as described above. Biomass in the 13th and 12th week (experiment 1 and 2 respectively) and in the 12th-17th and the 11th-16th weeks (experiment 2 and Santamaría & Van Vierssen, in prep., respectively) were compared using Student-t test and Three-Way ANOVA followed by multiple comparisons, as described above. Previous to these statistical comparisons between different experiments,  $\chi^2$  and Student-t test were performed between the control treatments of each of the experiments ( $20^\circ\text{C}$ , 16 h photoperiod) to assess the existence of significant differences attributable to lack of reproducibility, or to the one-week difference in age in the case of the biomass values. These tests were non-significant in all cases, and we thus decided to perform the subsequent statistical comparisons.

Biomass values were also used to fit a logistic curve for each treatment, assuming zero biomass for the seedlings if biomass was measured only twice along time. The resulting curves were compared for overall differences between the fits using the following F statistic (Vermaat & Hootsmans, 1994a):

$$F = \frac{[RSS_{1+2} - (RSS_1 + RSS_2)] / [df_{1+2} - (df_1 + df_2)]}{(RSS_1 + RSS_2) / (df_1 + df_2)} \quad (6)$$

where RSS stands for residual sum of squares and df for degrees of freedom,  $RSS_{1+2}$  is the RSS of the regression of the two data sets together, and  $RSS_1$  and  $RSS_2$  are the RSS of the separate regressions. The zero hypothesis is that the two sets of data pairs can be described best by one combined fit, the alternative is that two separate fits 'are better'. Despite the limited number of X-levels used for the logistic fits (3 to 6, including the added zero datapair), the number of data pairs was reasonably high in all regressions (always > 38). Previous comparisons between the control treatments from different experiments resulted, as for the ANOVA, in non-significant differences.

The above-described F-statistic (equation 6) was also used to test the existence of overall significant differences in the biomass dependence of tissue nutrient content of plants of different age (experiment 2). Relationship between tissue nutrient content and aboveground biomass was modelled by means of the following exponential equation:

$$NUT = A * e^{B * a.fdw} + C \quad (7)$$

where NUT stands for tissue nutrient content (total N or total P, in mg per g afdw of aboveground biomass) and afdw for the aboveground biomass, A is the Y-axis intercept of the curve (the maximum tissue nutrient content) and C the minimum asymptotic value (the minimum tissue nutrient content).

## 3.2. Results

### 3.2.1. Experiment 1: Acclimation to high temperatures

#### 3.2.1.1. Plant morphology

Exponential growth curves are shown in Fig.9. Relative growth rates from the exponential fits were significantly higher at 20 °C than at 30 °C for the variables total number of leaves per plant (NLE), total number of shoots per plant (NBU) and length of the longest leaf (LGL; Fig.10, t-tests:  $p < 0.001$ ). The actual values measured after 35 days of growth, however, were only significantly higher at 20 °C for the LGL (LGL35; t-test:  $p < 0.001$ ).

#### 3.2.1.2. Plant biomass

Temperature significantly affected total biomass and aboveground biomass yield, while belowground biomass and the AGB/BGB ratio remained unaffected (Table 6). Temperature and age interacted significantly for the total biomass. A non-surprising significant effect of age on all biomass variables was also detected.

While variances were not homogeneous for the ANOVA test, performing separate Student-t test within each of the different age categories resulted in homogeneous variances. Thus, multiple comparisons for all variables but AGB/BGB are based on such separate t tests (not based, thus, on the LSMEANS estimations from the two-way ANOVA). Significant differences between different temperatures were only found within the oldest plants (91 days of age) for the total and aboveground biomass, while they were found within both the 91 and the 49 days-old plants for the belowground biomass (Table 7). On the other hand, significant differences between the 28 days old and the older plant material were found for the

TABLE 6: ANOVA of biomass yield of *R. drepanensis* grown at two different temperatures (20 and 30 °C) after 28, 49 and 91 days of growth. Displayed values are probabilities and symbols refer to the levels of significance (NS: non-significant; \*:  $p < .05$ ; \*\*:  $p < .01$ ; \*\*\*:  $p < .001$ ).

	Total biomass	Belowground biomass (BGB)	Aboveground biomass (AGB)	AGB/BGB ratio
Temp	.0380*	.1298 <sup>NS</sup>	.0345*	.3153 <sup>NS</sup>
Age	.0001 <sup>***</sup>	.0001 <sup>***</sup>	.0001 <sup>***</sup>	.0001 <sup>***</sup>
Temp*Age	.0364*	.2826 <sup>NS</sup>	.2463 <sup>NS</sup>	.5702 <sup>NS</sup>

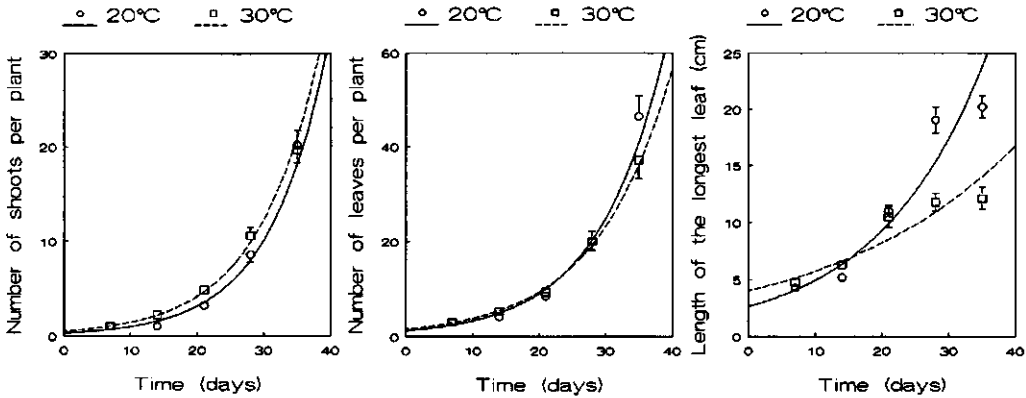


FIG.9: Effect of temperature on the early development of several morphometric parameters in *R. drepanensis*. Datapairs are the average of 20 replicate measurements. Lines showed in the figures are means of 20 separate exponential fits for the 20 replicate plants.

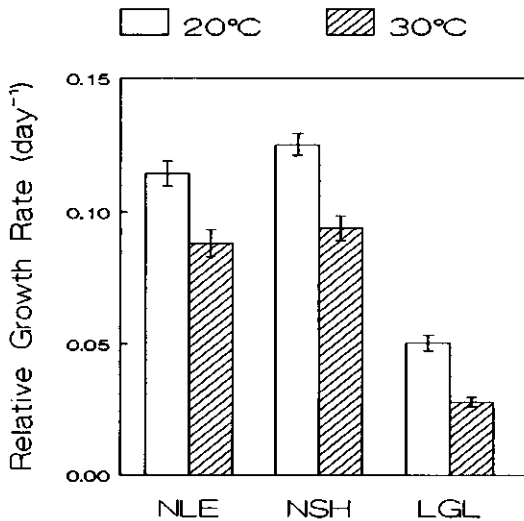


FIG.10: Exponential relative growth rate (left) for several morphometrical parameters of *R. drepanensis* plants grown at 20 and 30 °C. NLE: total number of leaves per plant. NSH: total number of shoots per plant. LGL: length of the longest leaf per plant (cm).

Table 7: Multiple comparisons for total, aboveground and belowground biomass and for the ratio between aboveground and belowground biomass (AGB/BGB). Small letters refer to horizontal comparisons, capital letters refer to vertical comparisons. Comparisons were not performed when the factor involved yielded non-significant results in the previous ANOVA. Such comparisons are thus not shown in the table.

Temp:	Total biomass		Aboveground biomass		Belowground biomass		AGB/BGB ratio	
	20°C	30°C	20°C	30°C	20°C	30°C	20°C	30°C
Age:								
28	a	a	a	a	a	a	A	C
49	b	b	b	c	b	b	B	CD
91	c	d	d	e	c	d	B	D

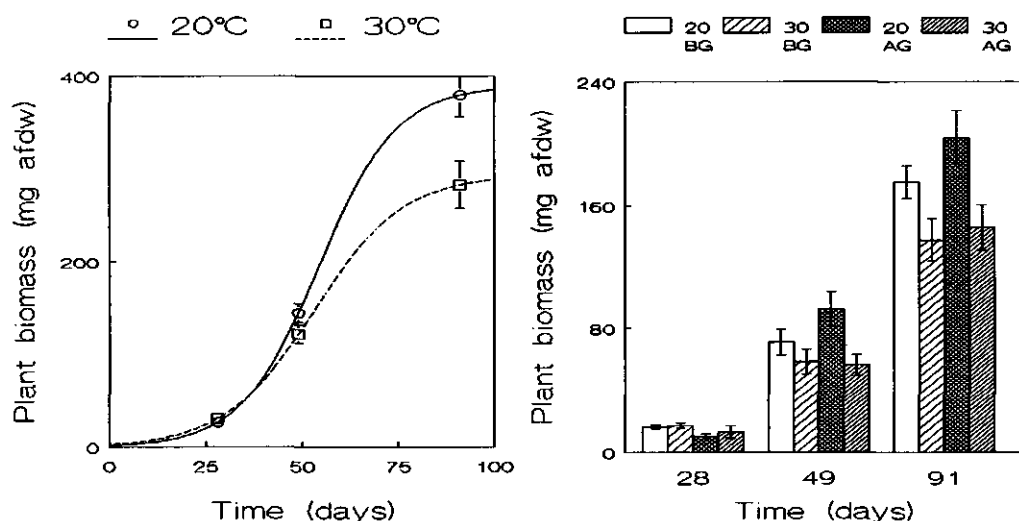


Fig.11: Development of individual plant biomass along time (left) and belowground and aboveground biomass yield (right; average  $\pm$  standard error) of *R. drepanensis* plants grown at two different temperatures. Datapoints are the average of 6 to 50 values. Logistic fits are also provided.

AGB/BGB biomass. As a general trend, both the above- and the belowground biomass (and thus also the total biomass) were lower at 30 °C (Fig.11), while the AGB/BGB ratio remained the same (Fig.12).

Basal and vertical shoot biomass were measured separately on the 91th day. Temperature had no significant effect on the ratio of vertical shoots to aboveground biomass (t-test,  $p=0.30$ ).

### 3.2.1.3. Nutrient content and chlorophyll concentration

Temperature, plant fraction and age, and also the interaction between plant fraction and age, all significantly affected total N content (Table 8). On the contrary, total P was only

TABLE 8: ANOVA and multiple comparisons for the effect of temperature ('temp'), plant fraction ('plfr'; above- versus belowground fraction) and age (49 vs. 91 days) on tissue nutrient content of *R. drepanensis*. ANOVA: Displayed values are probabilities and symbols refer to the levels of significance (NS: non-significant; \*:  $p < .05$ ; \*\*:  $p < .01$ ; \*\*\*:  $p < .001$ ). Multiple comparisons: Small letters refer to horizontal comparisons, capital letters refer to vertical comparisons. As no comparison between both temperatures was significant, they are not shown in the table.

ANOVA			Multiple comparisons					
	Total N	Total P	temp:	20°C		30°C		
			plfr:	above	below	above	below	
			age:					
temp	.0063 <sup>***</sup>	.4197 <sup>NS</sup>	total N	49	Aa	Ca	Ac	Cd
plfr	.0001 <sup>***</sup>	.3607 <sup>NS</sup>	91	Bb	Db	Be	Ce	
age	.0001 <sup>***</sup>	.0003 <sup>***</sup>	total P	49	Aa	Ca	Ac	Bc
temp*plfr	.3991 <sup>NS</sup>	.8077 <sup>NS</sup>	91	Bb	Cb	Ad	Bd	
plfr*age	.0397 <sup>*</sup>	.0274 <sup>*</sup>						
temp*age	.0734 <sup>NS</sup>	.0820 <sup>NS</sup>						
temp*plfr*age	.3900 <sup>NS</sup>	.4988 <sup>NS</sup>						

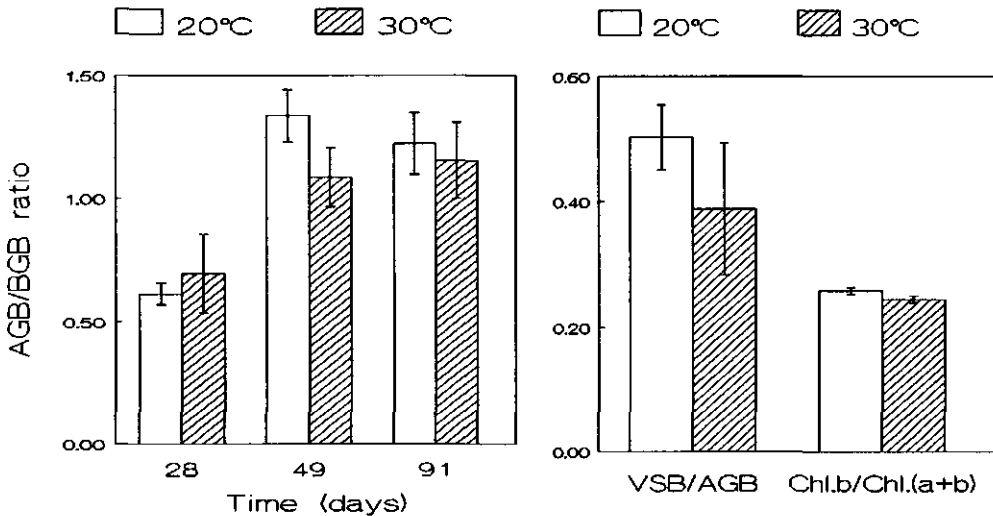


FIG. 12: Aboveground/Belowground biomass ratio (AGB/BGB, left graph), fraction of vertical shoot biomass relative to total aboveground biomass (VSB/AGB, right graph) and fraction of chlorophyll b relative to total chlorophyll (Chl.b/Chl.(a+b), right graph) in *R. drepanensis* plants grown at two different temperatures. Average  $\pm$  standard error.

significantly affected by age and its interaction with plant fraction.

Multiple comparisons are also shown in Table 8. As no comparison between temperatures was significant, they are not shown in the table. All age comparisons but one (within the belowground fraction at 30 °C) were significant for total N, while only one (within the aboveground fraction at 20 °C) was significant for total P. Only one comparison between both plant fractions was significant for total N (within the 49-days old plants grown

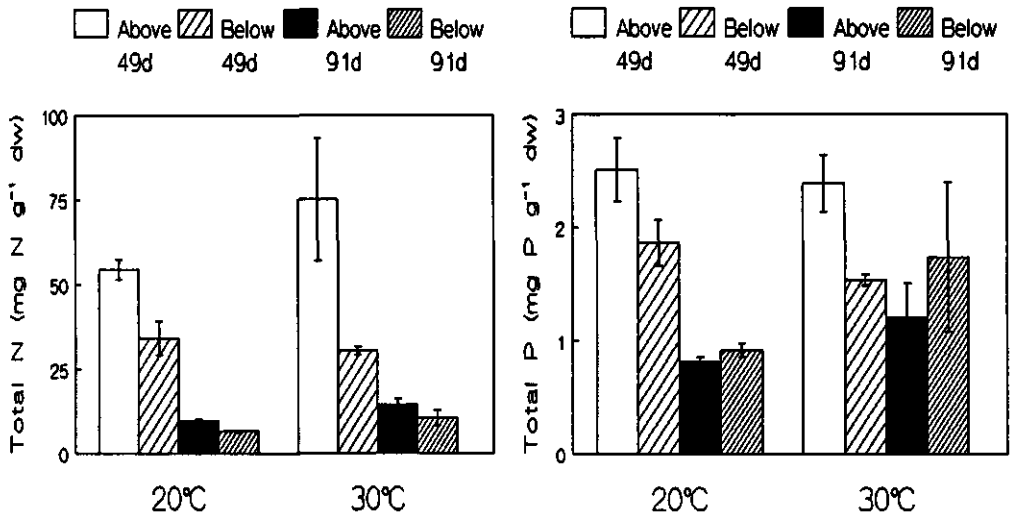


FIG.13: Tissue nutrient content of *R. drepanensis* plants grown at two different temperatures. 'Above': aboveground fraction. 'Below': belowground fraction. '49d': plants of 49 days of age. '91d': plants of 91 days of age.

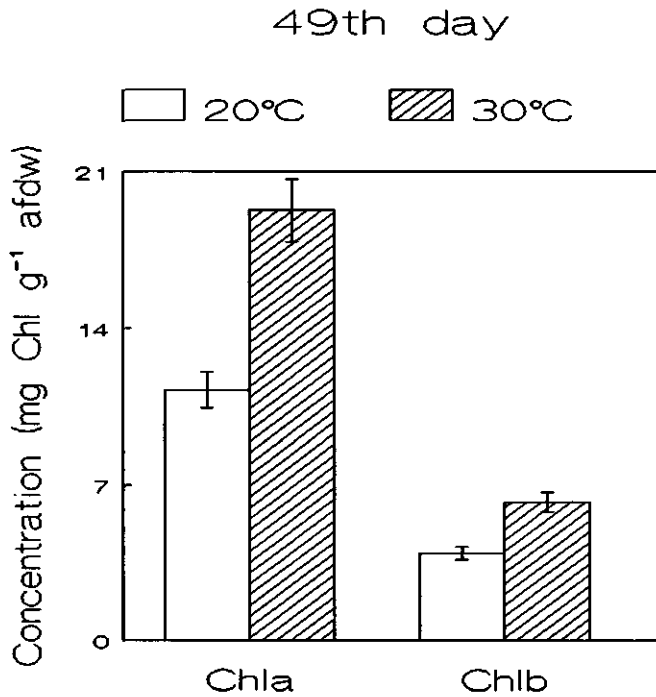


FIG.14: Leaf chlorophyll content of *R. drepanensis* plants grown during 49 days at two different temperatures. Average  $\pm$  standard error.

TABLE 9: ANOVA of parameter estimates and calculated parameter estimates derived from the biomass-related PI curves measured at different temperatures (ETEMP) using *R. drepanensis* plants grown at 20 and 30 °C (GTEMP). Displayed values are probabilities and symbols refer to the levels of significance (NS: non-significant; \*:  $p < .05$ ; \*\*:  $p < .01$ ; \*\*\*:  $p < .001$ ).  $P_m$ : maximum rate of gross photosynthesis.  $K_m$ : half-saturation constant. R: dark respiration.  $\alpha$ : apparent quantum yield. LCP: light compensation point.  $P_{500}$  and  $P_{250}$ : net photosynthesis at 500 and 250  $\mu\text{E m}^{-2} \text{s}^{-1}$ , respectively.

	$P_m$	$K_m$	R	$\alpha$	LCP	$P_{500}$	$P_{250}$
GTEMP	.3080 <sup>NS</sup>	.9517 <sup>NS</sup>	.8463 <sup>NS</sup>	.9001 <sup>NS</sup>	.7527 <sup>NS</sup>	.1583 <sup>NS</sup>	.2725 <sup>NS</sup>
ETEMP	.0535 <sup>NS</sup>	.0084 <sup>***</sup>	.0025 <sup>***</sup>	.0461 <sup>*</sup>	.0001 <sup>***</sup>	.0565 <sup>NS</sup>	.0104 <sup>*</sup>
GTEMP*ETEMP	.2039 <sup>NS</sup>	.4618 <sup>NS</sup>	.1169 <sup>NS</sup>	.4356 <sup>NS</sup>	.0168 <sup>*</sup>	.0326 <sup>*</sup>	.0817 <sup>NS</sup>

at 30 °C), and none of them was significant for total P.

Overall, nutrient concentration strongly decreased with increasing age, and tended to be lower for the belowground than for the aboveground fraction (Fig. 13). A slight tendency to higher nutrient concentration at higher temperatures was also found for total N. These differences are inversely proportional to those observed for the biomass yield.

Finally, chlorophyll-*a*, chlorophyll-*b* and total chlorophyll content were significantly higher at 30 °C than at 20 °C (t-test:  $p < 0.01$ ; Fig. 14), while the Chl(*b*)/Chl(*a*+*b*) ratio remained unaffected.

#### 3.2.1.4. Flowering and seed production

Only 4 of the 18 plants had flowered after 91 days of growth in the 30 °C treatment, and none of them developed seeds. Moreover, most of the inflorescences did not develop properly: the pollinic bags remained closed while already floating on the water surface, and the flowers became yellowish and progressively deteriorated.

On the other hand, 14 of the 21 plants had flowers in the 20 °C treatments (a significantly higher proportion according to a  $\chi^2$  test,  $p < 0.01$ ), and 8 of them showed fully-formed seeds. The average number of seeds per plant was significantly different from zero for the 20 °C treatment (t-test:  $p < 0.01$ ), indicating a significantly higher seed production at 20 °C than at 30 °C.

#### 3.2.1.5. Photosynthesis measurements

In total, 18 light-response curves were obtained. No datapoint was rejected as outlier, and while relatively great differences were found between replicate curves (see Fig. 15), each curve fitted its datapoints well (regression was significant in all cases).

Growth temperature (GTEMP) did not significantly affect any of the photosynthetic parameters tested (Table 9). Experimental temperature (ETEMP) significantly affected all parameters but the maximum gross production ( $P_m$ ) and the net production at 500  $\mu\text{E m}^{-2} \text{s}^{-1}$  ( $P_{500}$ ). Interaction was only significant for the light compensation point (LCP) and for  $P_{500}$ . None of the multiple comparisons between different levels of GTEMP was significant (Table 10). Two comparisons were significant for the dark respiration (R), the LCP and the net production at 250  $\mu\text{E m}^{-2} \text{s}^{-1}$  ( $P_{250}$ ), values at an ETEMP of 30 °C being significantly



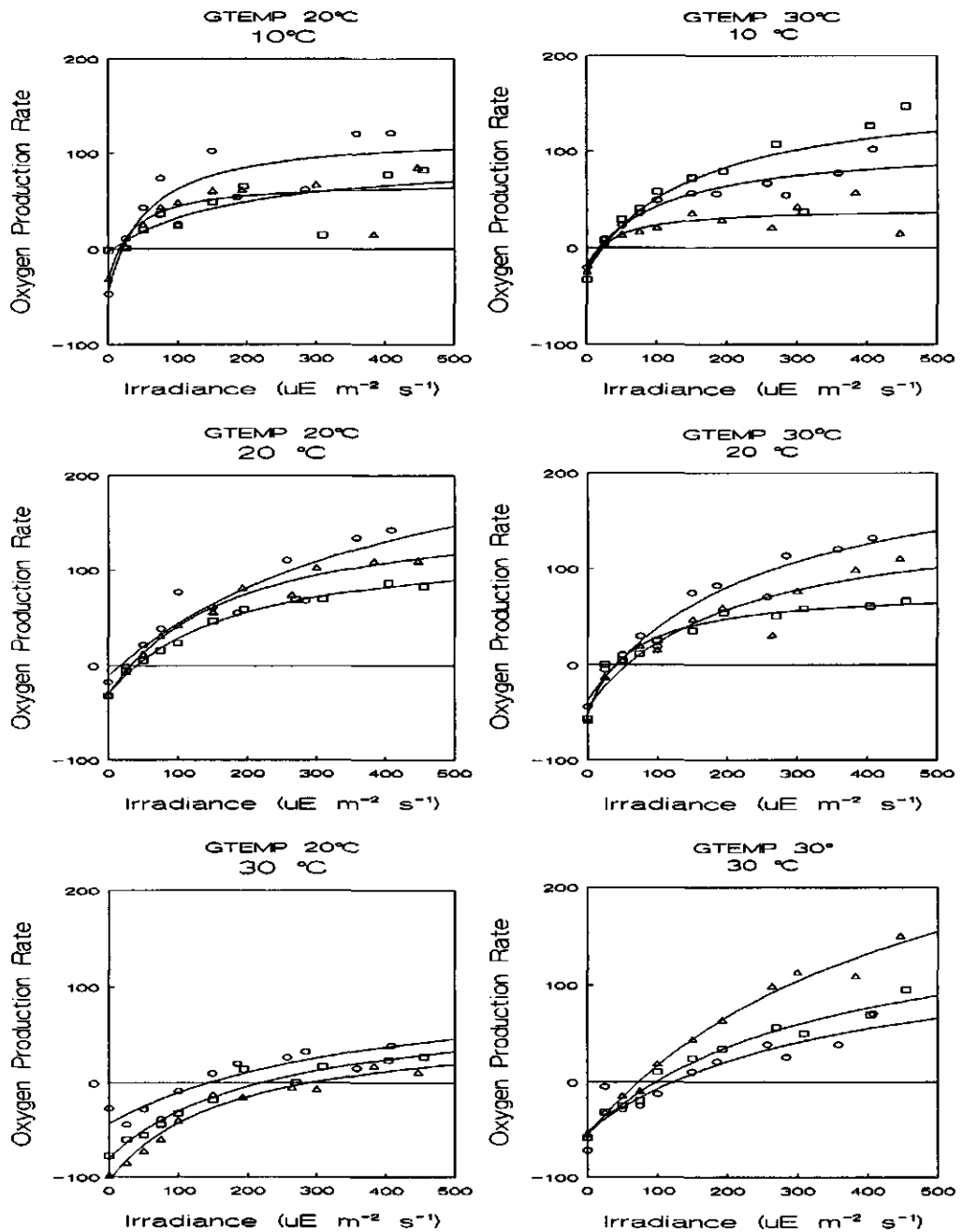


FIG. 15: Photosynthesis-Irradiance curves measured at different temperatures using *R. drepanensis* plants grown at 20 and 30 °C. Fitted lines using the rectangular hyperbola equation are also provided. GTEMP refers to the temperature at which plant material was grown; 10°C, 20°C and 30°C refer to the temperature at which photosynthesis was measured. Oxygen production rates in  $\mu\text{g O}_2 \text{g}^{-1} \text{afdw min}^{-1}$ .

TABLE 10: Multiple comparisons for the parameters estimates and calculated parameter estimates of biomass-related PI curves fitted by the rectangular hyperbola equation. GTEMP: temperature at which the plant material was grown. ETEMP: temperature at which the photosynthesis was measured. Small letters refer to horizontal comparisons, capital letters refer to vertical comparisons. Meaning of the parameter estimates as in Table 9.

	P <sub>m</sub>	K <sub>m</sub>	R	α	LCP	P <sub>500</sub>	P <sub>250</sub>
GTEMP (°C):	20 30	20 30	20 30	20 30	20 30	20 30	20 30
ETEMP:							
10°C	Aa Ba	Aa Ba	Aa Ca	Aa Ba	Aa Ca	ABa Ca	Aa Ca
20°C	Ab Bb	Ab Bb	Ab Cb	Ab Bb	Ab Cb	Ab Cb	Ab Cb
30°C	Ac Bc	Ac Bc	Bc Cc	Ac Bc	Bc Cc	Bc Cc	Bc Cc

TABLE 11: ANOVA of parameter estimates and calculated parameter estimates derived from the chlorophyll-related PI curves measured at different temperatures (ETEMP) using *R. drepanensis* plants grown at 20 and 30 °C (GTEMP). Displayed values are probabilities and symbols refer to the levels of significance (NS: non-significant; \*: p < .05; \*\*: p < .01; \*\*\*: p < .001). Meaning of the parameter estimates as in Table 9.

	P <sub>m</sub>	K <sub>m</sub>	R	α	LCP	P <sub>500</sub>	P <sub>250</sub>
GTEMP	.5453 <sup>NS</sup>	.9113 <sup>NS</sup>	.1592 <sup>NS</sup>	.3284 <sup>NS</sup>	.7073 <sup>NS</sup>	.9747 <sup>NS</sup>	.2725 <sup>NS</sup>
ETEMP	.0496*	.0088 <sup>***</sup>	.0045 <sup>***</sup>	.0500 <sup>NS</sup>	.0038 <sup>***</sup>	.0541 <sup>NS</sup>	.0104*
GTEMP*ETEMP	.2125 <sup>NS</sup>	.2366 <sup>NS</sup>	.0934 <sup>NS</sup>	.3581 <sup>NS</sup>	.0259*	.0336*	.0817 <sup>NS</sup>

different from those at 20 and 10 °C for the plants grown at a GTEMP of 20 °C. One comparison (ETEMP 20 vs. 30 °C, at GTEMP 20 °C) was significant for P<sub>500</sub>.

Overall, significant effects were generally discriminating the PI curves measured at 30 °C from those measured at 20 and 10 °C, for the plants grown at 20 °C. Photosynthesis measured at 30 °C was not significantly different from values measured at 20 and 10 °C when using plants grown at 30 °C. Curves measured at a given temperature did not differ significantly for the plant material grown at 20 °C or at 30 °C.

Respiration was higher and net production lower for the combination GTEMP 20°C, ETEMP 30 °C, which also resulted in higher K<sub>m</sub> and LCP values (Fig.16). Moreover, photosynthesis at the irradiance experienced during growth (P<sub>250</sub>) was dramatically lower for this GTEMP-ETEMP combination (Fig.17). In general, increased ETEMP resulted in increased net production values, increased dark respiration, decreased apparent quantum yield, and increased K<sub>m</sub> and LCP. Nevertheless, plants grown at 20 °C showed maximum P<sub>m</sub> and P<sub>500</sub> values at an ETEMP of 20 °C, while P<sub>m</sub> was maximum at an ETEMP of 30 °C for the plants grown at 30 °C. And, while R and LCP were much higher at 30 °C (ETEMP) for the plants grown at 20 °C, such increase in R and LCP was quite moderate for the plants grown at 30 °C.

ANOVA of the parameter estimates obtained from the chlorophyll-related curves yielded results almost identical to the ANOVA of the parameters from the biomass-related curves (Table 11). The only difference was that ETEMP had a significant effect also on P<sub>m</sub>, and no significant effect was detected on α (p=0.05). More significant differences were found in the subsequent multiple comparisons, but always following the same trends observed for the biomass-related parameter estimations (Table 12).

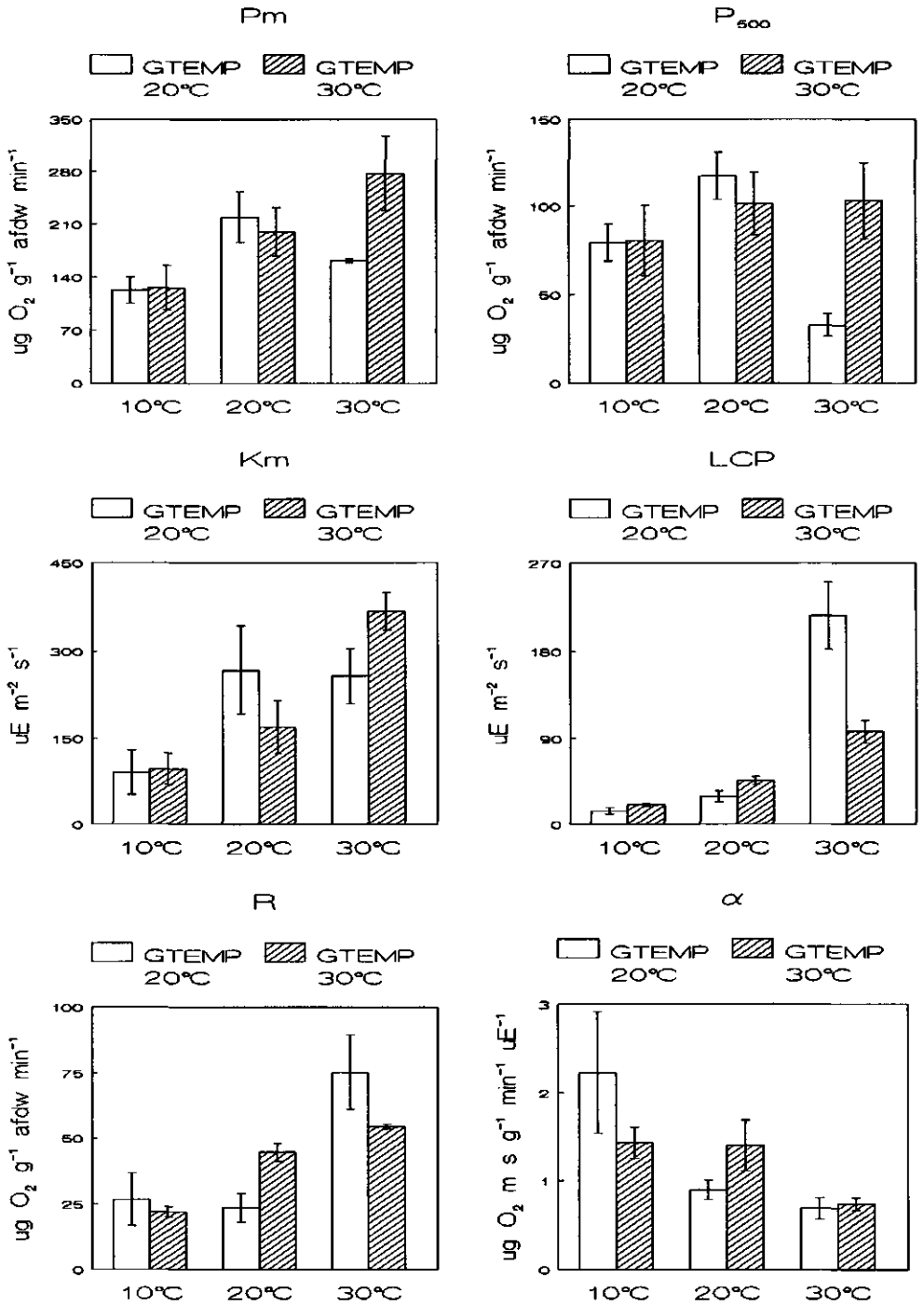


FIG.16: Effect of incubation temperature on the rectangular hyperbola parameter estimates (left) and derived parameter estimates (right) of the PI curves of *R. drepanensis* plants grown at two different temperatures. Temperature during growth is indicated by 'GTEMP'.

TABLE 12: Multiple comparisons for the parameters estimates and calculated parameter estimates of chlorophyll-related PI curves fitted by the rectangular hyperbola equation. GTEMP: temperature at which the plant material was grown. ETEMP: temperature at which the photosynthesis was measured. Results coded as in table 10. Meaning of the parameter estimates as in Table 9.

	$P_m$	$K_m$	R	$\alpha$	LCP	$P_{500}$	$P_{250}$
GTEMP (°C):	20 30	20 30	20 30	20 30	20 30	20 30	20 30
GTEMP:							
10°C	Aa Ba	Aa Ba	Aa Ca	Aa Ba	Aa Ca	ABa Ca	Aa Ca
20°C	Ab Bb	Ab BCb	Ab Cb	Ab Bb	Ab CDb	Ab Cb	Ab Cb
30°C	Ac Bc	Ac Cc	Bc Cc	Ac Bc	Bc Dc	Bc Cc	Bc Cc

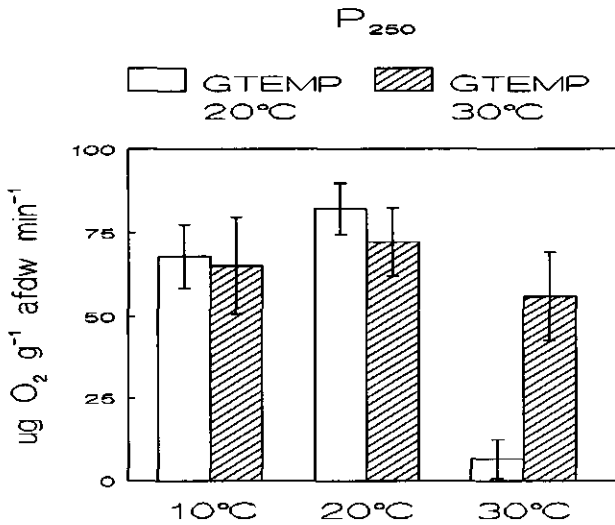


FIG.17: Effect of temperature on the estimated net photosynthetic production of *R. drepanensis* plants grown at two different temperatures.  $P_{250}$ : Net photosynthesis at  $250 \mu E m^{-2} s^{-1}$ , equivalent to the irradiance at which plants were grown.

3.2.2. Experiment 2: effect of low temperatures and seedling vernalization on flowering induction

3.2.2.1. Plant biomass

ANOVA was performed to test the effects of photoperiod, temperature and vernalization, and the two-way interactions including vernalization, on biomass and biomass allocation. Two separate ANOVAs were calculated for the 12th and the 17th week material, due to computer memory limitations. While photoperiod had a significant effect on all variables but the AGB/BGB ratio in the 12th week (Table 13), it only significantly affected the total biomass and the basal shoots biomass yields in the 17th week. Temperature affected significantly the aboveground, total and vertical shoots biomass at both ages tested. The AGB/BGB ratio was also affected by temperature for the 12th week measurements. The effect of vernalization was never significant, except for the total biomass in the 17th week. Interactions including vernalization were never significant.

TABLE 13: ANOVA of biomass yield and biomass allocation of *R. drepanensis* plants of 12 and 17 weeks of age, for the factors photoperiod (Phot, 16 and 10 h), vernalization (Vern, see text) and temperature (Temp, 14 and 20 °C). Displayed values are probabilities and symbols refer to the levels of significance (NS: non-significant; \*:  $p < .05$ ; \*\*:  $p < .01$ ; \*\*\*:  $p < .001$ ). 'AGB/BGB ratio': aboveground/belowground biomass ratio. 'VSB/AGB ratio': vertical shoots/aboveground biomass ratio.

	Below	Above	Total	Basal shoots	Vertical shoots	AGB/BGB ratio	VSB/AGB ratio	Number of flowers
12th week								
Phot	.0001 <sup>***</sup>	.0001 <sup>***</sup>	.0001 <sup>***</sup>	.0016 <sup>***</sup>	.0001 <sup>***</sup>	.1274 <sup>NS</sup>	.0001 <sup>***</sup>	1.000 <sup>NS</sup>
Vern	.3536 <sup>NS</sup>	.1011 <sup>NS</sup>	.1327 <sup>NS</sup>	.3221 <sup>NS</sup>	.1919 <sup>NS</sup>	.3130 <sup>NS</sup>	.9855 <sup>NS</sup>	.8165 <sup>NS</sup>
Temp	.9008 <sup>NS</sup>	.0001 <sup>***</sup>	.0004 <sup>***</sup>	.6593 <sup>NS</sup>	.0001 <sup>***</sup>	.0001 <sup>***</sup>	.1420 <sup>NS</sup>	.0001 <sup>***</sup>
Ph*Ve	.5617 <sup>NS</sup>	.8957 <sup>NS</sup>	.7206 <sup>NS</sup>	.7637 <sup>NS</sup>	.6994 <sup>NS</sup>	.7290 <sup>NS</sup>	.3489 <sup>NS</sup>	1.000 <sup>NS</sup>
Ve*Te	.1654 <sup>NS</sup>	.0772 <sup>NS</sup>	.0697 <sup>NS</sup>	.5044 <sup>NS</sup>	.0870 <sup>NS</sup>	.2917 <sup>NS</sup>	.5544 <sup>NS</sup>	.8093 <sup>NS</sup>
17th week								
Phot	.1868 <sup>NS</sup>	.1517 <sup>NS</sup>	.0285 <sup>*</sup>	.0349 <sup>*</sup>	.7034 <sup>NS</sup>	.5714 <sup>NS</sup>	.3499 <sup>NS</sup>	.9628 <sup>NS</sup>
Vern	.6084 <sup>NS</sup>	.0713 <sup>NS</sup>	.0363 <sup>*</sup>	.8181 <sup>NS</sup>	.1047 <sup>NS</sup>	.8057 <sup>NS</sup>	.2012 <sup>NS</sup>	.3926 <sup>NS</sup>
Temp	.0094 <sup>NS</sup>	.0001 <sup>***</sup>	.0001 <sup>***</sup>	.3997 <sup>NS</sup>	.0004 <sup>***</sup>	.7074 <sup>NS</sup>	.0980 <sup>NS</sup>	.0001 <sup>***</sup>
Ph*Ve	.4891 <sup>NS</sup>	.3016 <sup>NS</sup>	.1226 <sup>NS</sup>	.7125 <sup>NS</sup>	.3627 <sup>NS</sup>	.7065 <sup>NS</sup>	.3489 <sup>NS</sup>	.9628 <sup>NS</sup>
Ve*Te	.5924 <sup>NS</sup>	.4458 <sup>NS</sup>	.7811 <sup>NS</sup>	.5453 <sup>NS</sup>	.3612 <sup>NS</sup>	.2734 <sup>NS</sup>	.3866 <sup>NS</sup>	.7217 <sup>NS</sup>

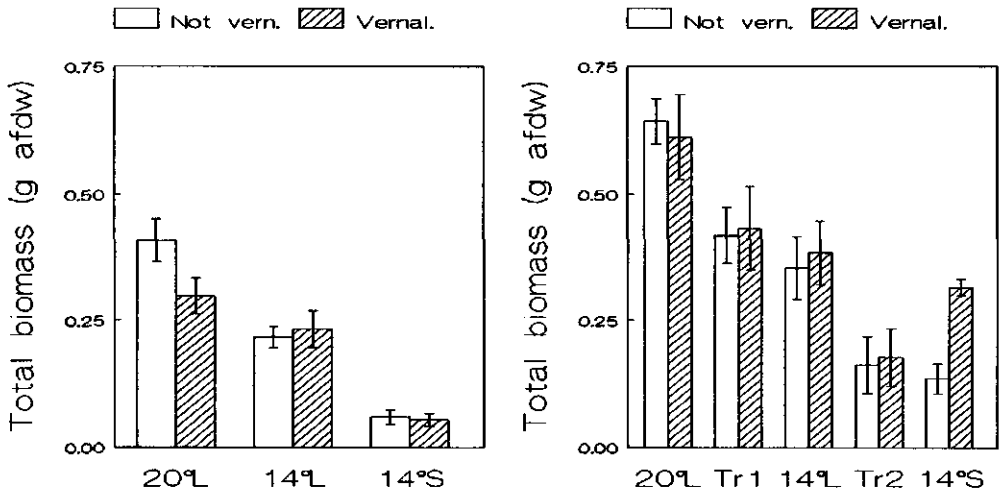


FIG. 18: Effect of the temperature and photoperiod treatments on the total biomass of *R. drepanensis* after 12 (left) and 17 (right) weeks of growth. 'L' stands for 'long' photoperiod (16 h), 'S' for 'short' photoperiod (10 h), 'Tr1' for plants transferred from 14 to 20 °C (long photoperiod) after 12 weeks of growth, 'Tr2' for plants transferred from short to long photoperiod (14 °C) after 12 weeks of growth.

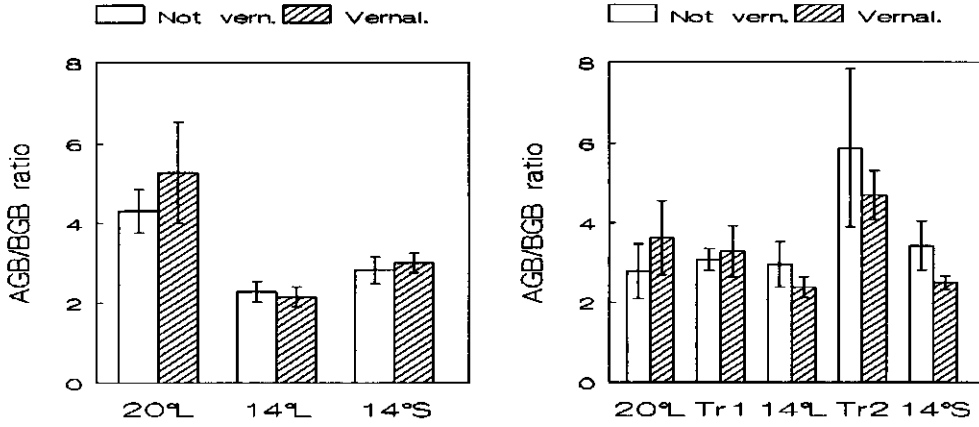


FIG.19: Effect of the temperature and photoperiod treatments on the aboveground/belowground biomass ratio (AGB/BGB ratio) of *R. drepanensis* plants after 12 (left) and 17 (right) weeks of growth. Legend as in Fig.18.

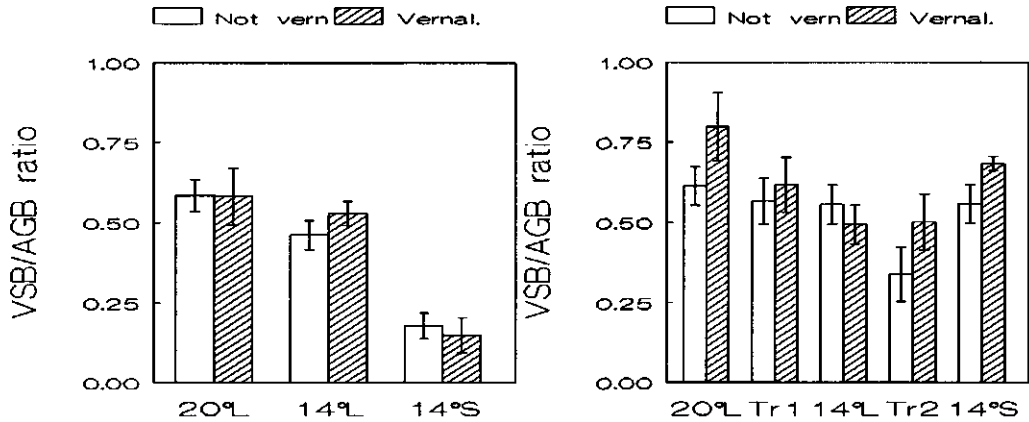


FIG.20: Effect of the temperature and photoperiod treatments on the vertical shoots to aboveground biomass ratio (VSB/AGB ratio) of *R. drepanensis* plants after 12 (left) and 17 (right) weeks of growth. Legend as in Fig.18.

For the 12th week values, both comparisons between different photoperiods (within the 14 °C temperature) were significant for all variables but the AGB/BGB ratio (no significant comparison) and the basal shoots biomass (only non-vernalised plants showed significant differences). Comparisons between different temperatures (within the 16 h photoperiod) were both significant for the variables aboveground biomass, total biomass, vertical shoot biomass and AGB/BGB ratio.

For the 17th week values, comparisons concerning temperature were significant for the variables aboveground biomass, total biomass and vertical shoot biomass (the latter only for the vernalised plants). All other comparisons were not significant.

Overall, total biomass decreased with decreasing temperature, and decreasing

TABLE 14: ANOVA of the tissue nutrient content of *R. drepanensis* plants grown at two different temperatures (14 and 20 °C) and photoperiods (16 and 10 h, referred as L and S respectively). 'Plant fraction' refers to tissue samples from the aboveground and belowground fraction of the plants. Displayed values are probabilities and symbols refer to the levels of significance (NS: non-significant; \*:  $p < .05$ ; \*\*:  $p < .01$ ; \*\*\*:  $p < .001$ ).

	12th week		17th week	
	Total N	Total P	Total N	Total P
Photoperiod	.0026 <sup>***</sup>	.0024 <sup>***</sup>	.0774 <sup>NS</sup>	.1296 <sup>NS</sup>
Temperature	.0001 <sup>***</sup>	.0005 <sup>***</sup>	.0001 <sup>***</sup>	.0001 <sup>***</sup>
Plant fraction	.0001 <sup>***</sup>	.0001 <sup>***</sup>	.0001 <sup>***</sup>	.0001 <sup>***</sup>
Phot*PFr	.8053 <sup>NS</sup>	.4916 <sup>NS</sup>	.7770 <sup>NS</sup>	.1020 <sup>NS</sup>
Temp*PFr	.0406 <sup>NS</sup>	.2973 <sup>NS</sup>	.0008 <sup>***</sup>	.0004 <sup>***</sup>
17th week, including transfer treatments				
	Aboveground		Belowground	
	Total N	Total P	Total N	Total P
Photoperiod	.2631 <sup>NS</sup>	.9973 <sup>NS</sup>	.2423 <sup>NS</sup>	.0005 <sup>***</sup>
Temperature	.0001 <sup>***</sup>	.0328 <sup>*</sup>	.0001 <sup>***</sup>	.0001 <sup>***</sup>

photoperiod resulted in a lower biomass (Fig. 18). While total biomass was initially lower for the vernalized plants in the control treatment, it was higher for the vernalized plants in the 14S treatment after 17 weeks of growth. AGB/BGB ratio was lower at 14 °C in the 12th week, and similar for both photoperiods at such temperature, but in the 17th week was similar for both temperatures and photoperiods (Fig.19). Finally, biomass allocation in vertical shoots (VSB/AGB biomass ratio) was lower at the 14S (14 °C, short photoperiod) in the 12th week, but it was already similar to the 20L and 14L treatments 5 weeks later (Fig.20).

### 3.2.2.2. Nutrient content

Temperature and plant fraction had a highly significant effect on total N and total P content both after 12 and 17 weeks of growth ( $p < 0.001$ ; Three-Way ANOVA, Table 14). Interaction of both factors was also significant for the 17th week dataset, while photoperiod resulted in significant differences only for the 12th week dataset.

Total N and total P content were significantly different for plants grown at 14 and 20 °C (except for the belowground fraction in the 12th week; multiple comparisons, Table 15). Plant fraction significantly affected both total N and total P content in all cases but one (total P content of the plants grown in the 14S treatment). No comparison was significant for the effect of photoperiod (14L vs 14S treatment).

Comparisons including the transfer treatments (17th week dataset) showed the total N content from the plants transferred from 14 to 20 °C to be significantly different from the 14L treatment (both for the above and belowground fractions), while its aboveground total P content differed significantly from both 14L and 20L. Photoperiod resulted in significant comparisons only for the aboveground total P content.

Overall, differences in nutrient concentration were inversely proportional to the observed differences in biomass yield. Both total N and total P increased with decreasing

TABLE 15: Multiple comparisons for tissue nutrient content. Small letters refer to comparisons between different plant fractions within the same treatment (horizontal comparisons), capital letters refer to treatment (either temperature or photoperiod) comparisons within the same plant fraction.

	12th week				17th week			
	Total N		Total P		Total N		Total P	
	Above	Below	Above	Below	Above	Below	Above	Below
Temp:								
14L	Aa	Cb	Aa	Cb	Aa	Cb	Aa	Cb
20L	Bc	Cd	Bc	Cd	Bc	Dd	Bc	Dd
Phot:								
14L	Aa	Cb	Aa	Cb	Aa	Cb	Aa	Cb
14S	Ae	Cf	Ae	Ce	Ae	Cf	Ae	Ce
	17th week, including transfer treatments							
	Total N		Total P		Total N		Total P	
	Above	Below	Above	Below	Above	Below	Above	Below
Temp:								
14L		A	A		A	A		
14L->20L		B	B		B	A		
20L		B	B		C	A		
Phot:								
14L		A	A		A	A		
14L->14S		A	A		D	A		
14S		A	A		E	A		

temperature and photoperiod (Fig.21), although the effect of photoperiod (at 14 °C) became undetectable for the 17th week dataset. And nutrient concentration tended to decrease with increasing plant age. Finally, aboveground fraction showed always higher nutrient concentrations than the belowground material.

### 3.2.2.3. Flowering and seed production

Development of the relative frequency of flowering plants with time is shown in Fig.22. Plants grown in the 20L treatment began to flower after 6 weeks of growth, and a maximum frequency of flowering plants of 80-90 % was already reached after 12 weeks of growth. On the other hand, plants grown at 14 °C (under both long and short photoperiods) only began to flower after 17 weeks of growth. Plants transferred from the 14L to the 20L treatment began to flower immediately, and the relative frequency quickly stabilised at 60 % (the number of replicate plants in this case was low, n=10, so relative frequencies have to be considered with some caution).

Accordingly, a  $\chi^2$  test performed on the frequencies of flowering plants after 12 weeks of growth resulted in significant differences between the 20L treatment and the 14L and 14S treatments. In the 17th week, the 20L treatment still showed a significantly higher frequency of flowering plants as compared with any of the treatments at 14 °C, the plants transferred from the 14L to the 20L showing intermediate values between both treatments



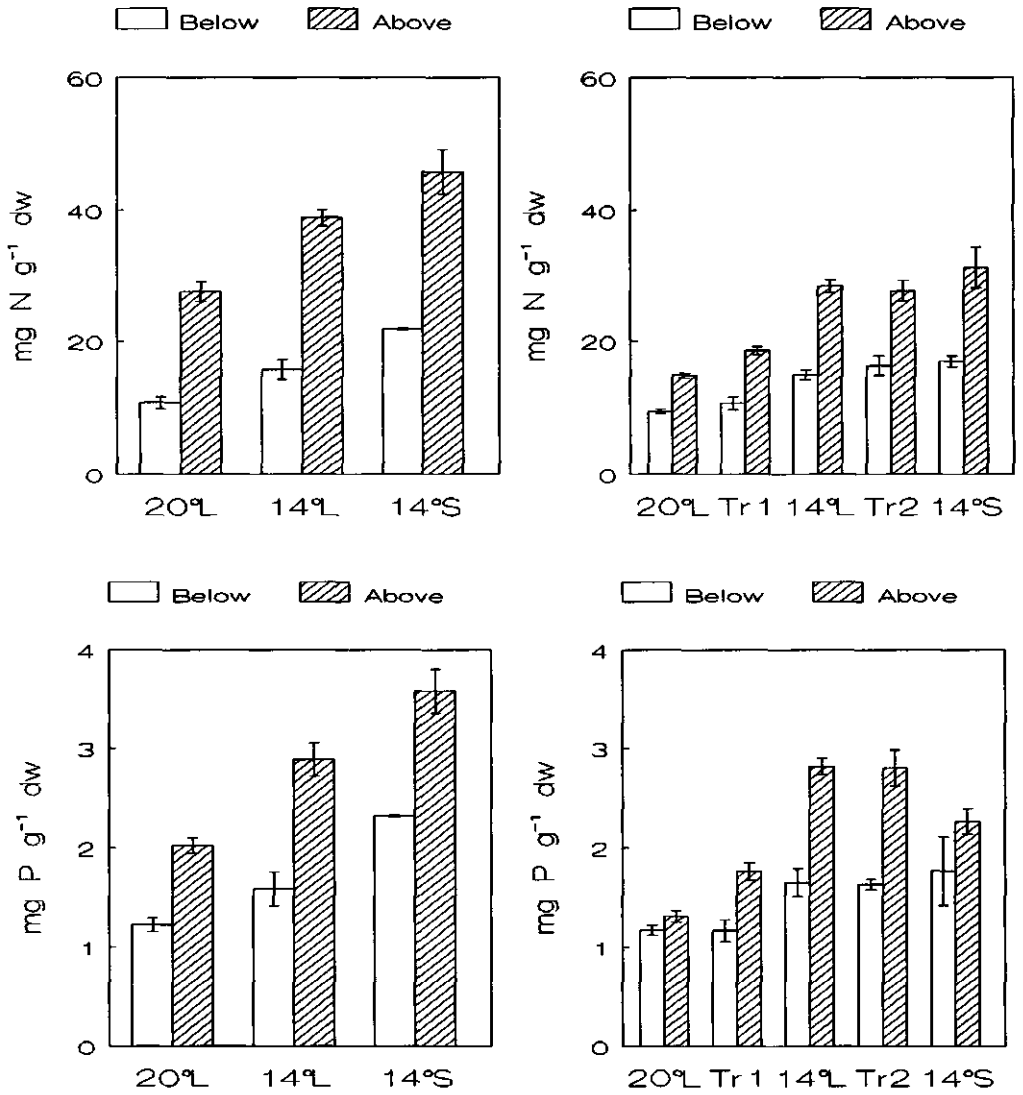


FIG.21: Effect of the temperature and photoperiod treatments on the tissue nutrient content of *R. drepanensis* plants after 12 (left) and 17 (right) weeks of growth. Legend as in Fig.18.

(not significantly different from any of them). However, neither the  $\chi^2$  test for differences due to the vernalization treatment on the frequency of flowering plants, nor the Student-based multiple comparisons for vernalization-induced differences in the number of flowers per plant yielded significant results.

## Effect of temperature

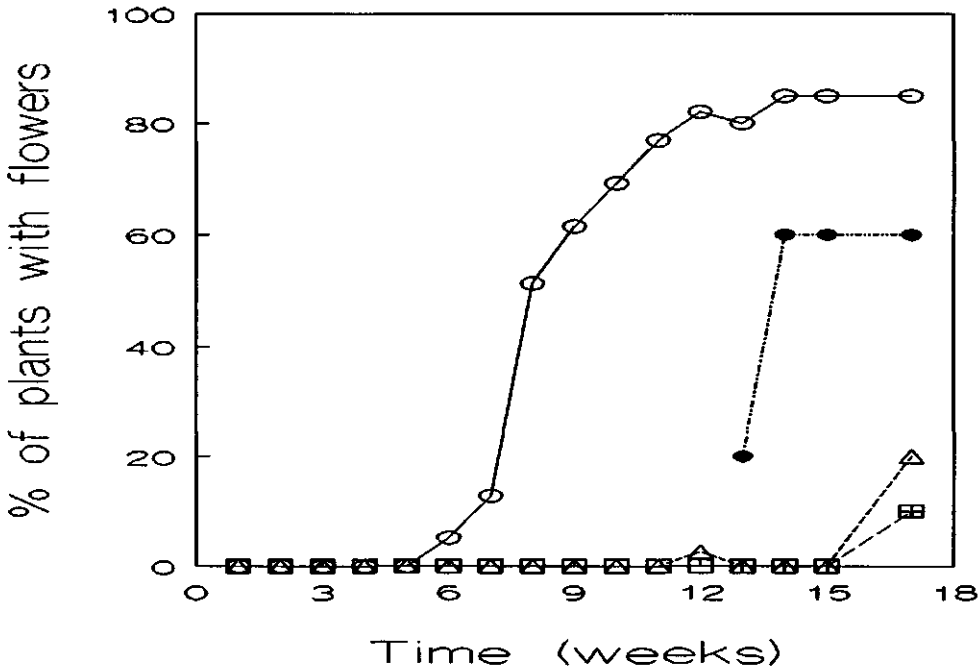


FIG.22:Development of the percentage of flowering plants with time for the different temperature and photoperiod treatments. Empty circles: control, temperature 20°C, photoperiod 16 hours. Triangles: 14°C, 16 hours photoperiod. Squares: 14°C, 10 hours photoperiod. Filled circles: plants transferred after 12 weeks from 14°C to 20°C, always under a 16 hours photoperiod. Crosses: plants transferred after 12 weeks from a 10 hours to a 16 hours photoperiod, always at a temperature of 14°C. n=40 during the first 12 weeks, then n=20 for the control and n=10 for all other treatments.

### 3.2.3. Biomass yield and RGR comparisons combining different experiments

#### 3.2.3.1. Combined temperature effect (experiments 1 and 2)

Total biomass values in the 12th (14 and 20 °C treatments) and 13th (20 and 30 °C treatments) week of growth were combined in a One-Way ANOVA. Both 20 °C treatments were entered separately. Treatment effect was highly significant ( $p < 0.001$ ), and subsequent multiple comparisons discriminated both 20 °C treatments from the 14 °C and the 30 °C, the latter two not differing significantly from each other.

Biomass values from both experiments are shown in Fig.23, together with the fitted logistic curves used for the F comparisons. Datapoints from the two 20 °C treatments were pooled in a common logistic fit, as their separate fits did not show a significantly lower RSS. Result of the 3 F-tests on the overall logistic fits were identical to those of the ANOVA on the 12th-13th week biomass, the 20 °C treatment differing significantly from both the 14 °C and the 30 °C treatments, which did not differ significantly from each other.

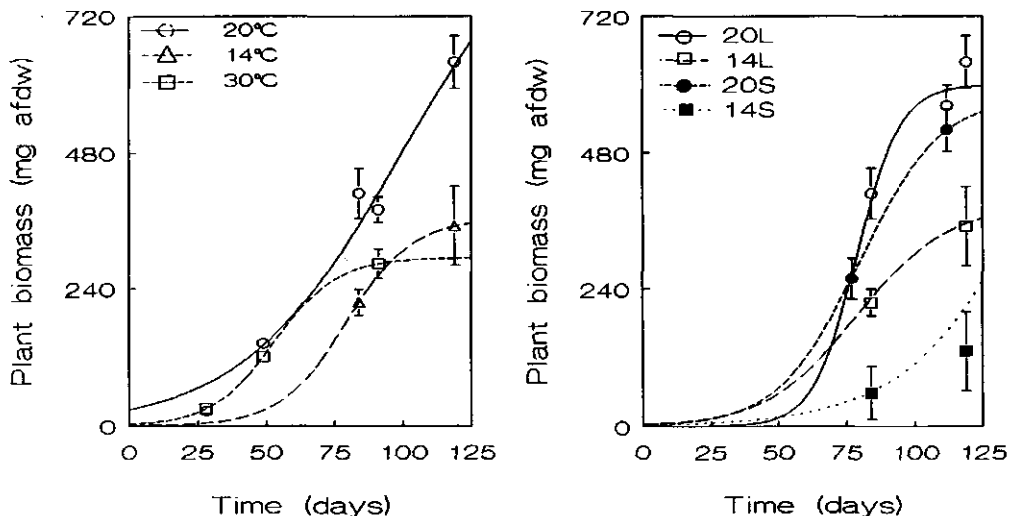


FIG.23: Effect of temperature and photoperiod on the biomass yield (total afdw per plant) of *R. drepanensis*. Left graph (effect of three different temperatures) combines the results of experiments 1 and 2. Right graph (effect of photoperiod and temperature) combines the results of experiment 2 with those from Santamaría & van Vierssen (in prep.).

### 3.2.3.2. Combined effect of temperature and photoperiod

Total biomass values in the 11th and the 16th week of growth (Santamaría & van Vierssen, in prep.) and in the 12th and the 17th week of growth (experiment 2) were combined in a Three-Way ANOVA to assess the effect of temperature (14 vs 20 °C), photoperiod (16 vs. 10 h) and age (11th-12th week vs. 16th-17th week) and their interactions. Results of the ANOVA have to be considered with caution, as residuals were neither homoscedastic nor normally distributed ( $p < 0.05$ ). The three factors showed a highly significant effect ( $p < 0.001$ ), and both interactions including temperature also had a significant effect ( $p < 0.05$ ).

A separate Two-Way ANOVA performed only on the 16th-17th week data (after  $\log_{10}$  transformation) showed homogeneity of the variances and normally distributed residuals ( $p > 0.05$ ). In this case, both temperature and photoperiod had a significant effect, while their interaction was not significant. Subsequent multiple comparisons showed significant differences only for the 14S versus 20S comparison. As a general trend, biomass decreased with decreased temperature and photoperiod.

Logistic curves fitted on the biomass values (Fig.23), again after pooling both 20L treatments in a common fit (separate fits were not significantly 'better'), showed significant differences for the comparisons '14L vs 14S', '20L vs. 14L' and '20S vs 14S', while the comparison '20L vs 20S' was not significant.

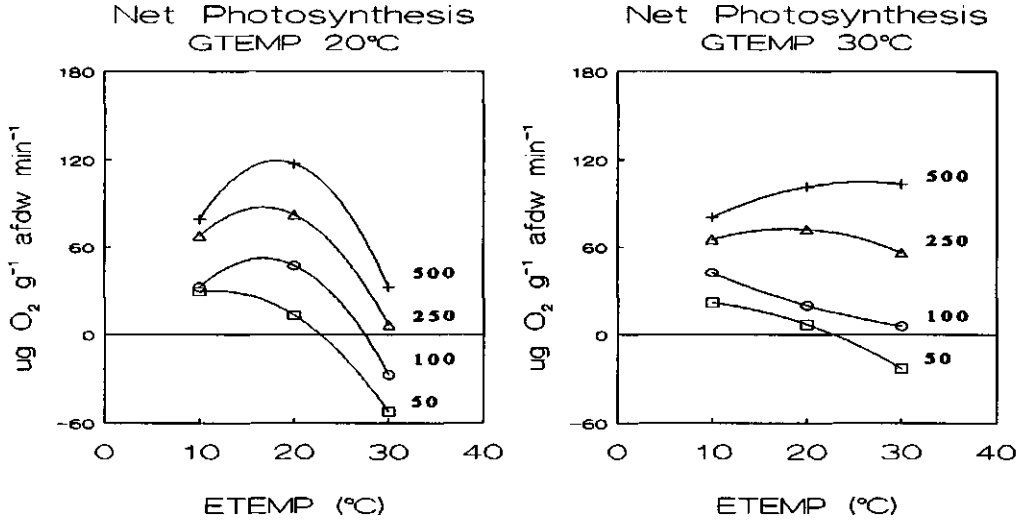


FIG.24: Effect of temperature on net photosynthesis at 4 different irradiances. Numbers inside the graphs indicate the irradiance in  $\mu\text{E m}^{-2} \text{s}^{-1}$ . GTEMP: temperature at which the plants were grown previous to photosynthesis measurements. Lines are second-order polynomial fits.

### 3.3. Discussion

#### 3.3.1. Photosynthetic acclimation in *R. drepanensis*

The temperature response of *R. drepanensis* agreed in general terms with what has been described previously for most of the submerged non-marine macrophytes:  $P_m$ ,  $K_m$ , LCP and R values tend to increase from 10 to 30 °C. As found for *M. salzigineum* (Orr *et al.*, 1988) and for the Florida population of *R. maritima* (Koch & Dawes, 1991),  $\alpha$  tended to decrease (significantly) with increasing temperature. Finally, net photosynthesis (both at 250 and at 500  $\mu\text{E m}^{-2} \text{s}^{-1}$ ) was not significantly affected by temperature, except for the interaction of growth and experimental temperatures (see below).

A first remark concerns the maximum gross production ( $P_m$ ), the only parameter not significantly affected by ETEMP in its biomass-related estimation. This is remarkable, as maximum temperature dependence is expected to occur at saturating irradiance. Now that  $P_m$  was not significantly affected, significant effects of temperature on  $P_{250}$  and  $P_{500}$  might well be attributed to the effect of temperature on respiration. Nevertheless,  $P_m$  was significantly affected by ETEMP once estimated in a chlorophyll-related dataset. It is also noteworthy that the chlorophyll-related estimation of  $\alpha$  is not significantly affected by ETEMP, indicating an inverse pattern of variation for  $P_m$  and  $\alpha$ .

The existence of a temperature acclimation effect is clear in this species: while the plants grown at 20 °C showed a significantly poorer photosynthetic performance at 30 °C, attributable mainly to a strong increase in the respiration rate (and probably also to a poorer light harvesting efficiency, reflected in a significantly lower  $\alpha$ ), those grown at 30 °C did

not show any significant difference in their photosynthetic performance at 10, 20 and 30 °C (with the exception of a significant increase in the chlorophyll-related LCP with temperature).

It is important to note that, while acclimation is clearly improving the photosynthetic performance of *R. drepanensis* at high temperatures (30 °C), it did not result in a poorer photosynthetic performance neither at intermediate (20 °C) nor at low temperatures (10 °C). As it may be observed from Fig.16, no parameter of the PI curve measured at 10 °C was strongly affected by GTEMP. In practice, this implies that the temperature response of net photosynthesis (at any irradiance, see Fig.24) does not show a shift in its temperature optimum, but an actual widening of the curve (contrary to the conclusions of Berry & Björkman, 1980). This response may be related to the particular conditions in the habitat of *R. drepanensis*: as they only have water in winter and spring, a relatively high photosynthetic performance at low temperatures is probably a rigidly established character of this species, while a certain capability to acclimate to high temperatures may only be of use in unusually wet years.

Another interesting feature of the temperature response of *R. drepanensis* may be appreciated in Fig.24. As described in Bulthuis (1987), the photosynthetic performance at lower irradiance is maximal at lower temperatures (the temperature optimum shifts from 20 to 10 °C, in plants grown at 20 °C; or from 30 to 10 °C, in plants grown at 30 °C, when lowering the irradiance from 500 to 50 °C  $\mu\text{E m}^{-2} \text{s}^{-1}$ ). This is mainly due to the lower dark respiration rates at low temperatures.

As low temperatures are coinciding with lower irradiance and shorter photoperiods along the annual cycle, it is tempting to suggest a link between the temperature acclimation and the photosynthetic light response. But along the spring season, light and temperature acclimation of *R. drepanensis*' photosynthesis follow two opposite trends: while  $P_m$  and R increase with increasing, 'summer' temperature,  $P_m$  decreases and R increases with increasing, 'summer' irradiance and photoperiod (Santamaría & van Vierssen, in prep.). It is thus obvious that a seasonal effect on respiration and photosynthesis is hardly attributable to an isolated temperature- or light-acclimation effect, but more to the interplay of both.

The temperature response of *R. drepanensis* grown at 20 °C strongly resembled that for the proposed winter-annual macrophytes (i.e., *R. maritima* from Koch & Dawes, 1991; Fig.1):  $P_m$  was optimal at (or close to) 20 °C, and decreased at 30 °C. For the plants grown at 30 °C,  $P_m$  behaved much more as described for the aquatic macrophytes proposed as 'summer-annuals', increasing from 10 to (at least) 30 °C. This stresses the importance of the temperature acclimation of *R. drepanensis* during its annual life-cycle: it allows for a degree of plasticity required in a dynamic environment.

Dark respiration followed the general trend found for the majority of aquatic macrophytes reviewed, increasing (more or less sharply, depending on temperature acclimation) with temperature from 10 to 30 °C. As a consequence, both  $K_m$  and LCP increased with temperature.

The decrease in  $\alpha$  with temperature is remarkable, or at least unexpected in the view of previous works (i.e., 'photosynthetic capacity does not change with temperature within the normal physiological limits', Bulthuis, 1987). But as stressed in the introduction, significant positive and negative changes in  $\alpha$  with temperature have been found in the literature (see Fig.6, right). Hence, the effect of temperature on the efficiency of the light harvesting system is a topic needing, in our opinion, more attention than it has received until now.

### 3.3.2. Temperature effect on growth: Interaction with photoperiod

Plants cultured at 20 °C had higher growth rates, both on a morphometric (number of shoots, number of leaves) and on a biomass basis, than those cultured at 30 °C. Thus, photosynthetic acclimation was not enough to assure the maintenance at 30 °C of growth rates similar to those at 20 °C. We attribute this to the higher respiratory rates displayed by the plants growing at 30 °C (a t-test comparing the PI parameter estimations between the combinations GTEMP=ETEMP=20°C and GTEMP=ETEMP=30°C yielded significant results only for R and LCP,  $p < 0.05$ ).

In fact, while the daily relative growth rate (RGR) computed from the net photosynthesis and dark respiration of 49-days old plants agreed relatively well with the instantaneous growth rate (IRGR, estimated from the logistic growth in afdw as in Santamaría & van Vierssen, in prep.) for the 20 °C treatment (RGR=0.056, IRGR=0.063 day<sup>-1</sup>), oxygen metabolism measurements considerably underestimated the IRGR for the 30 °C treatment (RGR=0.023, IRGR=0.051 day<sup>-1</sup>).

Berry & Björkman (1981) suggested that the apparent temperature dependence of respiration might lead to significant underestimations of the net carbon balance, due to the partitioning of respiration in a growth- and a maintenance-associated fraction. This hypothesis is in close agreement with our results, measured dark respiration rates resulting in a 50 % underestimation of the biomass growth rate when directly translated into primary production. But, as discussed above (see Section 2.3.1.), we believe that measured dark respiration can be used to directly estimate the effect of respiration on the carbon balance for the temperature at which it was measured, independent of the nature of the physiological reactions responsible for the increased respiratory rates. We believe respiratory and photosynthate partitioning to be the factors actually responsible for the observed differences in calculated growth rates, through their immediate effect on the PQ (we used a PQ=1 for our RGR calculations, both at 20 and 30 °C).

As found when analyzing the combined results from experiment 1 and 2, *R. drepanensis* showed an optimum growth at 20 °C, and lower growth rates when decreasing or increasing the temperature to 14 and 30 °C respectively. While significantly lower biomass yields for plants grown at 14 °C, as compared with those grown at 20 °C have also been reported for *E. canadensis*, *P. nodosus* and *V. americana* (Barko *et al.*, 1982), *H. verticillata* (Barko & Smart, 1981) and two populations of *R. maritima* (Koch & Dawes, 1991), a significant decrease in biomass when rising the temperature from 20 to 30 °C was found only for 1 of the 7 species reviewed (*E. densa*, which showed significantly lower biomass at 32 °C as compared with the yields at 20 and 28 °C, which were similar). Even *R. maritima*, with a photosynthetic performance resembling our findings for *R. drepanensis*, showed non-significantly different biomass yields at 22 and 30 °C.

Thus, despite its considerable photosynthetic plasticity at low temperatures (10 °C), its capacity to acclimate to high temperatures (30 °C), and its lower dependence on temperature for growth as compared with photosynthesis, *R. drepanensis* has a relatively narrow temperature range for optimum growth (biomass yield after 12 weeks of growth was only 70 % at 14 °C and 55 % at 30 °C, as compared with the biomass yield at 20 °C).

Finally, the combined results from experiment 2 and Santamaría & van Vierssen (in prep.) showed that both temperature and photoperiod significantly affected plant growth. Moreover, while a shorter photoperiod did not cause a significant decrease in biomass yield at 20 °C, it resulted in a 60 % lower biomass at 14 °C. Combination of short photoperiods

and low temperatures, as might be expected to occur during winter, thus resulted in a strong decrease in biomass production (average afdw per plant in the 14S treatment was only 27% of the yield in the 20L treatment, both after 16-17 weeks of growth).

### 3.3.3. Temperature effect on nutrient and chlorophyll content

Leaf chlorophyll concentration was higher at 30 °C than at 20 °C. As discussed in the introduction, this is in agreement with the results reported by Spencer & Ksander (1990) and Barko & Filbin (1983), who found chlorophyll concentration to increase with increasing temperature (in *P. gramineus*, and *E. canadensis* and *V. americana*, respectively), and in contrast with Vermaat & Hootsmans (1994b), who found a dilution effect of increasing temperatures on total chlorophyll concentration in *P. pectinatus*. Differences in the species' strategy, stressing morphological versus photosynthetic acclimation, might help to explain these conflicting results regarding the effect of temperature on chlorophyll concentration (as described for the light acclimation in Santamaría & van Vierssen, in prep.).

Significant differences found for the tissue nutrient content of the above- and belowground fractions are in agreement with Hootsmans & Vermaat (1994), and stress the necessity of separate analysis of above- and belowground material (van Vierssen, 1982).

Tissue nutrient content was significantly higher at low temperatures (14 °C as compared with 20 °C, experiment 2), while a further increase from 20 to 30 °C had little effect. Moreover, tissue nutrient content decreased strongly with increasing age, at all temperatures. Comparison with biomass values suggest all these differences to be attributable to a 'dilution' effect due to increasing biomass values, as suggested by Barko & Smart (1981) and Vermaat & Hootsmans (1994b) for a similar inverse relationship between biomass yield and chlorophyll content.

Plots of the total N and total P (aboveground) content versus the aboveground biomass for the individual plants from all different treatments are shown in Fig.25, together with fitted negative exponential curves. In general, tissue nutrient content tended to decrease with increasing biomass, until stabilising on a minimum asymptotic value. Further increases in biomass did not result in lower tissue nutrient concentration. Minimum asymptotic values found here (9.4-26.2 mg N g<sup>-1</sup> dw and 0.83-1.82 mg P g<sup>-1</sup> dw) were similar to the values suggested by Gerloff & Krombholz (1966; 13 mg N g<sup>-1</sup> dw and 1.3 mg P g<sup>-1</sup> dw) and Thursby (1984; 25-30 mg N g<sup>-1</sup> dw and 2.5-3.0 mg P g<sup>-1</sup> dw) to indicate the onset of nutrient limitation. Apparently, aboveground nutrient content decreases with plant biomass until reaching the minimum non-limiting nutrient concentration, which probably minimises the amount of energy spent by the plant on nutrient uptake.

Moreover, plants of similar age from all different treatments seem to follow a common trend, suggesting that significant differences in tissue nutrient content associated with different temperatures or photoperiods may be fully attributed to differences in biomass yield.

The relationship between total N and biomass changed significantly for different ages within the experiment 2 ( $p < 0.0001$ ), while the curve for total P-biomass was not significantly affected by age ( $p > 0.05$ ). Older plants showed a slightly sharper decrease in total N with increasing biomass, and a much lower asymptotic value (11 versus 26 mg N g<sup>-1</sup> dw). As the biomass range was comparable for both datasets, changes in the relationship are not attributable to further increases in plant biomass, but more to a generally lower N content of the 'older' plants. As this trend is followed also by the plants from the 14 °C treatments,

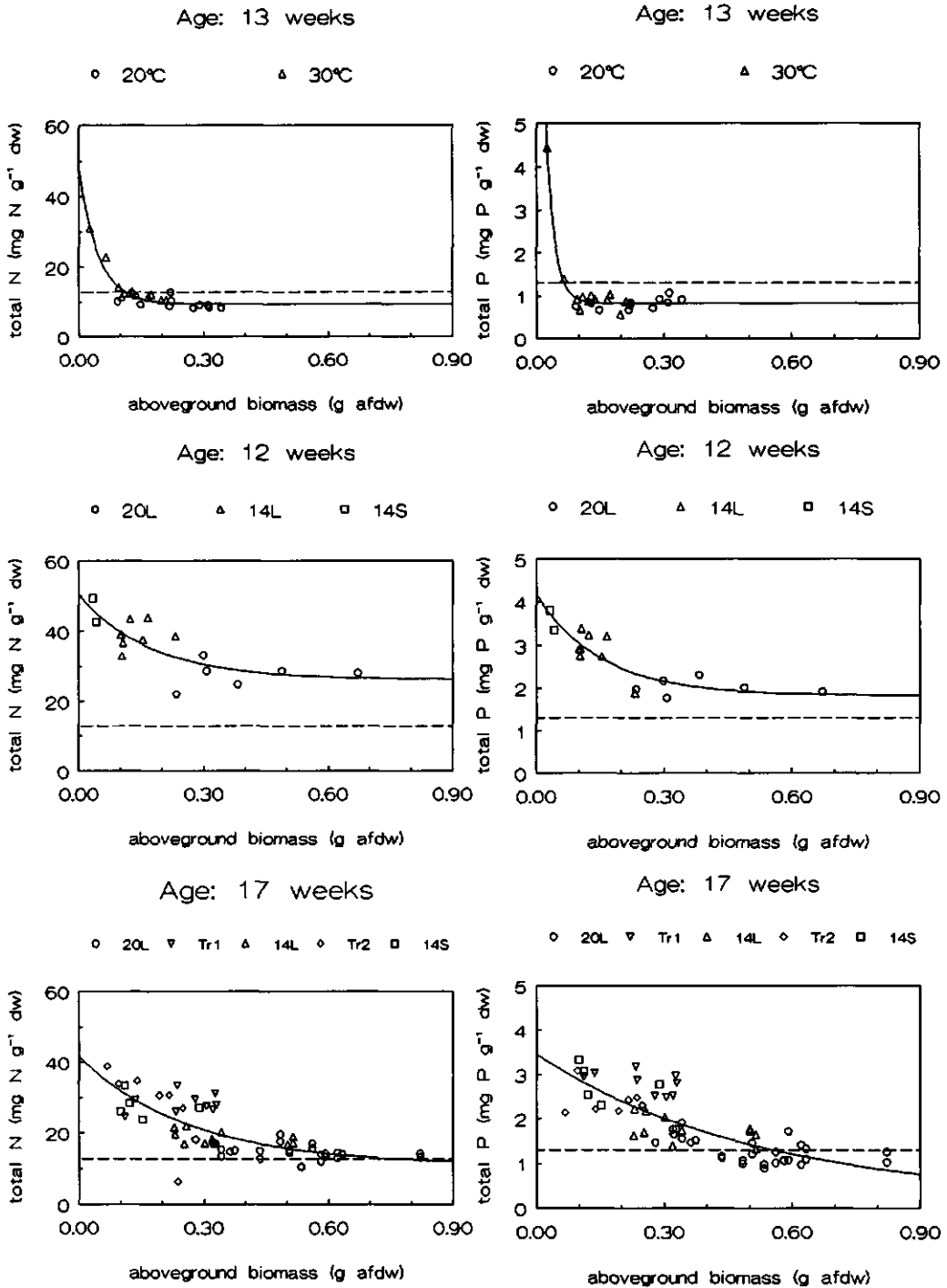


FIG.25: Relationship between aboveground plant biomass and aboveground nutrient tissue content of *R. drepanensis* plants grown at different conditions. Upper graphs: data from experiment 1. Central and lower graphs: data from experiment 2, coding of the treatments as in Fig.18.



the reduction in tissue nutrient content does not seem to be directly linked to an investment in reproduction, but more to an ageing effect. Leakage from senescent plant parts may probably explain these results.

The plants from experiment 1 showed a much sharper decrease in tissue nutrient content than those from experiment 2, and minimum asymptotic values similar to those from older plants from experiment 2. As all curves were nicely followed by the plants from the 20 °C treatments, they cannot be attributed to differences in growth temperature regimes. Moreover, the source of nutrients was similar for experiments 1 and 2. The origin of these differences in nutrient content remains thus unknown, although differences in the seed stocks may have accounted for them (the seeds from experiment 2, although coming from the same pool as those from experiment 1, were stored at 4 °C one year longer).

### 3.3.4. The induction of flowering: effect of temperature and photoperiod

Temperature had a quite dramatic effect on the induction of flowering and flower development of *R. drepanensis*. While 60 (experiment 1) to 90 % (experiment 2) of the plants had already flowered after 12 weeks of growth at 20 °C, only a few of them flowered at 30 °C, and none did flower at 14 °C. Moreover, flower development was strongly restricted at 30 °C, and as a consequence no seeds were formed.

A direct effect of temperature on flower induction was further tested by transferring 10 plants to 20 °C after 12 weeks of growth at 14 °C. Only two weeks after, 6 plants had already flowered, while only in the 17th week some plants had begun to flower at 14 °C.

Inhibition of flowering at 14 °C was not modified by photoperiod, as no plant flowered until the 17th week neither under short day (10 h) nor under long day (16 h) conditions. Moreover, transferring 10 plants from short to long day conditions (at 14 °C) in the 12th week had no effect on the induction of flowering.

The effect of temperature on seed production was altogether drastic: no seed was formed after 17 weeks of growth neither at 30 nor at 14 °C, while plants grown at 20 °C had already produced on average 5.6 seeds (and 22.6 flowers) per plant.

When comparing the strong effect of temperature on flower induction, and thus on seed production in *R. drepanensis*, with the relatively limited effect caused by photoperiod (in a comparable experimental design; Santamaría & Van Vierssen, in prep.), serious doubts arise regarding the relative importance of photoperiod in triggering flowering. Classically, temperature has been considered as a secondary factor in the induction of flowering, modifying only the photoperiodic response (see Salisbury, 1981). But *R. drepanensis* plants grown under long and short day conditions (10 and 16 h, respectively) only showed a limited postponing of flowering (significantly less plants had flowered after 11 weeks, but the proportion of flowering plants was 75 % under both photoperiods after 16 weeks; Santamaría & van Vierssen, in prep.), while almost no plant had flowered at 14 °C after 16-17 weeks of growth, neither under long nor under short day conditions. Moreover, while seed production was lower (less than half) under short day conditions (in close correlation, on the other hand, with the size of the plants), no seed had been produced after a similar period of time when plants were grown at 14 and 30 °C.

For *R. drepanensis*, results from experiment 2 confirmed the hypothesis outlined by Verhoeven (1979) for the European populations of *Ruppia cirrhosa* (Petagna) Grande and *Ruppia maritima* L. s.l.. He stated that temperatures below 15 °C inhibited the onset of flowering, and also suggested temperatures around 30 °C to inhibit both the induction of

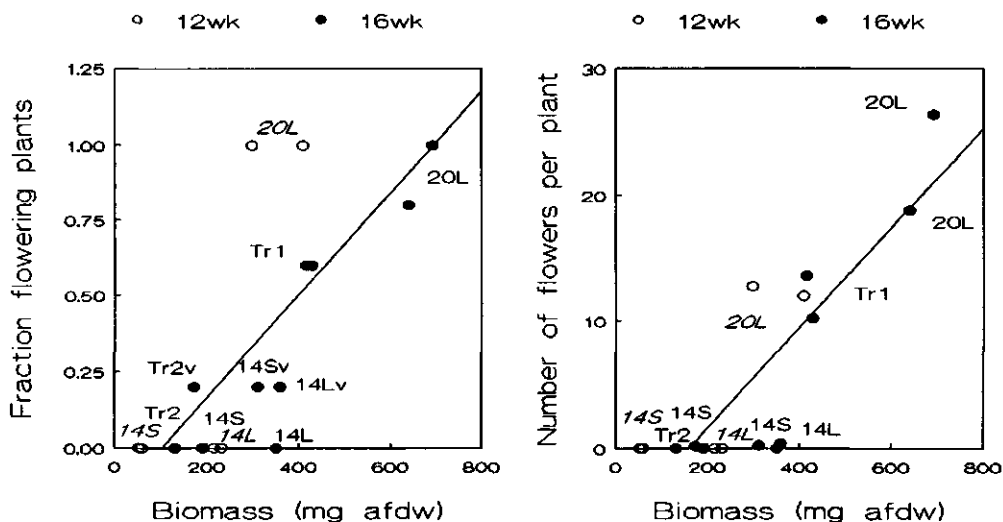


FIG.26: Relationship between the average plant biomass (in mg afdw) and the induction of flowering (proportion of flowering plants after 12 and 16 weeks of growth, left graph) and the flower abundance (average number of flowers per plant, right graph) for *R. drepanensis* plants from experiment 2. Labels indicate the treatments (each having 2 datapoints, corresponding to the vernalized and non-vernalized plants). Labels in italic refer to the 12th-week datapoints. In the left graph, 'v' refer to the vernalised plants.

flowering and flower development. On the other hand, our results differ from the temperature ranges hypothesised from field data of North American populations of *R. maritima*, as flowering in *R. drepanensis* was already observed at 20 °C (contrary to the threshold of 25 to 30 °C stated in Joanen & Glasgow, 1965 and Richardson, 1980).

The vernalization treatment did not result in significant differences neither in growth nor in flowering response of *R. drepanensis*. Although the 4 plants which flowered at 14 °C in the 17th week were all vernalized ones (2 flowered in the 14L treatment, 1 in the 14S and 1 in the Tr2; Fig.22), these proportions were too low to assess the significance of a vernalization effect on flowering. Unfortunately, the experiment did not continue long enough to confirm this; but, if vernalized plants have a capability for flowering at low temperatures, they do so with a significant delay (relative to 20 °C).

A relationship between plant biomass and flowering may be observed in Fig.26. While induction of flowering (as fraction of plants with flowers in the 12th and 16th week) correlated significantly with plant biomass ( $p < 0.01$ ),  $r^2$  was relatively low ( $r^2 = 0.57$ ). Indeed, both the position of the 12th-week 20L datapair (Fig.26, left) and the rapid flowering of the plants transferred from 14 to 20 °C (while biomass was not significantly higher than for the 14 °C treatment) strongly suggest temperature to have a biomass-independent effect on flower induction. Concerning flower production (as number of flowers per plant), correlation with biomass is better for the treatments for which a majority of plants had flowered, while the 16th-week 14L and 14S datapoints still remain far from the regression line (overall linear regression was highly significant,  $p < 0.001$ ,  $r^2 = 0.73$ ; Fig.26).

All in all, we conclude that temperature plays a major role in the induction of flowering of *R. drepanensis*. Nevertheless, photoperiod and irradiance also influence the

production of flowers and seeds. Low temperatures, short photoperiods and low irradiance result in a lower seed production, and high temperatures strongly inhibit the production of flowers.

The combination of these features result in a relatively narrow range of environmental conditions that occur only during late spring or early summer in the Mediterranean temporary wetlands. Thus, regarding the biomass dependence of seed yield (see also Santamaría & van Vierssen, in prep.) and the rapid decline of the vegetative apparatus of this species once reproduction has commenced, achieving a high biomass production during early spring is probably critical for the survivorship of *R. drepanensis* populations. Even if wetlands would not dry up at the end of spring, we do not expect its biomass and seed production to increase during the summer season.

### 3.4. Conclusions

Here we tested three hypotheses concerning the effect of temperature on the growth, photosynthetic performance and reproduction of a Mediterranean submerged macrophyte, *Ruppia drepanensis* Tineo.

Firstly, we hypothesised net photosynthesis and growth to be optimal at relatively low temperatures (10 to 20 °C) and to decrease at higher temperatures (30 °C). This was not fully confirmed by the measured growth rates, which were optimal at 20 °C and lower at 14 and 30 °C. The lower growth rates at 30 °C were attributed to higher respiratory rates, although calculations based on the measured rates of dark respiration and net photosynthesis strongly underestimated the increase in biomass (growth rate). On the other hand, low growth rates at 14 °C were not explained by significant differences in respiration and photosynthesis, although they probably result from the poorer irradiance-saturated photosynthetic performance (i.e., lower  $P_m$ ) at such temperatures. Moreover, temperature and photoperiod interacted in their effect on biomass yield, short photoperiod resulting in significantly lower biomass only at low temperatures.

Secondly, we hypothesised acclimation to high temperatures (30 °C) to result in an enhanced photosynthetic performance at such temperatures. According to our results, its acclimation capacity allowed *R. drepanensis* to improve its photosynthetic performance when grown at high temperatures (30 °C). This was achieved by widening the temperature response curve to reach higher net photosynthesis values at high temperatures. However, acclimation was not enough to maintain a growth rate similar to those of plants grown at 20 °C.

The third hypothesis predicted temperatures below 15 °C to inhibit flower induction, and temperatures above 30 °C to inhibit flower development. This was confirmed by our results. Temperature proved to have a major influence on the induction of flowering and on flower development, and no seed was produced by the plants grown at 14 and 30 °C. Photoperiod, irradiance and probably plant size further modified flowering once it was triggered by temperatures above 14 °C and below 30 °C.

Finally, some caution is recommended when calculating biomass yields from photosynthesis data, as high temperatures were demonstrated to have a more pronounced effect on respiration than on actual biomass gain, and low temperatures resulted in biomass yields lower than expected on a photosynthesis basis. As stressed in Santamaría & van Vierssen (in prep.), an increased effort in understanding the effect of environmental variables on the photosynthate and respiration partitioning will probably be needed if the accuracy of

models calculating biomass yields from photosynthetic and respiratory data is to be improved.

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## Chapter 4

### The influence of ammonia on the growth and photosynthesis of *Ruppia drepanensis* Tineo from Doñana National Park (SW Spain)

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**ABSTRACT:** In a laboratory experiment, *Ruppia drepanensis* Tineo seedlings from a brackish marsh in Southern Spain were grown at 20 and 30 °C, at three different nitrogen levels. These levels were obtained by the addition of a slow release fertilizer (23%  $\text{NH}_4\text{NO}_3$  by weight) to a sediment mixture of sand and clay (3:1). Several morphometric parameters were recorded during the first five weeks of the experiment, and photosynthesis and respiration were measured after 7 weeks of growth. Results showed a significant reduction of growth and development with increasing nitrogen and temperature levels. Dark respiration increased strongly at high nitrogen levels. At the same time, net photosynthesis at 250 and 500  $\mu\text{E m}^{-2} \text{s}^{-1}$ , Pm, Km and LCP were not affected by either factor. We attribute these phenomena to ammonia toxicity, since relatively high total ammonia levels ( $\text{NH}_3 + \text{NH}_4^+$ ) were found in the interstitial water.



## 1. INTRODUCTION

The effects of increasing nutrient loads on aquatic ecosystems have been described relatively well. It seems clear that there is a causal relation between increasing periphyton and algal blooms, and macrophyte decline and disappearance (e.g. Phillips *et al.*, 1978; De Nie, 1987). But apart from changes in the dominant primary producers through shading effects, high nitrogen levels in the water and sediment may also directly affect the physiology and thus the growth dynamics of submersed macrophytes through toxic effects of ammonia (Mattes & Kreeb, 1974).

The aim of this research was to study the effect of different temperatures and high nitrogen levels on the growth, development and photosynthesis of the submersed macrophyte *Ruppia drepanensis* Tineo under laboratory conditions. This species is the dominant aquatic plant in the brackish area of the Doñana marsh (Southern Spain). This wetland is surrounded by agricultural land used for rice culture and other cereal crops. The high use of fertilizers there can be expected to cause an increase in the concentration of nitrogen inside the marsh area.

We hypothesized that flowering may be triggered by decreased nitrogen availability, and in that case increased nitrogen levels could postpone flowering and reproduction and lead to a longer growth period. Although there was no direct support in literature for this assumption, it seemed a reasonable mechanism. If this should in fact occur in a temporary wetland like the Doñana brackish waterbodies, where aquatic macrophytes survive the dry period by producing drought-resistant seeds, population survival of these annual macrophytes could be threatened.

Related to the temperature effect, we expected plant development to be faster at higher temperatures (Barko & Smart, 1981; Barko *et al.*, 1982), together with earlier flowering and reproduction (Richardson, 1980). We also expected an increase in the rates of net photosynthesis and respiration with increasing temperature, as reviewed in Hootsmans & Vermaat (1991).

## 2. MATERIALS AND METHODS

### 2.1. Experimental set-up: Plant material cultivation

Seeds attached to plant material were collected in an old channel of the Salinas de San Isidoro (Doñana National Park, SW Spain), at the end of the growing season (June 1991). A mixed population of *R. drepanensis* and *Althenia orientalis* (Tzvelev) García-Murillo & Talavera occurs in this locality. Seeds and wet plant material were stored together in plastic bags at 4 °C, until October 1991.

The seeds were germinated in tap water, in the dark and at room temperature (20 °C). After three days, germinated seedlings were selected and transferred to containers with brackish water (0.5 g l<sup>-1</sup> artificial sea salt), where they stayed for one week under low light conditions (10 to 20  $\mu\text{E m}^{-2} \text{s}^{-1}$ ) and room temperature. Previous experience had shown that this treatment resulted in the best survival percentage. After one week the seedlings, of between 3 and 5 cm size, were randomly distributed over the treatments and subsequently planted.

Two levels of temperature (20 $\pm$ 0.5 and 30 $\pm$ 0.5 °C) and three levels of nitrogen

(referred from now on as "N0", "N1" and "N2") were combined in six treatments. Each treatment consisted of one aquarium (60\*40\*40 cm<sup>3</sup>), with the exception of the "N0" nitrogen level that had two aquaria for each temperature. The eight aquaria were placed in two phytotrons. The aquaria had independent pumping systems for cooling and heating, and a common light system.

Temperature was maintained at the required levels by a system of two pumps with independent thermostats, one connected to a cooling system and one equipped with a heating system. To ensure that the water was homogeneously mixed, the heating pump was working continuously, although not constantly heating. Demineralised water was used to replenish the aquaria when necessary.

As a light source 17 fluorescent tube lamps (Philips, colour 84, 36 W) were used in each phytotron, providing a light intensity of 240  $\mu\text{E m}^{-2} \text{s}^{-1}$  at 1 cm below the water surface (measured with a LICOR LI-192S sensor). The photoperiod was 16 hours.

Each aquarium contained 39 plastic cups (8 cm height) filled with 100 ml of a mixture of sand and clay (3:1 by weight) and covered by 1 cm of washed sand. Space among the cups was also filled with washed sand. The remaining volume of the aquaria was filled with tap water one week before planting the seedlings.

The three levels of nitrogen were obtained by adding a slow release fertilizer (Osmocote® pellets) to the sediment mixture. The pellets contained 23 % of ammonium nitrate by weight, and were placed at 1 and 3 cm above the bottom of the cups.

The clay used in the sediment mixture and clay samples from Doñana National Park (lucio Vetas Altas Chico, July 1991) were analyzed to determine the total nitrogen content (Novozamsky *et al.*, 1983). The values obtained (clay used in the sediment mixture:  $938 \pm 434 \text{ mg N kg}^{-1}$ , average  $\pm$  standard deviation; Doñana clay:  $1592 \text{ mg N kg}^{-1}$ ) were used to determine the three nitrogen treatments. Nitrogen level N1 was comparable to the nitrogen content of Doñana clay (108.8 mg N per sediment cup), N0 was half of it (54.4 mg N / cup), and N2 had twice as much (228 mg N / cup).

Samples of surface and interstitial water were taken for analysis of alkalinity and total ammonia ( $\text{NH}_3 + \text{NH}_4^+$ ) 1, 2 and 4 weeks after starting the experiment. Two replicate samples were taken in polyethylene flasks. The interstitial water was sampled each time from different, randomly-selected plant cups, using a semipermeable stick (70\*3 mm) connected to a 10 ml syringe (RHIZON SSS, Rhizosphere Research Products, NL). Interstitial water samples were taken overnight. Alkalinity was determined by titrimetry with 0.02 N sulphuric acid, using methyl orange and phenolphthalein as end-point indicators (APHA, 1985). Total ammonia was determined by potentiometric measurements (METHROM Titroprocessor 636 dosimeter, ORION 95-10 specific electrode).

## 2.2. Characterization of plant material

During the first five weeks of the experiment the following parameters were recorded in 20 randomly-selected plants from each treatment: number of shoots, number of leaves per shoot, length of the largest leaf and number of flowers per plant. The same plants were measured each week. In the "N0" treatment, 10 different plants were randomly selected from each of the two aquaria at each temperature treatment.

After four weeks, six plants were harvested from each treatment to determine their biomass (above and below-ground fractions). Fresh weight and dry weight (70 °C, 24 h) were recorded.

Plants collected after 7 weeks for the photosynthesis measurements were also used for biomass determination (fresh, dry and ash-free dry weights, the last after 3 h at 520°C). Subsamples were taken to determine the aboveground to belowground biomass ratio. Plants from the treatment "30 °C, N2" could not be used for biomass measurements, due to their very small size.

Ash free dry weight of the 4-weeks plant material was calculated from the dry weight values by using the dw/afdwt regression line fitted using the 7-weeks plant material.

### 2.3. Experimental set-up for photosynthesis measurements

All photosynthesis measurements were done in a 96 l aquarium connected to a cooling system which kept the temperature during the incubation at  $\pm 1^\circ\text{C}$  of the desired value. Together with this system, three heating coils were used for the measurements at 30 °C. The temperature selected was the same as that at which the plants did grow (20 and 30 °C respectively). The aquarium was filled with tap water and 20 g  $\text{NaHCO}_3$  was added to arrive at saturating inorganic carbon levels (3.7 mM  $\text{HCO}_3^-$ ; Sand-Jensen, 1983). Nitrogen was bubbled in the aquarium prior to the oxygen production measurements, in order to reduce the concentration of dissolved oxygen.

Three independent, replicate systems were used. Each one consisted of an electrode chamber and a 5 cm diameter perspex tube interconnected with pvc tubing. A peristaltic pump (Watson Marlow 504U) was used to circulate the water. Temperature and dissolved oxygen were recorded every 10 seconds with a Campbell 21X datalogging set connected to three WTW EO-196 oxygen electrodes and WTW OXY 196 electrode meters. Light was provided by a Philips 400 W HPIT metal halide lamp. Different light levels were created by varying the distance between lamp and water surface and using a neutral density net. A shallow perspex flow-through waterbath was suspended beneath the lamp to absorb the infrared radiation.

Flow rate was  $0.75 \text{ l min}^{-1}$  (equivalent to  $6.5 \text{ mm s}^{-1}$  in the perspex tube). This is about half of the flow rate used by Hootsmans & Vermaat (1991) and Sand-Jensen (1983). Nevertheless, since in a comparable incubation chamber Westlake (1967) found that rates of photosynthesis of *Potamogeton pectinatus* L. did not increase further above flow rates of  $0.4 \text{ mm s}^{-1}$ , we expect that the used flow rate had no appreciable limiting effect on photosynthesis.

Five to six randomly-selected, intact plants of seven weeks old were used per tube. Plants were carefully washed out of the sediment, and kept overnight in darkness in order to permit acclimation and to deplete lacunar oxygen reserves. Next morning, dark respiration of the plant material was measured during 30 to 45 minutes. Subsequently, the tubes were exposed to the different light levels (15 to 45 min, until at least a  $0.5 \text{ mg l}^{-1}$  increase in dissolved oxygen concentration was reached), starting with the lowest intensity and ending with the highest. Between each measurement the tubes were opened and the medium inside was completely replenished with the surrounding water.

As the light field could not be held homogeneous for the light levels above  $150 \mu\text{E m}^{-2} \text{ s}^{-1}$ , we measured light on three points along each tube and used the average intensity per tube for further calculations. Light intensities at 1 cm below the water surface outside the tubes were on average 25, 50, 75, 100, 150, 190, 265, 300, 380 and  $435 \mu\text{E m}^{-2} \text{ s}^{-1}$  respectively. For N1 and N2 plant material, only respiration and production at 265 and  $435 \mu\text{E m}^{-2} \text{ s}^{-1}$  were measured.

It was not possible to measure a PI curve for the plants grown at 30°C and nitrogen level N2. Due to their very low biomass, these plants did not produce a sufficiently large increase in oxygen concentration during the time available for the measurements.

### 3. CALCULATIONS AND STATISTICAL ANALYSIS

#### 3.1. Morphometrical data

Statistical analysis of the morphometrical data followed Vermaat & Hootsmans (1991), with minor modifications, and were done with the SAS statistical package (SAS Institute Inc., 1988). An exponential curve was fitted for the morphometric variables using a non-linear iterative technique based on the Marquardt algorithm (Conway *et al.*, 1970), since the inflexion point of a logistic growth curve was not reached during the experimental time. To facilitate computation, four curves per aquarium were fitted, each one with data from five, randomly-selected plants.

Parameters of the curves were compared by means of an Analysis of Variance (ANOVA) followed by multiple comparisons among treatments. ANOVA tests were done with the General Linear Models (GLM) procedure, after  $\log_{10}$  transformation if residuals were not normally distributed. Homogeneity of residual variances was checked with a plot of predicted versus residual values.

For multiple comparisons, we used the LSMEANS option in SAS. Comparisonwise error rates (CER) for each comparison were adjusted to maintain an experimentwise error rate (EER) of 0.05.

#### 3.2. Photosynthesis data

Each data set relating oxygen concentration to time for a particular light level was checked for measurement errors, and lag phases were excluded. Oxygen exchange rates were calculated by linear regression and expressed in  $\mu\text{g O}_2$  per g afdw of plant tissue per minute ( $\mu\text{g O}_2 \text{ g afdw}^{-1} \text{ min}^{-1}$ ).

The resulting data set for each treatment replica consisted of the experimental light levels and the corresponding  $\text{O}_2$  exchange rates. A rectangular hyperbola (Michaelis-Menten model) was fitted to each data set using the non-linear regression (NLIN) procedure of the SAS statistical package (SAS Institute Inc., 1988). The resulting model parameter estimates were used for ANOVA and multiple comparisons (as above), together with one derived parameter, the light compensation point (LCP). Estimated net oxygen production rates at 250 and 500  $\mu\text{E m}^{-2} \text{ s}^{-1}$  ( $P_{250}$  and  $P_{500}$ ) were also used, as sometimes the estimated maximum rate of gross photosynthesis (parameter  $P_m$ ) was reached beyond the light level range used in the experiment.

A total of 6 light-response (PI) curves was obtained from the experiment, for the plants grown at the zero nitrogen level (3 replicate curves at 20 °C, and 3 at 30 °C). For comparison with other PI curves from literature (see Discussion), only these curves will be used.

PI curves measured for the plants grown at the nitrogen levels N1 and N2 were based on the respiration rate and 2 production rates (at 265 and 435  $\mu\text{E m}^{-2} \text{ s}^{-1}$  light intensities). Two different data sets were used for the statistical analyses: the first one included the

TABLE 1: Ammonia concentration in the water column and porewater ( $\text{mg NH}_4^+ \text{-N l}^{-1}$ ). Results shown in the table are the average value of two replicate samples (\*: no replicate samples). N0, N1 and N2 represent the three nitrogen levels.

Weeks:	Water column			Porewater		
	1st	2nd	4th	1st	2nd	4th
20°C,N0	0.11	0.12	0.06	3.5*	0.9	0.6
20°C,N0	0.08	0.09	0.07	1.1	2.9	1.9
20°C,N1	0.09	0.12	0.38	14.4	9.8	39.5
20°C,N2	0.23	0.42	0.27	297.9	9.6	235.0
30°C,N0	0.10	0.35	--	2.3	1.4	3.0
30°C,N0	0.06	0.07	0.28	1.6	1.1*	2.8
30°C,N1	0.14	0.13	0.16	4.9	4.4	11.0
30°C,N2	1.96	0.18	0.37	24.9	17.1	22.0

measured respiration and photosynthetic rates (RESP, P265 and P435), and the second one included the parameter estimates (Pm, Km and R) and the production rates ( $P_{250}$  and  $P_{500}$ ) estimated with the fitted curves.

## 4. RESULTS

### 4.1. Water chemistry

Initial bicarbonate alkalinity of the tap water used to fill the aquaria was 1.2 mM. Bicarbonate alkalinity decreased over time in all aquaria, from initial values of about 0.9 mM (1st week) to values of 0.4 to 0.7 mM. Carbonate alkalinity was not detectable in any of the samples.

Total ammonia concentration in the surface water showed a general increase with increasing nitrogen level (Table 1), but it never exceeded  $0.5 \text{ mg NH}_4^+ \text{-N l}^{-1}$  (except in one case, the first week in the "30°C,N2" aquarium). Total ammonia concentration in the interstitial water showed a more clear increase with increasing nitrogen level.  $\text{NH}_4^+ \text{-N}$  ranged from 0.5 to  $3.5 \text{ mg l}^{-1}$  for the N0 treatments (at both temperatures), 5 to  $40 \text{ mg l}^{-1}$  for the N1 treatments, and above  $15 \text{ mg l}^{-1}$  (but extremely variable at 20°C, from 9 to  $300 \text{ mg l}^{-1}$ ) for the N2 treatments.

### 4.2. Characterization of plant material

#### 4.2.1. Plant morphometry

Only a few plants flowered in some of the treatments during the 7 weeks experiment. Thus, the parameter "number of flowers per plant" was not included in the statistical tests.

A Two Way ANOVA test was done to compare the effects of growth temperature (GTEMP) and nitrogen level (NITR) on the estimated relative growth rates of the variables "number of leaves" (RLV), "number of shoots" (RSH) and "length of the largest leaf" (RLL). GTEMP and NITR effects were significant for all three parameters tested. The

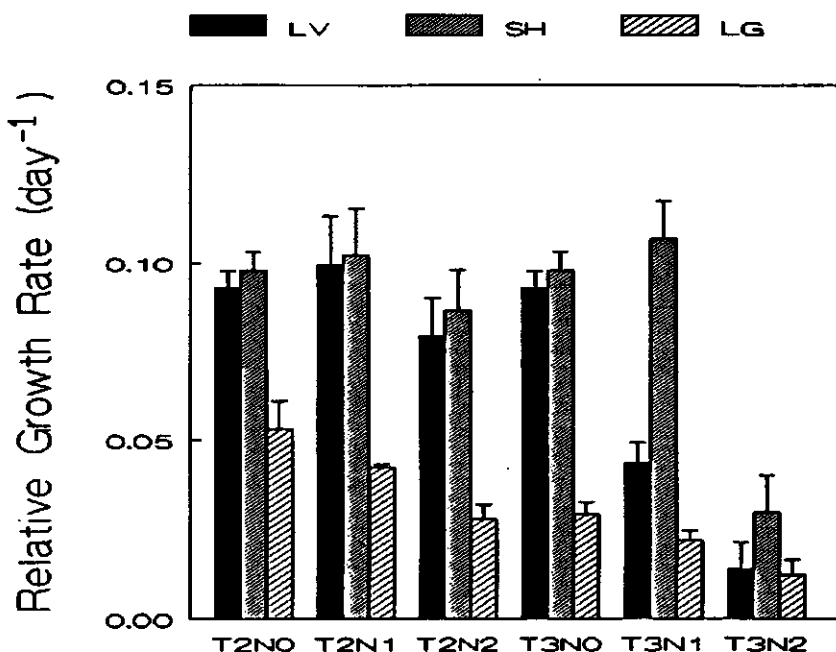


FIG. 1: Means and standard deviations of relative growth rates (day<sup>-1</sup>) of the number of leaves (LV, filled bars), number of shoots (SH, densely hatched bars) and the length of the longest leaf (LG, thinly hatched bars). T2, T3 represent the temperatures 20 and 30 °C, N0, N1 and N2 represent the three nitrogen levels.

interaction of nitrogen and temperature was also significant for RLV and RSH.

All three relative growth rates decreased with increasing temperature and increasing nitrogen level (Fig. 1). Multiple comparisons were done among the 9 combinations of GTEMP and NITR (comparisons for interaction between both factors are excluded; EER = 0.05, CER = 0.0056). For RLV, all comparisons except "N0 vs N1, 20°C" and "N1 vs N2, 20°C" were significant. For RSH and RLL, a significant nitrogen level effect was only present between N0 and N2 at both temperatures. Temperature effect was significant in all comparisons but one (N1 level of RSH) for all three parameters tested.

The derived parameters number of leaves, number of shoots and largest leaf length on the 30th day of age (LV30, SH30 and LL30, respectively) were calculated with the fitted curves. Two Way ANOVA (after log<sub>10</sub> transformation) yielded significant results for both growth temperature and nitrogen level ( $p < 0.001$ ), as well as their interaction ( $p < 0.01$ ), on LV30 and SH30. LL30 was significantly affected by GTEMP and NITR ( $p < 0.001$ ), but not by their interaction ( $p = 0.194$ ). All three parameters (LV30, SH30 and LL30) decreased with increasing temperature and nitrogen level. The subsequent multiple comparisons showed that the temperature effect did not result in significant differences inside the zero nitrogen level (N0), and the nitrogen effect did not lead to significant differences at 20 °C. Length of the largest leaf (LL30) showed significant differences in all comparisons but one (N1 vs N2, 30°C).

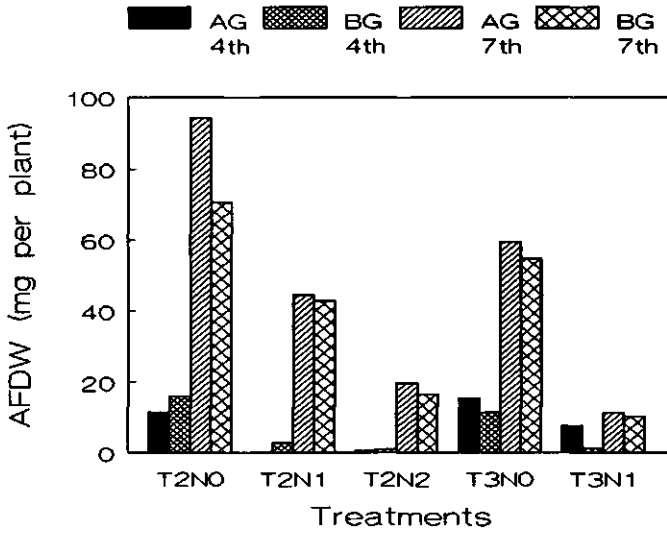


FIG.2: Individual plant biomass (mg afdw) after 4 and 7 weeks. For the coding of treatments, see Fig. 1. AG = aboveground biomass, BG = belowground biomass.

#### 4.2.2. Plant biomass

Biomass after 4 and 7 weeks is shown in Fig.2. There was a decrease in aboveground (AGB) and belowground (BGB) biomass with increasing temperature and increasing nitrogen level, although the temperature effect was only evident for the 7 weeks values. An increase of biomass with time was also noticed, especially for the zero nitrogen (N0) treatment.

Higher AGB/BGB ratios were found in the 30°C treatments for the 4th week, with very high values observed for the "N2, 30°C" treatment. AGB/BGB ratios increased with time for the 20 °C treatments, but decreased with time for the 30 °C treatments, both converging to a similar 7th-week value.

Two Way ANOVA was performed to compare the effects of growth temperature (GTEMP) and nitrogen level (NITR) and their interaction on the aboveground, belowground and total biomass (TOTB), and on the ratios aboveground/belowground biomass (AGB/BGB), aboveground biomass/number of leaves (AGB/LV) and aboveground biomass/number of shoots (AGB/SH), of plants of 7 weeks.

The overall F-value was not significant for the AGB/BGB ratio. Nitrogen level had a significant effect on all parameters tested but the AGB/BGB ratio. Growth temperature significantly affected all parameters except the AGB/BGB and AGB/LV ratios. The interaction effect of both factors was only significant for the AGB/SH ratio. Aboveground, belowground and total biomass decreased with increasing growth temperature and nitrogen level. AGB/LV and AGB/SH ratios decreased with increasing nitrogen levels, and were unaffected by growth temperature.

Multiple comparisons were done among five combinations of the factors GTEMP and NITR (comparisons for interaction between both factors are excluded; EER=0.05, CER=0.010). The results are shown in Table 2.

TABLE 2. Multiple comparisons for aboveground (AGB), belowground (BGB) and total biomass (TOTB), and for the ratio of aboveground biomass per number of leaves (AGB/LV) and per number of shoots (AGB/SH). N0, N1 and N2 represent the three nitrogen levels. Small lettering shows comparisons between nitrogen levels within each temperature, capitals do the same for comparisons between temperatures within each nitrogen level. Significant differences are indicated by different letters.

	TOTB			AGB			BGB			AGB/LV			AGB/SH		
	N0	N1	N2	N0	N1	N2	N0	N1	N2	N0	N1	N2	N0	N1	N2
20 °C	aA	bC	c	aA	bC	c	aA	aB	b	aA	bB	b	aA	bB	c
30 °C	dB	eD	-	dB	eD	-	cA	dC	-	cA	dB	-	dA	dB	-

#### 4.3. Photosynthesis measurements

The fitted photosynthesis-light curves are shown in Fig.3, and the parameters calculated by curve fitting are presented in Table 3. The non-linear fitting procedure resulted in negative Km values for three replicate curves (one from each of the treatments "N1,20°C", "N2,20°C" and "N1,30°C", respectively). The results of this three curves were not included when performing the ANOVA of the derived parameters, but the actual datapoints were included in the ANOVA of the actually measured rates.

Differences between replicate curves, as found by Hootsmans & Vermaat (1991) were also observed. Although these differences existed, each resulting curve presented a good fit of its respective datapoints. The F-test was significant for all curves.

Results of the Two Way ANOVA tests were similar for both data sets. Both growth temperature and nitrogen level, and their interaction had a significant effect only on dark respiration (RESP,  $n=15$ ,  $p<0.05$ ; and R,  $n=12$ ,  $p<0.005$ ). For Pm and Km parameter estimations and for production rates P<sub>265</sub>, P<sub>250</sub>, P<sub>435</sub> and P<sub>500</sub>, neither GTEMP nor NITR nor their interaction had a significant effect. Respiration increased with increasing growth temperature and increasing nitrogen level.

## 5. DISCUSSION

### 5.1. The effect of temperature and nitrogen level on growth

Both temperature and nitrogen level affected the growth of *R. drepanensis* in a qualitative and quantitative way. The quantitative effects of both factors are described in relation to plant biomass and to the relative growth rate.

#### 5.1.1. Temperature effect

Comparing the biomass results at 20 and 30 °C (Fig.2), it can be concluded that *R. drepanensis* grew better at 20°C than at 30°C, independent of the nitrogen level. This contrasts with Barko & Smart (1981) and Barko *et al.* (1982), who found that growth of different species (*Elodea canadensis* Michx., *Potamogeton nodosus* Poiret and *Vallisneria*



TABLE 3: Parameter estimates (mean  $\pm$  standard deviation) from the fitted photosynthesis-light curves. Pm = maximum rate of gross production ( $\mu\text{g O}_2 \text{ g afdw}^{-1} \text{ min}^{-1}$ ), Km = half saturation constant ( $\mu\text{E m}^{-2} \text{ s}^{-1}$ ), R = dark respiration ( $\mu\text{g O}_2 \text{ g afdw}^{-1} \text{ min}^{-1}$ ), LCP = light compensation point ( $\mu\text{E m}^{-2} \text{ s}^{-1}$ ), n = number of replicates.

Treatment	n	Pm	Km	R	LCP
N0, 20 °C	3	219.4 $\pm$ 71.5	267.7 $\pm$ 159.6	23.3 $\pm$ 11.5	29.0 $\pm$ 12.4
N0, 30 °C	3	277.5 $\pm$ 105.4	367.7 $\pm$ 67.3	54.3 $\pm$ 2.1	96.7 $\pm$ 24.1
N1, 20 °C	2	226.0 $\pm$ 26.9	412.0 $\pm$ 219.2	29.0 $\pm$ 0.0	59.0 $\pm$ 24.2
N1, 30 °C	2	386.5 $\pm$ 153.4	111.0 $\pm$ 117.4	202.5 $\pm$ 60.1	150.3 $\pm$ 81.5
N2, 20 °C	2	302.5 $\pm$ 64.3	160.0 $\pm$ 190.9	89.0 $\pm$ 26.9	50.1 $\pm$ 37.0

*americana* Michx. in Barko & Smart, 1981; *Egeria densa* Planch, *Hydrilla verticillata* Royle and *Myriophyllum spicatum* L. in Barko *et al.*, 1982) increased with increasing temperature to at least 28 °C. On the other hand, Richardson (1980) found and reviewed temperature optima for vegetative growth of *Ruppia maritima* L. at 20 to 25 °C, with higher temperatures leading to flowering and seed production. However, his information is not convincing, as in fact his optima are simple correlations between the various stages in plant development and the temperature cycle in field.

The relative growth rates of leaves and shoots were higher at 20°C than at 30°C. Plants grown at 30°C had lower number of leaves and smaller leaf length than plants grown at 20°C, independent of the nitrogen level. A temperature effect on the number of shoots per plant was clear at nitrogen levels N1 and N2, but no significant effect could be found at the zero nitrogen level.

The relative growth rate of the parameter "number of leaves" found in this experiment is comparable to the values found by Vermaat & Hootsmans (1991) for *P. pectinatus* using logistic fitting. They reported a growth rate of 0.08 to 0.10 day<sup>-1</sup> (plants grown at 18°C, 200  $\mu\text{E m}^{-2} \text{ s}^{-1}$ ), while in this study a growth rate of 0.11 day<sup>-1</sup> was found (20°C, 240  $\mu\text{E m}^{-2} \text{ s}^{-1}$  and nitrogen level N0).

On first sight, it is remarkable that a Mediterranean species showed a growth optimum at 20°C, while a temperature of 30 °C is easily reached in this region during late spring and summer. This may be due to the fact that *R. drepanensis* inhabits temporary wetlands, which dry out before the temperature reaches up to 30 °C. A more detailed discussion of this hypothesis is presented below (see Temperature effect on photosynthesis).

### 5.1.2. Nitrogen effect

The levels of total nitrogen fertilization used in this study and mentioned in the literature were converted to a common unit (g N m<sup>-2</sup> sediment surface area) for comparison. In the present work, the total amount of nitrogen present was 10.9 g N m<sup>-2</sup> in the N0 level, 21.8 g N m<sup>-2</sup> in the N1 level, and 43.6 g N m<sup>-2</sup> in the N2 level.

In a fertilization experiment also using Osmocote pellets (10% nitrate + 9% ammonium), Pulich Jr. (1985) found that *R. maritima* plants showed a significant positive response to

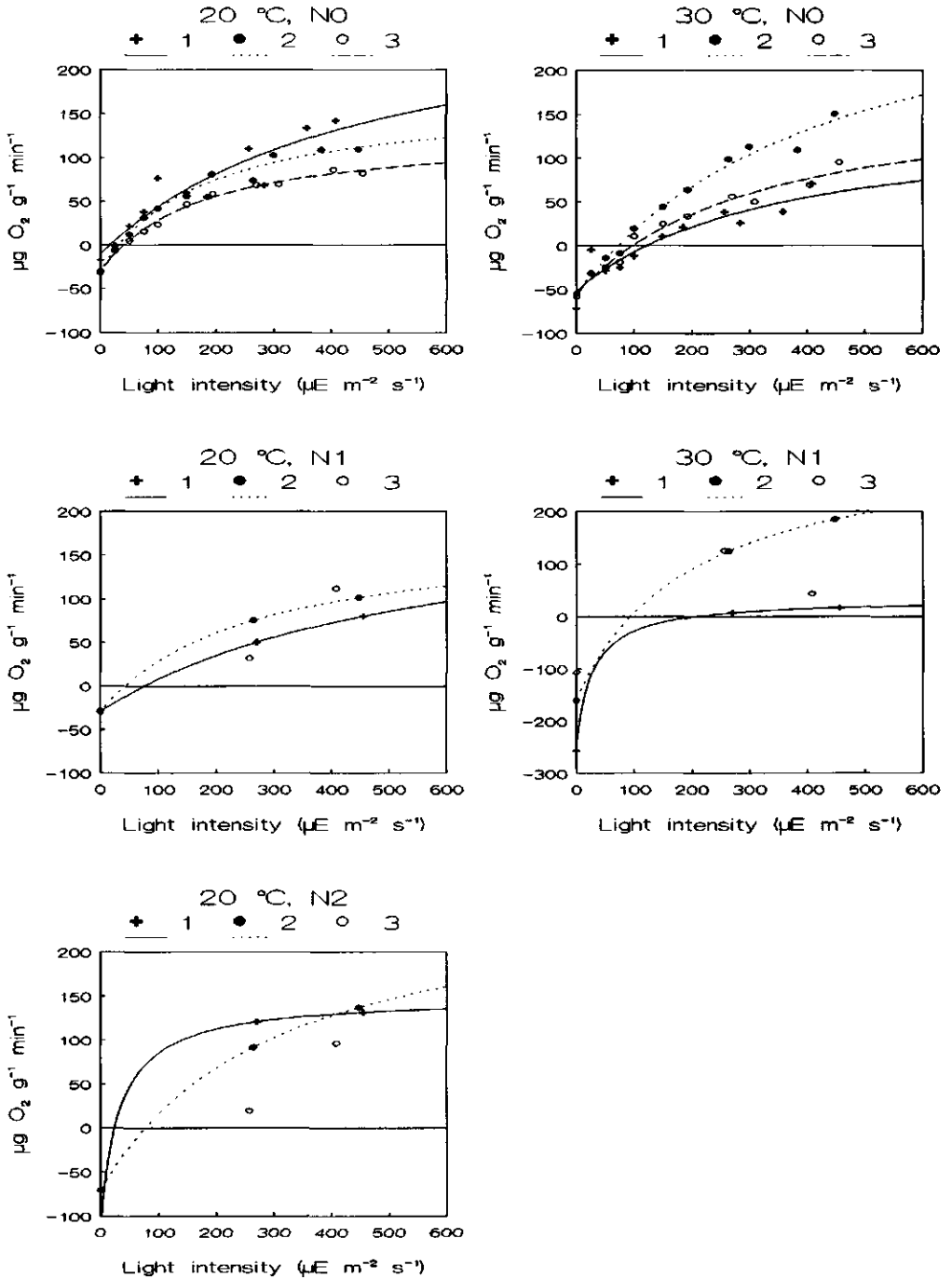


FIG.3: Replicate photosynthesis-light curves fitted with the rectangular hyperbola (Michaelis-Menten equation). Nitrogen levels are indicated with NO, N1, N2. Note difference in the vertical scale for the "30°C,N1" treatment.

fertilization. Addition of 20 g N m<sup>-2</sup> (a value comparable to our N1 nitrogen level) caused an increase in shoot biomass, but no apparent change in underground biomass.

Various authors did fertilization experiments using different species and higher and lower fertilization rates than the one we used: 100 g N m<sup>-2</sup> ammonium and *Heterozostera tasmanica* (Martens ex Aschers) den Hartog in Bulthuis & Woelkerling (1981); 12.5, 25 and 50 g N m<sup>-2</sup> ammonium nitrate (Roberts *et al.*, 1984) and 64 and 128 g N m<sup>-2</sup> nitrate (Orth, 1977), both using *Zostera marina* L. All found a stimulating effect of nitrogen addition on different growth parameters.

All these results contrast with our findings. Using 23% ammonium nitrate Osmocote pellets as fertilizer, we observed a limiting effect on growth and development of *R. drepanensis* with increasing nitrogen levels. Independent of the two temperatures used, plants showed a significant decrease in above, below and total biomass, and also in the number of leaves, number of shoots and largest leaf size, with increasing nitrogen levels.

To explain these results, a toxic effect of the compounds used as fertilizer on *R. drepanensis* can be suggested. Although the references cited above show that the addition of ammonium can positively influence shoot length, leaf area and biomass of submerged vascular plants (also in Short, 1987, working with *Z. marina*), and ammonia is referred to as the preferred source of nitrogen for both leaves and roots of *Z. marina* in Short & McRoy (1984), ammonium salts have also been reported to be injurious to plants when placed very close to the root zone (Lorenz *et al.*, 1955 and Grogan *et al.*, 1956, as cited in Vines & Wedding, 1960), and Bloemendaal & Roelofs (1988) cite ammonia as a common pollutant with a toxic effect on aquatic plants.

In an experiment using *Potamogeton densus* plants cultivated in solutions of ammonium, Mattes & Kreeb (1974) found that growth and net assimilation rate were negatively affected by increasing ammonium concentrations, and they concluded a minimum toxic level of 5 mg NH<sub>4</sub><sup>+</sup> l<sup>-1</sup> (equivalent to 3.9 mg NH<sub>4</sub><sup>+</sup>-N l<sup>-1</sup>). Using the percentage of decaying plant parts as an indicator of plant vitality, Roelofs (1991) reported a toxic effect on *Stratiotes aloides* L. plants cultivated during 10 weeks at NH<sub>4</sub>Cl concentrations equal or higher than 50 μM (equivalent to 0.7 mg NH<sub>4</sub><sup>+</sup>-N l<sup>-1</sup>).

Hence, the high levels of total ammonia measured in the interstitial water (Table 1) during our experiment could have caused an inhibition of plant growth. This hypothesis is supported by the fact that ammonia supply in the root zone has been reported to inhibit nitrate uptake by leaves and to almost suppress nitrate uptake by roots in *R. maritima* (Thursby & Harlin, 1984). Similar results have been described for *Z. marina* (Iizumi & Hattori, 1982) and for *S. aloides* (Roelofs, 1991).

The toxicity of ammonia is dependent on temperature, pH and on the plant species itself (Bloemendaal & Roelofs, 1988). Significance of the interaction of growth temperature and nitrogen level in the present experiment could reflect such an influence of temperature on the ammonia toxic effect.

The pH has often been suggested as the basic cause of toxicity to plants resulting from ammonia applications. The amount of non-dissociated ammonia increases with increasing pH. This form can cross the cell barriers easily and in high amounts it can be an effective inhibitor of respiration (Vines & Wedding, 1960). This explanation fits with the fact that potassium depletion together with the presence of high levels of ammonia has been reported to cause severe injuries to cultured terrestrial plants. The absence of potassium leads to a low pH inside the cell and a higher pH outside, favouring the passage of ammonia into the cells (Warren, 1962).

Measurements of pH were done in the 6th week of the experiment, in the water column

and in samples of interstitial water. High values (10.1) were only found in the water column of the N0 aquarium; pH values in the N1 and N2 aquaria were in a range from 7 to 8. Interstitial water samples presented values varying from 7.2 to 7.6, in which the predominant form of ammonia is the ion  $\text{NH}_4^+$ .

In the sediment of Lake Wingra, Irisimah *et al.* (1976) found levels of total ammonia in a range of 4.9 to 8.9  $\text{mg NH}_4^+\text{-N l}^{-1}$ . Søndergaard (1990) reported ammonium concentrations up to 15  $\text{mg NH}_4^+\text{-N l}^{-1}$  in the upper 20 cm of the sediment of a shallow hypertrophic lake, Lake Søbygaard. Brinkman & Raaphorst (1986) reviewed ammonium "concentrations in the porewater of the aerobic layer ... in the order of 0.1 to 3  $\text{mg NH}_4^+\text{-N l}^{-1}$  (...), although they can also be much higher (10 to 50  $\text{mg NH}_4^+\text{-N l}^{-1}$ )". Molongoski & Klug (1980) reported porewater ammonia concentrations between 20 and 40  $\text{mg NH}_4^+\text{-N l}^{-1}$  in the sediment surface of a shallow hypereutrophic lake, Wintergreen Lake. All these values are comparable to the total ammonia concentrations found in the interstitial water of the N1 level during our experiment (4.4 to 39.5  $\text{mg NH}_4^+\text{-N l}^{-1}$ ). Consequently, extrapolation of the results found here to field conditions seems reasonable.

## 5.2. The effect of growth temperature and nitrogen level on photosynthesis

### 5.2.1. Comparison with literature data

The information on *R. drepanensis* photosynthesis available in literature is very limited. García *et al.* (1991), using *R. drepanensis* plants from a shallow lake in South Spain (Laguna de Fuente de Piedra), found a maximum rate of net photosynthesis of 0.55  $\text{mg C g dw}^{-1} \text{h}^{-1}$  at an experimental temperature of 25 °C, equivalent after conversion to a maximum rate of gross photosynthesis of 51  $\mu\text{g O}_2 \text{ g afdw}^{-1} \text{min}^{-1}$  (assuming  $\text{PQ}=1$  and  $\text{afdw}=0.8\text{dw}$ , and a recalculated rate of dark respiration of 0.36  $\text{mg C g dw}^{-1} \text{h}^{-1}$ ). Pm values of 219 and 278  $\mu\text{g O}_2 \text{ g afdw}^{-1} \text{min}^{-1}$  were obtained in our measurements at 20 and 30 °C respectively (N0 treatments), four to five times higher than the one reported above.

The same authors found a light compensation point (LCP) of 86  $\mu\text{E m}^{-2} \text{s}^{-1}$ , and a half saturation constant (Km) of 236  $\mu\text{E m}^{-2} \text{s}^{-1}$ . Both values are within the ranges found in our measurements (Table 3; N0 treatments).

Comparing these results, it seems that large variation exists. We attribute this mainly to differences in plant age or restricted carbon availability. Working with water and plant material from a shallow lake in the Doñana brackish marsh area, Pm values were found to decrease with age (before vs. after seed production) from 116 to 59  $\mu\text{g O}_2 \text{ g afdw}^{-1} \text{min}^{-1}$  (assuming  $\text{afdw}=0.8\text{dw}$ ), and to increase from 116 to 223  $\mu\text{g O}_2 \text{ g afdw}^{-1} \text{min}^{-1}$  when increasing the bicarbonate concentration from 0.02 to 3.75 mM (Santamaría *et al.*, 1992). These measurements were done at 20 to 24 °C, and the Pm estimation with "young" material and 3.75 mM bicarbonate is quite similar to the Pm found in the present experiment at 20 °C. The Pm value obtained using low DIC concentrations and old plant material approaches very much the value found by García *et al.* (1991), who used water and plants obtained at the end of the growing season (April).

Santamaría *et al.* (1992) concluded that plant age and bicarbonate concentration did not significantly affect the Km value, which agrees with the similarity between the Km and LCP values found by García *et al.* (1991) and those presented here.

### 5.2.2. Temperature effect

Respiration was the only parameter affected by temperature and nitrogen level, and also by their interaction. Hootsmans & Vermaat's (1991) review on the effect of temperature on photosynthesis mentions an increase of both photosynthetic activity and respiration with increasing temperature in all references cited. Although the quantitative effect of temperature seems to be rather variable, they conclude that "... up till a certain level (30 °C), increasing temperatures will lead to increased Pm, Km, R and LCP...".

Several of the references mentioned in Hootsmans & Vermaat (1991) dealt with species from (or collected from) temperate regions, where the chance of being exposed to temperatures of 30 °C is less than in the Mediterranean region. It seems remarkable that *R. drepanensis*, a plant distributed all around the Western Mediterranean, does not show a similar increase in photosynthetic activity from 20 to 30 °C. This may be caused by the fact that *R. drepanensis* plants in their natural habitats (temporary shallow lakes) are not subjected to constant high temperatures throughout their life cycle. Temporary wetlands in Central and Southern Spain fill up in autumn, have water all along winter and spring, and dry out in late spring when temperatures reach levels above 25 to 30 °C (unpubl. data; also in García *et al.*, 1991). Thus, life cycle of *R. drepanensis* usually develops in a range between 10 to 25 °C. High temperatures are associated with the end of the growing season, and may be used to induce seed production to overcome the dry period. Temperatures of 25 to 30 °C are cited to induce seed production (Richardson, 1980) for the American populations of *R. maritima*, although such an effect was not found for *R. drepanensis* in this study.

### 5.2.3. Nitrogen level effect

Nitrogen level during growth affected respiration, which increased with increasing nitrogen level. On the other hand, maximum gross production (Pm), half saturation constant (Km) and light compensation point (LCP) all remained unaffected.

Van der Eerden (1982) proposed a double mechanism for ammonia toxicity: first, it acts as an inhibitor of photosynthetic phosphorylation, reducing plant growth; second, it also acts by saturating the lipids in the cell membrane, increasing its permeability and decreasing its flexibility. He further suggested that ammonia can be detoxified by conversion into aminoacids, if metabolic activity is high enough and if enough carbohydrates are available.

These mechanisms could explain our results, assuming that the negative effect of increased nitrogen level on growth and photosynthetic activity of the plants is also due to ammonia toxicity. NH<sub>4</sub><sup>+</sup>-induced uncoupling of photophosphorylation does not limit photosynthetic oxygen production (as the electron transport is not affected), but it does severely restrict the ATP production in leaves and consequently reduces the associated carbon assimilation. This may explain why plants grown under the N1 and N2 treatments grew slower, but did not show lower values of net oxygen production. On the other hand, detoxification of ammonia by conversion of stored carbohydrates into aminoacids consumes ATP, possibly resulting in the higher respiration rates and the reduced growth rates found in the plants grown under the N1 and N2 treatments.

Temperature interacted significantly with the nitrogen effect on respiration. A temperature effect on both the ammonia passage of the cell membrane and on the several reactions involved in the ammonia detoxification could account for this effect.

## 6. CONCLUSIONS

*R. drepanensis* plants cultured at 30 °C grew slower and showed a higher respiratory rate than those cultured at 20 °C, although both had similar rates of net photosynthesis. The temperature optimum below 30 °C can be attributed to the particular characteristics of Mediterranean temporary wetlands. These have water only until the end of the spring season, thus not reaching temperatures above 25 °C during most of the growing season of the plants.

Nitrogen fertilization in the form of ammonium nitrate in the root zone resulted in a slower growth of the plants, together with an increase in dark respiration. Net rate of photosynthesis remained unaffected. We attribute this effect to ammonia toxicity, which by uncoupling ATP formation during the photophosphorylation and the respiratory phosphorylation, reduces carbon fixation and thus plant growth without affecting photosynthetic oxygen production.

The total nitrogen levels used in the experiment were within the range of those present in the sediments of the Doñana brackish marsh waterbodies. Thus, the negative effects of high nitrogen contents observed in our experiments can be expected in the field also. Further work is necessary to examine the actual concentrations of ammonia in the sediments of this area, although values from the literature suggest that the total ammonia concentrations used here are within the range of those present in eutrophic sediments.

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## Chapter 5

### Flowering time as influenced by nitrate fertilization in *Ruppia drepanensis* Tineo.

L. Santamaría, M.J.M. Hootsmans and W. van Vierssen

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**ABSTRACT:** In a laboratory experiment, *Ruppia drepanensis* Tineo seedlings from a brackish marsh in Southern Spain were grown with and without potassium nitrate fertilization ( $1.4 \text{ g N week}^{-1} \text{ m}^{-2}$  added to the water column), both under nutrient-rich (clay:sand mixture) and nutrient-poor (sand) sediment conditions. Potassium nitrate fertilization delayed flower initiation within both types of sediment, but it also resulted in increased flower abundance under nutrient-poor sediment conditions. Besides, plant-to-plant differences in flower production were positively correlated with the above- to belowground biomass ratio. We thus propose that nutrient supply and biomass allocation should be incorporated to the age- and size-dependent models for plant reproductive effort. These models should also consider separately the induction of flowering and the production of flowers (flower abundance). Nitrogen and phosphorus concentrations in the plant tissue tended to reach a certain minimum value ( $15 \text{ to } 25 \text{ mg N g}^{-1} \text{ dw}$ ,  $1 \text{ to } 2 \text{ mg P g}^{-1} \text{ dw}$ ), both under limiting and non-limiting nitrogen conditions. Tissue nutrient concentrations gave thus ambiguous results when used to determine possible nutrient limitation. Finally, significant plastic responses to nutrient supply were found at three different levels: in the different characters (biomass yield, biomass allocation, tissue nitrogen and phosphorus concentration and flower production), in the variation associated to these characters, and in the correlation between characters.

## 1. INTRODUCTION

Increased nutrient loads in aquatic ecosystems have been extensively reported to result in low-diverse, phytoplankton-dominated communities, often referred to as the 'turbid state'. It seems clear that the decline of submerged vegetation plays a key role in triggering the change of events switching the equilibrium from the high-diverse, 'clear' state to the 'turbid' state (Phillips *et al.*, 1978; De Nie, 1987). Increased shading by periphyton and phytoplankton causes macrophytes to decline, and subsequent changes in the faunal community result in the stabilization of the phytoplankton-dominated state (Moss, 1990; Scheffer, 1990).

In non-tropical brackish and freshwater ecosystems, phosphorus is considered to be the main limiting nutrient, and eutrophication abatement programmes concentrate on reducing the phosphorus loading. But, apart from changes in the dominant primary producers resulting from increased phosphorus loadings, high nitrogen levels in the water and sediment may also directly affect the physiology and thus the growth dynamics and reproduction of submersed macrophytes. The toxic effect of ammonia has been reported to limit plant photosynthesis (Dendène *et al.*, 1993) and growth (Mattes & Kreeb, 1974), and moderately high levels of ammonia in the sediment caused limited growth and inhibited reproduction in *Ruppia drepanensis* Tineo (Santamaría *et al.*, 1994). Besides the effect of ammonia, high nitrogen levels may also affect the induction of flowering (Evans, 1969).

The aim of this study was to determine the effect of high nitrogen levels on the flowering and reproduction of *R. drepanensis*. From field observations, we hypothesised that flowering is triggered by decreased nitrogen availability, and that an increase in nitrogen levels will postpone flowering and reproduction and lead to a longer growth period. Support for this hypothesis has been found in several physiological studies (Wada & Totsuka, 1982; Wada & Shinozaki, 1985; Tanaka *et al.*, 1986). Some indirect support was also found in Verhoeven (1979), who reported that the combination of a nutrient-rich sediment (organic mud versus sand) and a relatively high salinity (18.5 versus 3.5 g l<sup>-1</sup> Cl<sup>-</sup>) inhibited the flowering of *Ruppia cirrhosa* (Petagna) Grande and *Ruppia maritima* L.

Because we previously found ammonia toxicity to negatively affect *R. drepanensis* growth and reproduction (Santamaría *et al.*, 1994), we tested our hypothesis by means of additions of potassium nitrate to the water column. Interaction with two levels of sediment fertility was also studied to assess whether the hypothesised effect of nitrogen fertilization occurred also in combination with nitrogen-poor sediment conditions. This would provide more information about the nitrogen pathway leading to the observed effects.

## 2. MATERIALS AND METHODS

Seeds attached to plant material were collected in an old channel of the Salinas de San Isidoro (Doñana National Park, SW Spain), at the end of the growing season (June 1991). A mixed population of *R. drepanensis* and *Athenia orientalis* (Tzevelev) García-Murillo & Talavera occurs in this locality. Seeds and plant material were stored in opaque plastic bags at 4 °C, until December 1992.

The seeds were germinated in tap water, in the dark and at room temperature (20 °C). After three days, germinated seedlings were pretreated with brackish water (4 days at 0.5 g l<sup>-1</sup> artificial sea salt), randomly selected and distributed, and planted. This germination

TABLE 1: Summary of the experimental conditions. N and P weekly addition in grams added to each 100 l aquarium. Nitrogen and phosphorus concentrations of the clay used for the mixture were  $938 \pm 434$  mg N kg<sup>-1</sup> dw and  $239 \pm 56$  mg P kg<sup>-1</sup> dw (mean  $\pm$  standard error, n=3). N and P concentration of the washed sand was below the detection limit of the analysis.

Treatment	Sediment	N addition (g KNO <sub>3</sub> wk <sup>-1</sup> )	P addition (g KH <sub>2</sub> PO <sub>4</sub> wk <sup>-1</sup> )	Total N load (mg N plant <sup>-1</sup> )	Total P load (mg P plant <sup>-1</sup> )
'clay'	clay + sand	-	-	55	14
'clay+N'	clay + sand	2.0	-	200	14
'sand'	sand	-	0.24	< 5	21
'sand+N'	sand	2.0	0.24	145	21

procedure was previously found to result in the highest number of viable seedlings.

Two types of sediment (1:3 clay-sand mixture, hereafter referred to as the clay sediment; and pure sand, hereafter referred to as the sand sediment) and two nitrate levels in the water column (tap water, and tap water plus a weekly addition of 2.0 g KNO<sub>3</sub> per tank) were combined in four treatments (hereafter referred to as 'clay', 'clay+N', 'sand' and 'sand+N'; see also Table 1). Sand-sediment treatments ('sand' and 'sand+N') were also supplied with a weekly addition of 0.24 g KH<sub>2</sub>PO<sub>4</sub> per tank in the water column, to avoid P limitation.

The experimental setup was as described in Santamaría *et al.* (1994). Each treatment consisted of one aquarium (60 x 40 x 40 cm<sup>3</sup>) containing 39 plastic cups (8 cm height) filled with 100 ml of sediment covered by 1 cm washed sand. One seedling was planted in each separate cup. Each treatment consisted thus of 39 plants, except for the 'clay' treatment, which consisted of only 20 plants. The aquaria were filled with tap water one week before planting the seedlings, and replenished with demineralised water during the experiment. The four aquaria were placed in one phytotron, where they had independent pumping systems for cooling and heating (temperature:  $20 \pm 0.5$  °C) and a common light system (irradiance: 250  $\mu$ E m<sup>-2</sup> s<sup>-1</sup> at 1 cm below the water surface).

Nitrate and orthophosphate concentrations in the water column and porewater were measured monthly during the experiment. Measurements were always done one hour prior to the weekly addition of N and P. Duplicate samples were taken in polyethylene flasks, and stored at -20 °C until further analysis. Porewater was sampled each time from different, randomly selected plant cups using a semipermeable stick (70 x 3 mm) connected to a 10 ml syringe (RHIZON SSS<sup>®</sup>). Dissolved orthophosphate was measured in an Aquatec<sup>®</sup> auto-analyzer, and nitrate+nitrite (hereafter referred to as 'nitrate') was measured according to the sulphanilic acid method following nitrate reduction in a column of cadmium (APHA *et al.*, 1992).

The number of plants with flowers was recorded weekly. Criterion for flowering was the appearance of expanded sheaths in the leaf bundles of the vertical shoots (Gamerro, 1968; Posluszny & Sattler, 1974). After 12 weeks, half of the plants were randomly selected and harvested for the determination of biomass, nitrogen and phosphorus concentration in the plant tissue, and number of flowers per plant. Half of the harvested plants were divided in aboveground (shoots and leaves) and belowground (rhizomes and roots) parts, dried (70 °C, 24 h) and stored until further determination of their tissue N and P concentration. The

remaining plants were divided in belowground parts, basal shoots and vertical shoots, dried (110 °C, 24 h) and ashed (520 °C, 3 h) to calculate the ash-free dry weight (afdwt). An afdwt/dw regression line fitted to the latter dataset was used to calculate the afdwt of the plant material from the 'N and P' subsample.

Determination of the tissue N and P concentration followed Novozamsky *et al.* (1983), and consisted of a digestion of the plant material in a mixture of selenium, hydrogen peroxide, salicylic acid and sulphuric acid, followed by a determination of the total N and total P using an Aquatec<sup>®</sup> auto-analyzer. Every plant was analyzed separately, but in some of the treatments several plants had to be pooled to reach a minimum biomass of 150 mg dw.

After this first harvest, the 20 plants that remained in the 'sand+N' treatment were randomly divided into two groups: one was kept under the same conditions, while the other was transferred to the 'sand' treatment (this group will hereafter be referred to as the 'transfer' treatment, 'Tr'). The plants that remained in all the other treatments were kept under the same conditions. Again, the number of plants with flowers were recorded weekly, and plants were harvested in the 17th week for the same determinations as described above.

Statistical analysis followed Santamaría *et al.* (1994), and was performed with the SAS statistical package (SAS Institute Inc., 1988). Number of flowers, biomass and tissue N and P concentrations were compared by means of two- and three-way Analysis of Variance and Student-t tests, after log<sub>10</sub> transformation if the residuals were not normally distributed or not homoscedastic. Three-way ANOVAs were followed by multiple comparisons among treatments, adjusting the comparisonwise error rates (CER) to maintain an experimentwise error rate (EER) of 0.05.

Strong residual heteroscedasticity prevented the use of multiple comparisons using the LSMEANS estimates for the variable 'number of flowers per plant'. Following preliminary F-tests for the homogeneity of the variances, separate t-tests were performed for each comparison (Steel & Torrie, 1981). In one of the treatments, the number of flowers was zero for all the plants. Comparisons involving this treatment were performed using a one-tailed Student-t test to falsify the null hypothesis 'the average number of flowers per plant (of the treatment having flowered) is zero'.

The relation between the correlation structure of traits (characters) and their plasticity was analyzed using correlation networks (Schlichting, 1986) based on multiple correlation matrices obtained from the calculated Pearson correlation coefficients. Correlation networks for the traits measured in each of the treatments (environments) were used to detect changes in correlation structure across environments. Plasticity correlation networks, representing the correlations of the plastic responses of pairs of characters across environments, were used to measure the degree of similarity of the plastic responses of each pair of characters.

The number of flowering plants after 12 and 17 weeks of growth was compared by means of  $\chi^2$  tests, adjusting the CER to maintain an EER of 0.05. The development of the percentage of flowering plants with time was fitted to the following logistic formula

$$\%FP = \frac{K}{1 + q * e^{-r * days}} \quad (1)$$

where %FP is the percentage of flowering plants, K is the asymptotic maximum value for %FP, q is an integration constant, and r is the instantaneous growth rate. The resulting curves were compared for differences between the overall fits by using a F statistic as described in Vermaat & Hootsmans (1994).

TABLE 2: Nitrate concentration (in mg N l<sup>-1</sup>) in the water and porewater for the four different treatments. Measurements were done one hour prior to the weekly fertilization, except for the porewater from the 28th day, which was measured 3 days after the weekly fertilization.

Water column					Porewater				
Day	clay	clay+N	sand	sand+N	Day	clay	clay+N	sand	sand+N
31	1.42	24.2	3.95	26.0	28	-	0.19	-	0.19
66	0.26	26.9	0.20	29.4	31	0.19	0.20	0.19	0.19
90	0.02	37.4	-	27.7					

### 3. RESULTS

Nitrate concentration in the water column was higher in the fertilized treatments and comparable for the clay and sand sediments (Table 2). Values from the 'clay+N' and 'sand+N' treatments suggest that a certain equilibrium was achieved at concentrations of 25 to 35 mg N l<sup>-1</sup>, independent of the different biomass of the plants (which varied both with age and sediment type, see below). Nitrate concentration in the porewater was similar in all four treatments (Table 2).

Orthophosphate was undetectable in most of the cases, and always lower than 0.5 mg P l<sup>-1</sup>. In spite of the weekly addition of K<sub>2</sub>HPO<sub>4</sub> in the sand sediment treatments, orthophosphate concentration was similar in all different treatments (data not shown).

Potassium nitrate fertilization in the water column resulted in a postponed flowering in both types of sediment: while the percentage of flowering plants did not differ within sediment types after 12 and 17 weeks ( $\chi^2$  tests yielded non-significant results), the 'N-fertilized' plants flowered 2-3 weeks later in the clay sediment, and 4-5 weeks later in the sand sediment (Fig.1). F-tests performed on the fitted logistic curves resulted in highly significant results for the four comparisons tested ('clay' vs 'clay+N', 'sand' vs 'sand+N', 'clay' vs 'sand' and 'clay+N' vs 'sand+N';  $p < 0.001$ ).

The number of flowers per plant after 12 weeks was reduced by the nitrate fertilization (Fig.2; t-tests:  $p < 0.01$  in the clay sediment,  $p < 0.05$  in the sand sediment). This trend was maintained in the clay sediment after 17 weeks, although it became non-significant probably due to the high plant-to-plant variability (t-test:  $p = 0.21$ ). In the sand sediment, on the contrary, potassium nitrate addition resulted in a higher flower production per flowering plant (Fig.2, right). Nevertheless, differences in flower abundance were not significant when considering the average of the whole 20 plants (Fig.2, left; t-test:  $p = 0.89$ ).

Plant biomass did not increase with nitrate fertilization in the clay sediment (Fig.3), while it increased slightly with increased nitrate in the sand sediment. Besides this, biomass yield was much lower in the sand than in the clay sediment. Results of the ANOVAs on biomass yield and biomass allocation are shown in Table 3, although some care is recommended due to the lack of homoscedasticity and normality of the residuals, only partially solved by performing separate two-way ANOVAs. Overall, sediment and age seem to have highly significant effects on aboveground (AGB), belowground (BGB) and total plant biomass, while nitrate addition resulted in significant biomass increases only within the sand sediment.

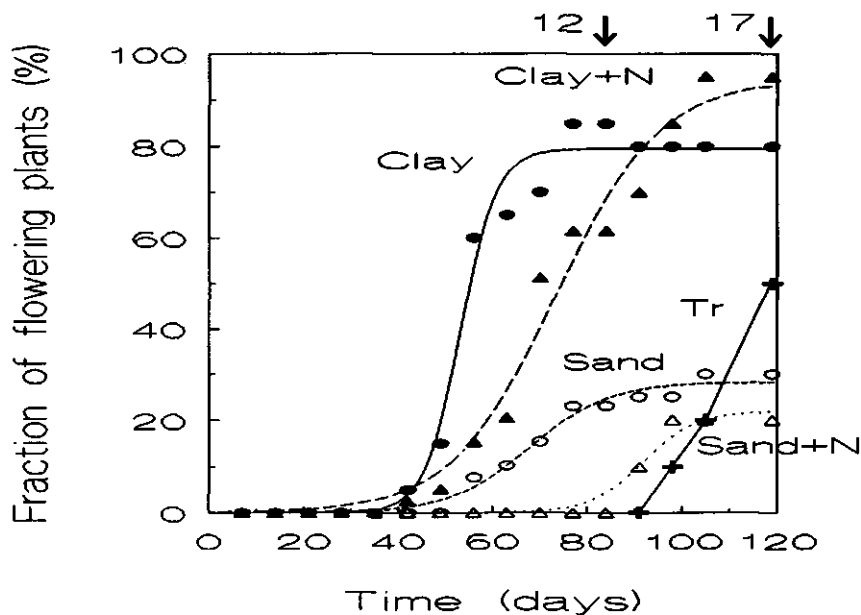


FIG.1: Percentage of flowering plants along time for the different treatments. Coding of the treatments as in Table 1. 'Tr': plants transferred from 'Sand+N' to 'Sand' after 12 weeks of growth.  $n=40$  for the first 12 weeks (except for the 'Clay' treatment,  $n=20$ ), then  $n=20$  for the treatments 'Clay+N' and 'Sand' and  $n=10$  for 'Clay', 'Sand+N' and 'Tr'. Curves are fits of the logistic equation, except for 'Tr'.

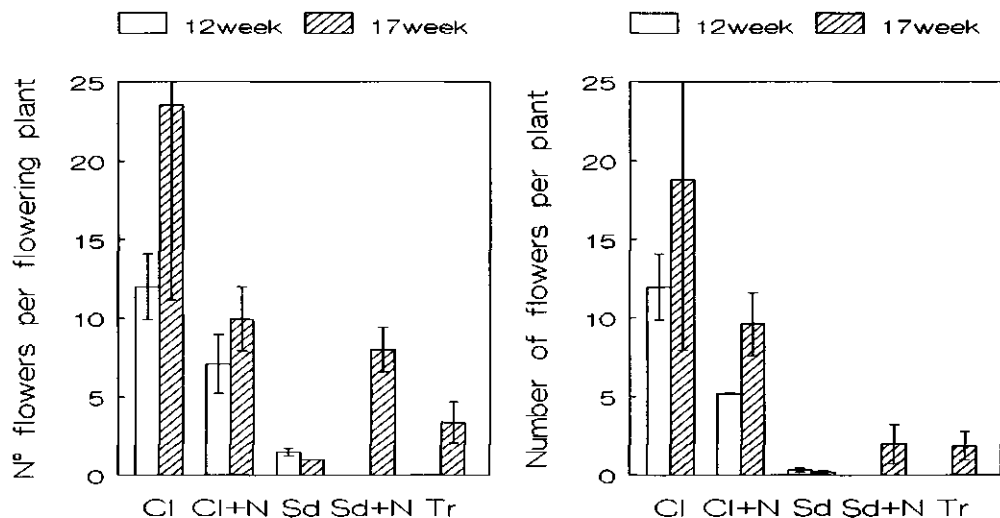


FIG.2: Average number of flowers per plant (left) and per flowering plant (thus excluding the zero values; right) for the different treatments. Mean  $\pm$  standard error. Coding of the treatments as in Table 1.

Left graph:  $n=20$ , except for 'Cl', 'Tr' and 'Sd+N' in the 17th week, where  $n=10$ .

Right graph: 12th week:  $n=10$  for 'Cl',  $n=14$  for 'Cl+N',  $n=4$  for 'Sd'.

17th week:  $n=8$  for 'Cl',  $n=19$  for 'Cl+N',  $n=4$  for 'Sd',  $n=2$  for 'Sd+N',  $n=5$  for 'Tr'.

TABLE 3: Results of the ANOVA for the effect of nitrate concentration in the water ('nitrate'), sediment type ('sediment'; clay:sand versus sand) and age (12 versus 17 weeks) on biomass yield and biomass allocation in *R. drepanensis*. Values shown are the associated probability levels of the F-tests. \*, \*\* and \*\*\* indicate 0.05, 0.01 and 0.001 significance levels respectively, 'NS' indicates non-significant results. 'log<sub>10</sub>' indicates ANOVAs performed on log-transformed data, 'NH' non-homoscedastic residuals, and 'NN' residuals for which normal distribution could not be assumed ( $p < .01$ ).

Three-Way ANOVA						
	BGB	AGB	TOTB	AGB/BGB	VSGB/AGB	
Sediment	.0001 <sup>***</sup>	.0001 <sup>***</sup>	.0001 <sup>***</sup>	.0001 <sup>***</sup>	.0001 <sup>***</sup>	
Nitrate	.8791 <sup>NS</sup>	.1567 <sup>NS</sup>	.4502 <sup>NS</sup>	.0001 <sup>***</sup>	.1067 <sup>NS</sup>	
Age	.0011 <sup>**</sup>	.0001 <sup>***</sup>	.0001 <sup>***</sup>	.1720 <sup>NS</sup>	.0010 <sup>**</sup>	
Nitr*Sed	.8303 <sup>NS</sup>	.9985 <sup>NS</sup>	.8780 <sup>NS</sup>	.0001 <sup>***</sup>	.8291 <sup>NS</sup>	
Nitr*Age	.8631 <sup>NS</sup>	.2702 <sup>NS</sup>	.4278 <sup>NS</sup>	.0001 <sup>***</sup>	.1136 <sup>NS</sup>	
Sed*Age	.0034 <sup>**</sup>	.0012 <sup>**</sup>	.0001 <sup>***</sup>	.0001 <sup>***</sup>	.0025 <sup>**</sup>	
Sed*Nitr*Age	.8244 <sup>NS</sup>	.6927 <sup>NS</sup>	.9343 <sup>NS</sup>	.0036 <sup>**</sup>	.0459 <sup>*</sup>	
Remarks:	NN,NH	NN,NH	NN,NH	log <sub>10</sub>		
Separate Two-way ANOVAs						
	clay sediment			sand sediment		
	BGB	AGB	TOTB	BGB	AGB	TOTB
Nitrate	.8999 <sup>NS</sup>	.4836 <sup>NS</sup>	.7095 <sup>NS</sup>	.0006 <sup>***</sup>	.0001 <sup>***</sup>	.0241 <sup>*</sup>
Age	.0006 <sup>***</sup>	.0001 <sup>***</sup>	.0001 <sup>***</sup>	.0005 <sup>***</sup>	.0001 <sup>***</sup>	.0001 <sup>***</sup>
Nitr*Age	.9586 <sup>NS</sup>	.7265 <sup>NS</sup>	.8284 <sup>NS</sup>	.6240 <sup>NS</sup>	.0001 <sup>***</sup>	.0013 <sup>**</sup>
Remarks:	log <sub>10</sub> ,NN	log <sub>10</sub>		NN,NH	NN,NH	

Above- to belowground biomass ratio (AGB/BGB) was significantly affected by sediment type and age, and all (two- and three-way) interactions were also significant (see Table 3). On the other hand, vertical shoots to aboveground biomass ratio (VSGB/AGB) was significantly affected by sediment type and age, by their interaction and by the three-order interaction 'sediment \* age \* nitrate'. In the multiple comparisons, AGB/BGB was significantly higher for the nitrate-fertilized treatment only within the sand sediment, and the plants grown in the sand sediment had significantly lower AGB/BGB ratios than those grown in the clay sediment (except for the nitrate-fertilized treatments in the 17th week, Fig.3). Moreover, the 'sand+N' treatment was the only one in which AGB/BGB ratio increased between the 12th and the 17th week. The VSGB/AGB ratio tended also to be higher in the clay sediment (Fig.3), and increased with age only in the 'sand+N' treatment.

Tissue N concentration of the belowground fraction was significantly affected only by nitrate fertilization and age, while both two-way interactions including sediment type also significantly affected the tissue N concentration of the aboveground fraction (Table 4, above). Tissue N concentration tended to increase with nitrate fertilization, and to decrease with age (Figs. 4 and 5).

Pooled above- and belowground tissue N concentrations was significantly affected by almost all factors (sediment type, nitrate fertilization, plant fraction and their two- and three-

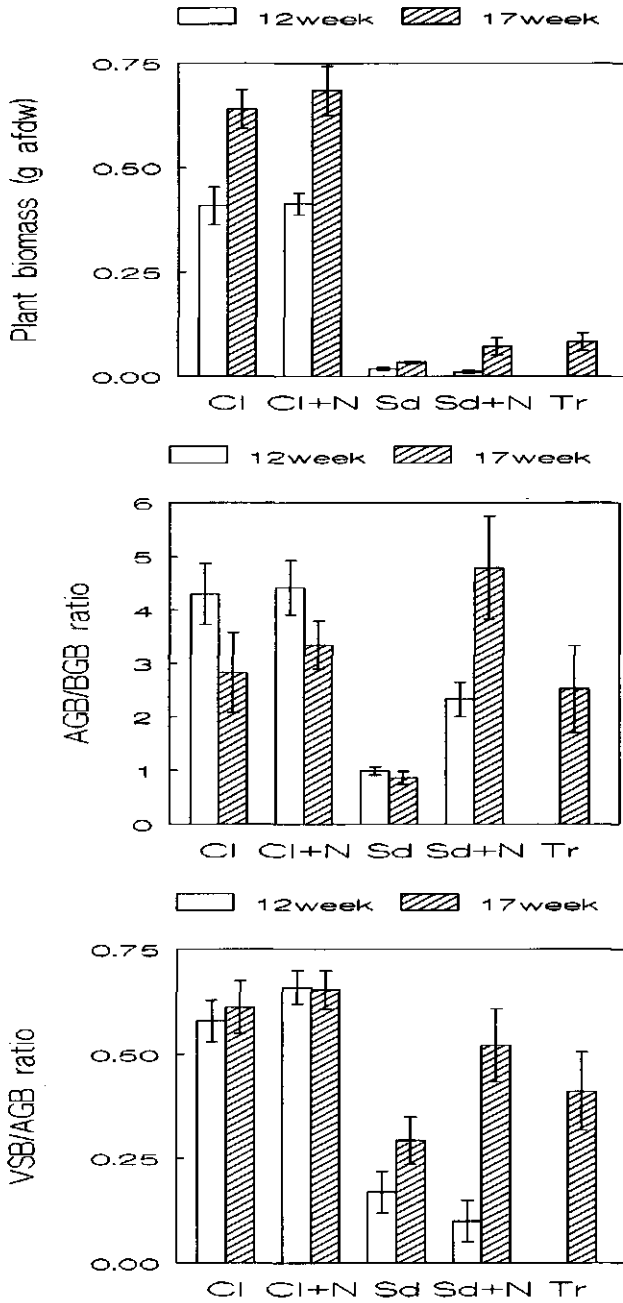


FIG.3: Effect of the type of sediment and nutrient supply in the water column on the total biomass yield (above) and biomass allocation (centre and below) of *R. drepanensis*. 'AGB/BGB' ratio: aboveground- to belowground biomass ratio. 'VSB/AGB ratio': vertical shoots to aboveground biomass ratio. Mean  $\pm$  standard error. Coding of the treatments as in Table 1.



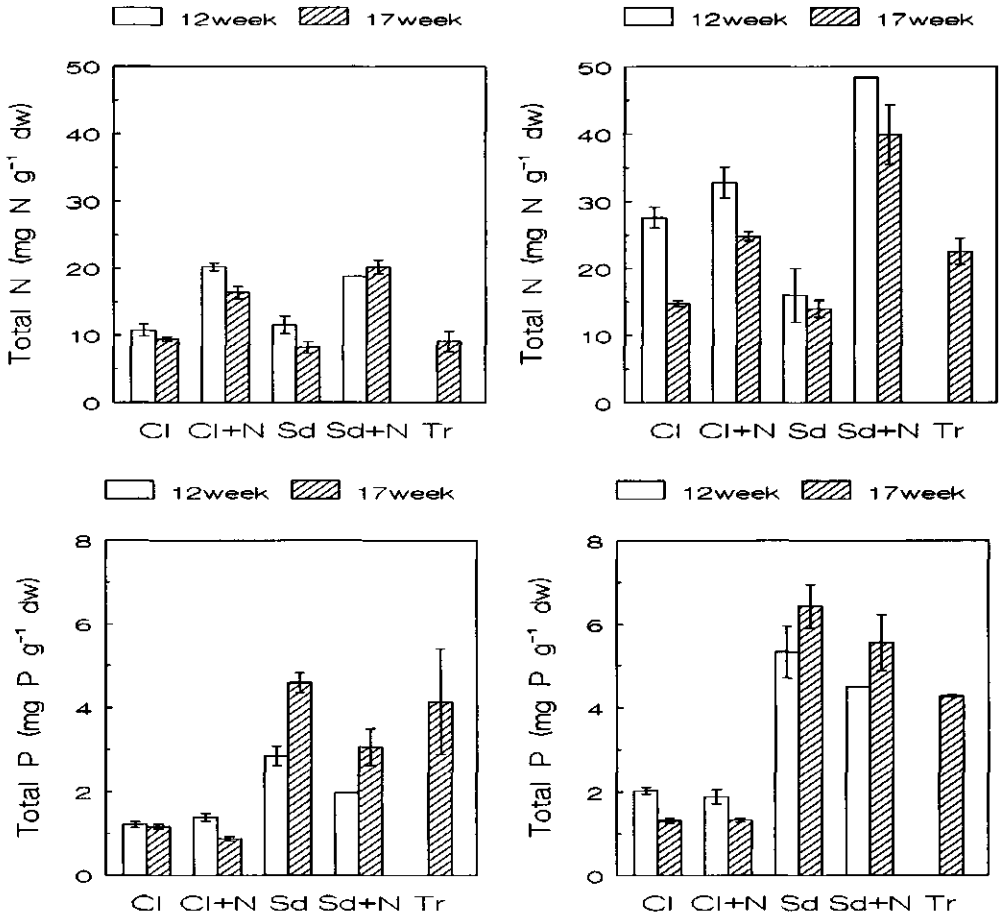


FIG. 4: Nutrient content of the belowground (left) and aboveground (right) tissue of *R. drepanensis* plants grown at different levels of nutrient supply. Coding of the treatments as in Table 1. Mean  $\pm$  standard error.

order interactions) both for the 12th and the 17th week datasets (Table 4, below). Results of the multiple comparisons overall were similar to the above-described, with the additional remarks of a scarcity of significant differences within the 12th week dataset, and the significantly higher tissue N concentrations of the aboveground fraction (as compared with the belowground fraction) in all treatments but the 'sand' one.

Tissue P concentration was significantly affected by sediment type, nitrate fertilization and age, and by the interaction of sediment type and age (Table 4, above). The nitrate\*sediment interaction was also significant for the belowground fraction. Pooled above- and belowground tissue P concentration was significantly affected by sediment type, nitrate fertilization and plant fraction, and by several of their two-order interactions (Table 4, below).

Overall, tissue P concentration tended to be higher in the treatments with sand sediment than in those with clay (sand sediment treatments had been supplemented with a

TABLE 4: Results of the ANOVAs for the effect of nitrate concentration in the water ('nitrate'), sediment type ('sediment'; clay:sand versus sand), age (12 versus 17 weeks) and plant fraction ('plfr', above- versus belowground fractions) on the tissue nitrogen and phosphorus concentration of *R. drepanensis*. Values shown are the associated probability levels of the F-tests. \*, \*\* and \*\*\* indicate 0.05, 0.01 and 0.001 significance levels respectively, 'NS' indicates non-significant results. 'log<sub>10</sub>' indicates ANOVAs performed on log-transformed data.

	Belowground fraction		Aboveground fraction	
	Total N	Total P	Total N	Total P
Sediment	.7140 <sup>NS</sup>	.0001 <sup>***</sup>	.3014 <sup>NS</sup>	.0001 <sup>***</sup>
Nitrate	.0001 <sup>***</sup>	.0038 <sup>**</sup>	.0001 <sup>***</sup>	.0109 <sup>***</sup>
Age	.0200 <sup>*</sup>	.0311 <sup>*</sup>	.0001 <sup>***</sup>	.4654 <sup>NS</sup>
Nitr*Sed	.3898 <sup>NS</sup>	.0088 <sup>**</sup>	.0001 <sup>***</sup>	.3130 <sup>NS</sup>
Nitr*Age	.2336 <sup>NS</sup>	.1427 <sup>NS</sup>	.3414 <sup>NS</sup>	.6862 <sup>NS</sup>
Sed*Age	.7405 <sup>NS</sup>	.0001 <sup>***</sup>	.0357 <sup>*</sup>	.0002 <sup>***</sup>
Sed*Nitr*Age	.0503 <sup>NS</sup>	.4020 <sup>NS</sup>	.1017 <sup>NS</sup>	.7684 <sup>NS</sup>
Remarks	log <sub>10</sub>	log <sub>10</sub>	log <sub>10</sub>	log <sub>10</sub>
	Age = 12 weeks		Age = 17 weeks	
	Total N	Total P	Total N	Total P
Sediment	.7438 <sup>NS</sup>	.0001 <sup>***</sup>	.0001 <sup>***</sup>	.0001 <sup>***</sup>
Nitrate	.0001 <sup>***</sup>	.0462 <sup>*</sup>	.0001 <sup>***</sup>	.0003 <sup>***</sup>
Plfr	.0001 <sup>***</sup>	.0001 <sup>***</sup>	.0001 <sup>***</sup>	.0001 <sup>***</sup>
Nitr*Sed	.0188 <sup>*</sup>	.0390 <sup>*</sup>	.0001 <sup>***</sup>	.0516 <sup>NS</sup>
Nitr*Plfr	.0466 <sup>*</sup>	.7609 <sup>NS</sup>	.0001 <sup>***</sup>	.0105 <sup>*</sup>
Sed*Plfr	.6559 <sup>NS</sup>	.0001 <sup>***</sup>	.0023 <sup>**</sup>	.0026 <sup>**</sup>
Sed*Nitr*Plfr	.0067 <sup>**</sup>	.6489 <sup>NS</sup>	.0044 <sup>**</sup>	.7947 <sup>NS</sup>
Remarks				log <sub>10</sub>

weekly addition of 0.24 g K<sub>2</sub>HPO<sub>4</sub>). Tissue P concentration of the belowground fraction also tended to decrease with nitrate fertilization for the 17-weeks old plant material, and increased with increasing age for the aboveground fraction of the nitrate-fertilized treatments (Fig.5). Tissue P concentration was significantly higher for the aboveground fraction as compared with the belowground fraction only in the nitrate-fertilized treatments (both for the 12- and 17-weeks datasets).

Correlation networks among characters within the 'clay' and 'clay+N' treatments are shown in Fig.6. A multiple correlation matrix was not calculated for the 'sand' and 'sand+N' treatments because of the low number of replicates resulting from de pooling of plant material needed for the analysis of the tissue N and P concentrations. Positive correlations were found between the flower abundance and the above- to belowground biomass (AGB/BGB) ratio in both treatments. Number of flowers and tissue N concentration were negatively correlated within the 'clay+N' treatment. Finally, biomass and tissue P concentration were negatively correlated within the 'clay' treatment.

Multiple regression analysis showed that the AGB/BGB ratio was the only variable which explained a significant portion of the variance associated to the flower abundance.

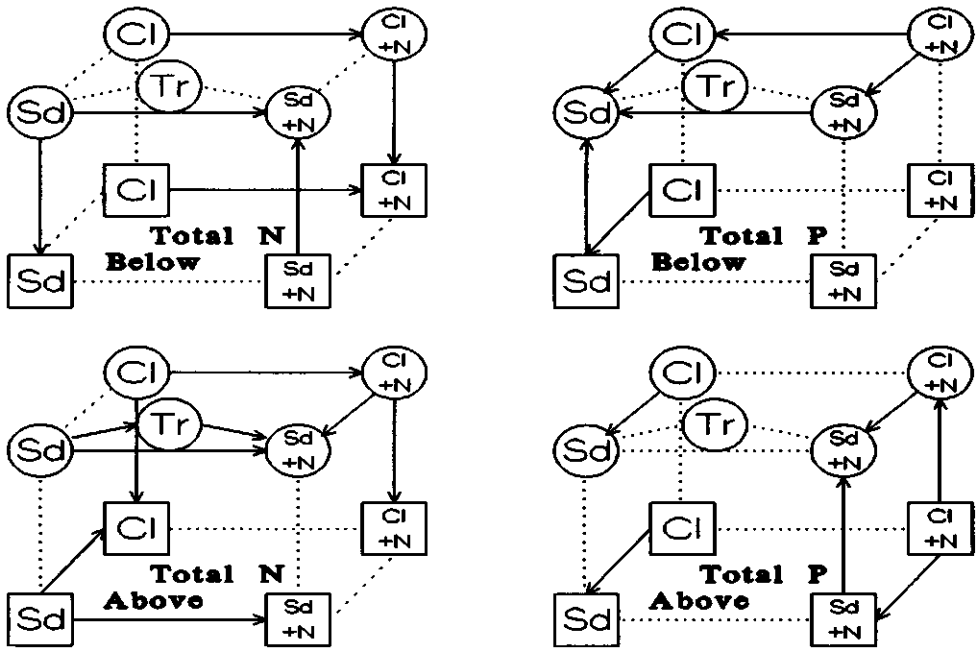


FIG.5: Results of the multiple comparisons for the tissue nutrient content of the plants grown under different nutrient regimes. Broken lines indicate non-significant comparisons, arrows point to significantly higher values (EER=0.05). Squares: age=12 weeks. Circles: age=17 weeks. Below: belowground fraction. Above: aboveground fraction.

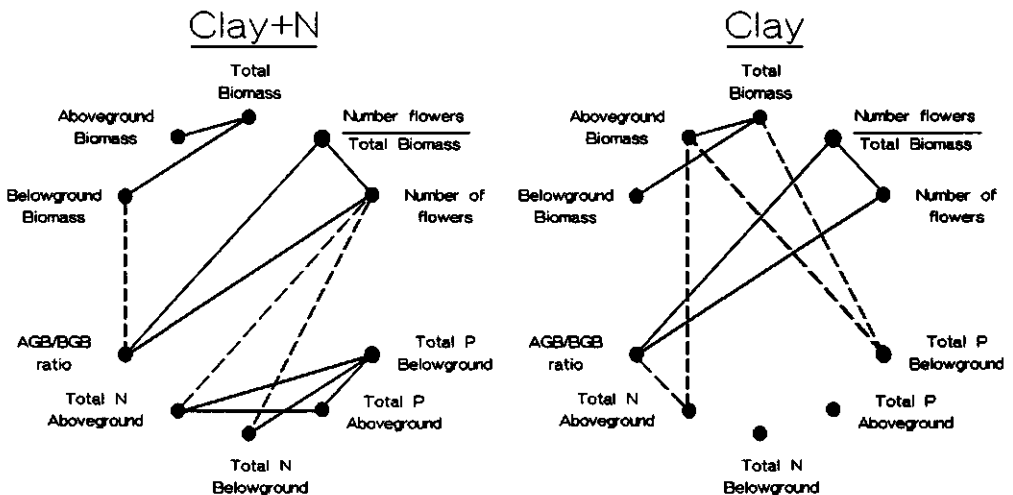


FIG.6: Correlation networks representing the correlations among characters of 17-weeks old *R. drepanensis* plants grown in two treatments differing in nitrogen supply. 'Clay': clay-sand sediment (n=8). 'Clay+N': id. plus addition of potassium nitrate to the water column (n=15). Solid lines indicate significant positive Pearson correlations, dashed lines indicate significant negative correlations (p < 0.05).

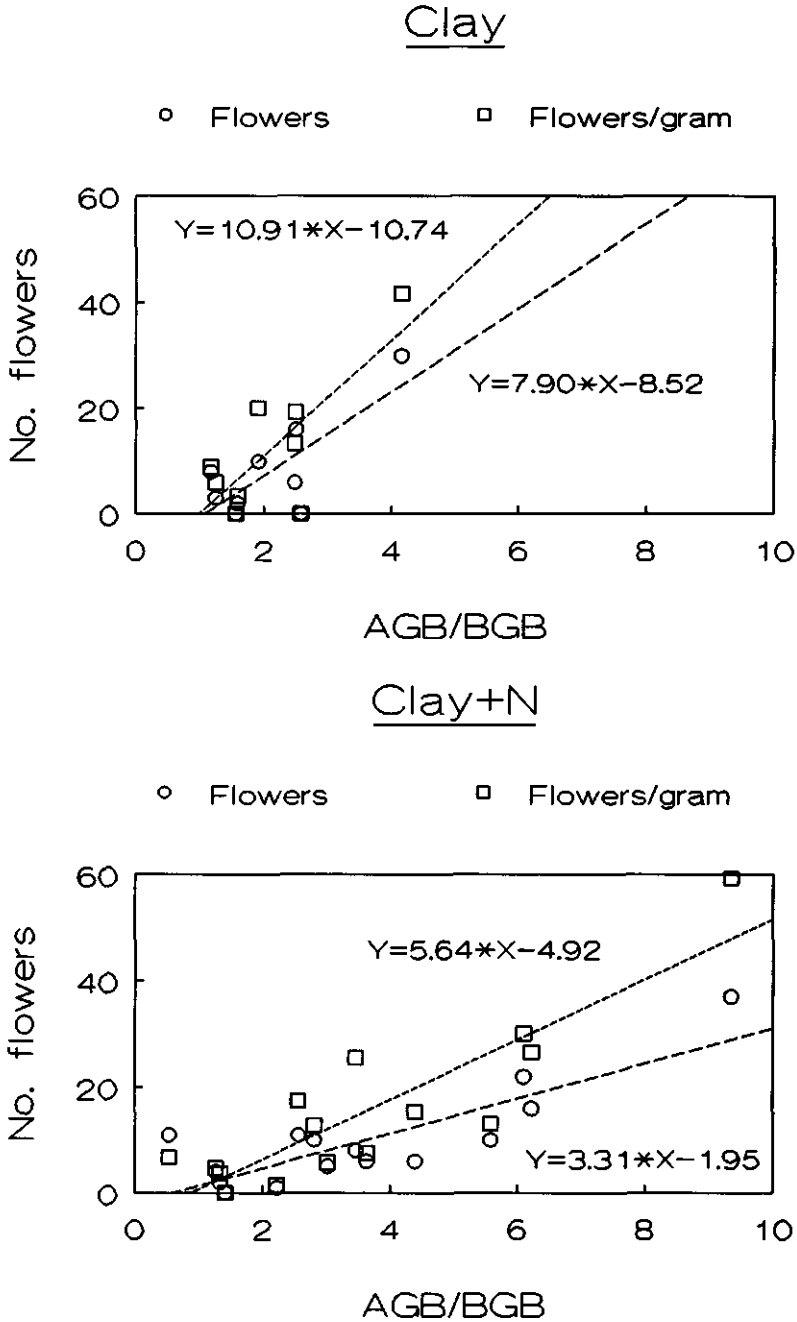


FIG.7: Linear regression describing the relationship between the aboveground to belowground biomass ratio (AGB/BGB) and the number of flowers per plant ('Flowers') and number of flowers per gram afdw ('Flowers/gram') from *R. drepanensis* plants grown in two separate environments differing in nitrogen supply (see text).

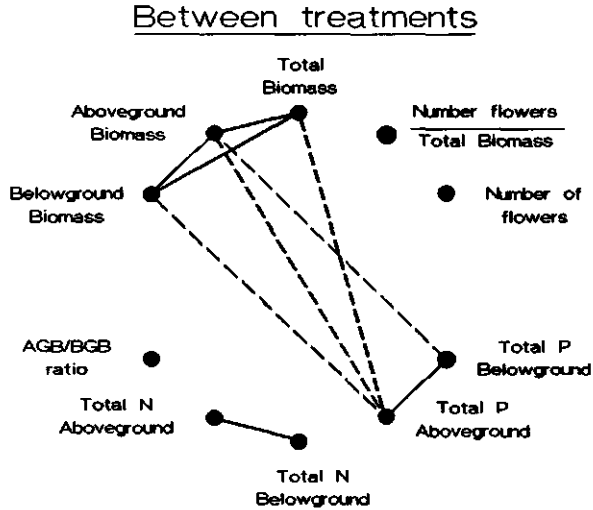


FIG. 8: Correlation networks representing the correlated responses of characters (plasticity correlations) across four treatments differing in nutrient status, for 17-weeks old *R. drepanensis* plants ( $n=4$ ). Solid lines indicate significant positive Pearson correlations, dashed lines indicate significant negative correlations ( $p < 0.05$ ). Values of the variables used for the multiple correlation analysis were the average of 10 to 20 replicate plants.

Linear fits were significant both for the 'clay' and the 'clay+N' treatments ('clay':  $r^2 = .58$  and  $.59$ , 'clay+N':  $r^2 = .70$  and  $.77$ , for the no. flowers and no. flowers  $g^{-1}$  afdw total biomass, respectively; see Fig. 7).

Multiple correlation analysis performed for the plasticity correlations across the four treatments (Fig. 8) showed that no character was significantly correlated with flower production. Once we excluded the correlations among characters representing similar characteristics, only biomass and tissue P concentration showed significant (negative) correlations.

#### 4. DISCUSSION

##### 4.1. Nutrient supply, tissue nitrogen and phosphorus concentration and plant biomass

Nitrate fertilization resulted in a higher tissue nitrogen concentration with both types of sediment. Culture on clay resulted only temporarily (12th week samples) in a higher tissue N concentration. Higher nitrate supply and higher tissue N concentration did not result in a higher biomass yield in the clay sediment: plant growth was thus not N-limited in the 'clay' treatment. Nitrate fertilization resulted in a significantly higher biomass yield (17th week) in the sand sediment: plant growth was thus N-limited in the 'sand' treatment. Other factors, presumably the deficient mineral nutrition, further limited plant growth in the 'sand+N' treatment, as its biomass yield was much lower than in the 'clay' treatment.

Tissue N concentration was higher in the 'sand+N' than in the 'clay+N' treatment. As nitrate supply was higher in the 'clay+N' treatment (see Table 1), these differences in

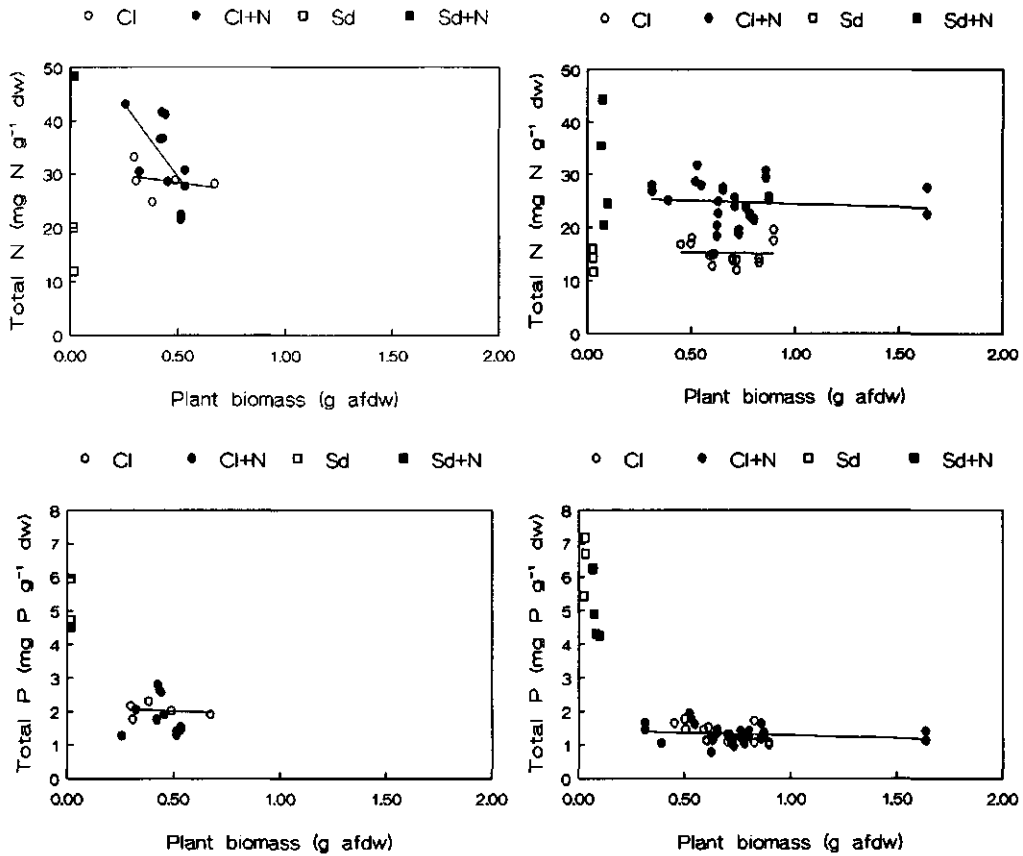


FIG.9: Relationship between total plant biomass and aboveground tissue nutrient content for *R. drepanensis* plants grown under different nutrient regimes during 12 and 17 weeks (left and right graph, respectively). Datapairs represent the individual plants. Separate (total N) and pooled (total P) linear fits are also provided for the 'Cl' and 'Cl+N' datasets. Coding of the treatments as in Table 1.

tissue N concentration cannot be attributed to differences in the N supply, but more to a dilution effect of increasing biomass on tissue nutrient concentration (similar to the findings of Barko & Smart, 1981 and Hootsmans & Vermaat, 1994). As plants from the 'sand+N' treatment stopped growing earlier, their tissue N concentration could be maintained at a higher level than in the 'clay+N' treatment.

The relationship between total plant biomass and N and P concentration in the aboveground tissue is shown in Fig.9. Tissue N and P concentrations tended to stabilise around a certain value, differing for the different treatments, following biomass increase with age. For the 17 weeks dataset, tissue N and P concentration of the 'clay' and 'clay+N' treatments was independent of plant biomass.

Stabilization around a certain tissue N and P concentration is probably dictated by the energetic cost of nutrient uptake, balanced with the plant's nutritional needs. As it may be concluded from the 'sand' treatment, plants growing in a very restricted nitrogen supply do

not grow, but try to maintain this minimum value (10-15 mg N g<sup>-1</sup> dw). But this tissue concentration may still allow for plant growth which is not N-limited, as in the 'clay' treatment.

Minimum asymptotic values found here are similar to the findings of Gerloff & Kromholz (1966; 13 mg N g<sup>-1</sup> dw, 1.3 mg P g<sup>-1</sup> dw) and Thursby (1984; 25-30 mg N g<sup>-1</sup> dw, 2.5-3 mg P g<sup>-1</sup> dw). But a major difference is that, although low nitrogen levels supplied to the plants in the 'sand' treatment limited their growth significantly, measured N concentrations in the aboveground tissue were not below Gerloff & Kromholz's (1966) critical values. Moreover, our results show that under medium to low nutrient supply conditions (59.5 mg N per plant in the 'clay' treatment), tissue nutrient values tended to converge around this 'critical value', and nutrient limitation will only be indicated by little (if any) departures from it. Tissue nutrient concentration thus may be useful in rejecting nutrient limitation (if clearly suprathreshold values are found), but values close to the threshold may not indicate nor falsify the existence of nutrient limitation if concomitant data on growth are absent (see also Chambers & Fourqurean, 1991).

Finally, differences in biomass allocation into above- and belowground organs may reflect a response to nutrient conditions. While AGB/BGB ratio decreased with age in the 'clay' treatments (similar to Santamaría & Hootsmans, in prep.), it increased in the 'sand+N' treatment. While in both 'clay' treatments the main source of nutrients was the sediment, both phosphorus and nitrogen were supplied in the water column for the 'sand+N' treatment. Thus, the increased AGB/BGB ratio may well be understood as the response of the plants to the available source of nitrogen, localized in the water column instead of in the sediment.

#### 4.2. Induction of flower and flower production

The addition of potassium nitrate to the water column significantly delayed the flowering of *R. drepanensis*. Nevertheless, the percentage of flowering plants was similar at the end of the experiment. This effect was maintained at very low nutrient levels: the plants grown in the sand sediment also flowered several weeks later if potassium nitrate was added.

While nitrate fertilization resulted in a higher flower production in the sand sediment, flower production did not increase with nitrate supply in the clay sediment (in which plant growth was not nitrogen-limited, see above). Plants transferred from the 'sand+N' to the 'sand' treatment in the 12th week showed more flowering plants but less flowers per flowering plant than the 'sand+N' treatment 5 weeks later (see Figs. 1 and 2). Nitrogen limitation resulted thus in a lower production of flowers, and our results suggest that the effect of nitrate fertilization on flower induction and on flower abundance are independent. A comparable effect has been reported for *Fragaria* sp. (Guttridge, 1969), high nutrient supply postponing flowering but low nutrient supply resulting in flower inhibition.

Several studies have reported a significant effect of nitrogen supply on flower induction. Two kind of responses have been described: while in *Pharbitis nil* Choisy (Wada & Shinozaki, 1985), *Lemna perpusilla* Torr. (Hillman, 1969), *Fragaria* sp. (Guttridge, 1969), *Chenopodium rubrum* L. and *Chenopodium foetidum* (Cumming, 1969), *Pisum sativum* L. (Haupt, 1969), *Sinapis alba* L. (Bernier, 1969) and *Brassica campestris* L. (Friend, 1969) low nitrogen conditions stimulated flowering, and in some cases they even replaced photoperiodic requirements (*Perilla crispa* (Thunb.) Tanaka, in Wada & Totsuka, 1982; and *Lemna paucicostata*, in Tanaka *et al.*, 1986), high nutrient supply promoted

flowering in *Xanthium strumarium* L. (Salisbury, 1969), *Perilla nankinensis* (Lour.) Decne (Zeevaart, 1969) and *Lycopersicon sculentum* Mill. (Wittver & Aung, 1969).

We have found *R. drepanensis* flowering to be responsive to photoperiod (as a quantitative long-day plant) and to factors affecting plant size (such as the irradiance; Santamaría & Van Vierssen, in prep.). Temperature had a major role in influencing flower induction, which was fully inhibited at 14 °C, optimal at 20 °C and very reduced at 30 °C (Santamaría & Hootsmans, in prep.). In this study, an increased nitrogen supply has been found to delay flowering.

*R. drepanensis* is a winter-annual submerged macrophyte occurring in Mediterranean temporary wetlands. Its life-cycle is based on a rapid vegetative growth followed by an intensive flower and seed production. High variability in climatic conditions makes the timing of inundation and desiccation of the wetlands relatively unpredictable. Under such conditions, temperature probably is a more suitable predictor of the coming climatic (and thus hydrological) conditions than photoperiod. But a restricted nutrient availability, as probably any other condition limiting growth, is also able to shift plant resources to reproduction.

The concept underlying our discussion is the allocation of reproductive effort in an obligate annual species. Two strategies are optional at every moment of the plant's life cycle: continuing vegetative growth, as greater biomass will allow for a greater seed production in the future, or beginning to reproduce, which assures seed production before the wetland's drying, but limits seed production to what is determined by the present biomass. In *R. drepanensis*, a short-living annual, once flowering has been triggered a full investment in reproduction is quickly achieved (Santamaría & Van Vierssen, in prep.).

Weiner (1988) proposed a model in which there is a minimum size for sexual reproduction, above which the relationship between output and size is linear. Plant size and age have been also referred to affect flower induction in the submerged angiosperms *Vallisneria americana* Michx. (Titus & Hoover, 1991), *Lobelia dortmanna* L. (Farmer & Spence, 1987), *Thalassia testudinum* Banks ex König (Gallegos *et al.*, 1992) and *Zostera marina* L. (Olesen, 1993). But, as Schmid & Weiner (1993) stressed, biomass may not be the most important factor determining plant reproduction. If the onset of reproduction is to be understood in the framework of the plant's resources allocation, nutrient availability and its relationship with the nutrients already stored in the plant's tissues must interact with plant biomass in determining the threshold for flowering.

Multiple correlation analysis performed on the different plant variables within the 'clay' and 'clay+N' treatments showed that plant-to-plant variability in flower abundance was not significantly correlated with plant biomass (Fig.6). Flower production did show highly significant, positive correlations with the aboveground to belowground biomass ratio (AGB/BGB). More interesting, the tissue N concentration was negatively correlated with the flower production within the 'clay+N' treatment.

Positive correlations between AGB/BGB ratio and flower production suggest that not only biomass, but also biomass allocation may have a significant influence on plant reproduction. While a relatively lower nitrogen supply results in an early reproduction, investment in a higher belowground biomass fraction as needed to assure a balance between growth and nitrogen supply (including thus the energy expenses of nitrogen uptake) may result in a lower reproductive effort. In such case, the slope of the no. flowers *versus* AGB/BGB relationship should be higher for the 'clay' than for the 'clay+N' treatment. This is because in the 'clay' treatment the nitrogen availability is lower, and restricted to the sediment, and thus the energy expended in nitrogen uptake as related with the belowground fraction may be expected to be higher. The results of the linear fits of flower abundance



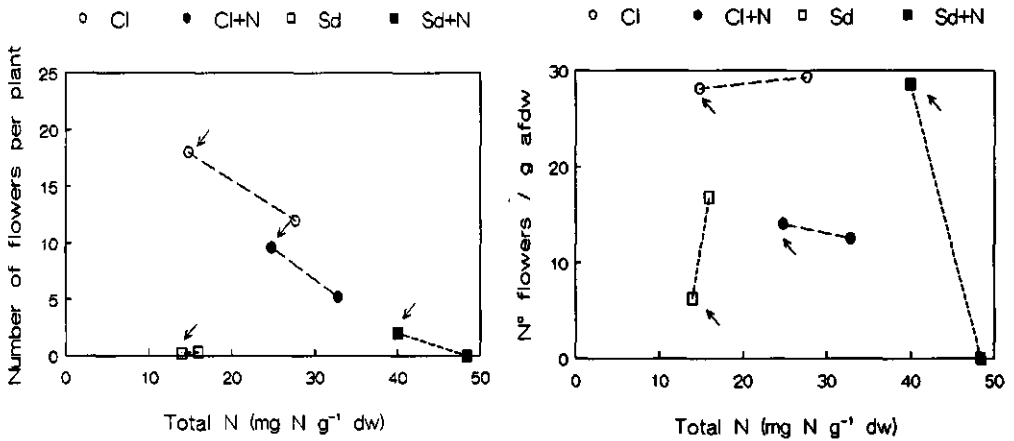


FIG.10: Relationship between the tissue nitrogen content and flower production (relative to plant biomass, above; per individual plant, below) in *R. drepanensis* plants of 12 and 17 weeks of age. Arrows indicate the 17th-week datapairs. Datapairs are averages of 10 to 20 replicate plants. Coding of the treatments as in Table 1.

(Fig.7) are consistent with this prediction: the slope of the 'clay' fit is higher than the slope of the 'clay+N' fit.

We interpret the negative correlation between flower production and tissue N concentration within the 'clay+N' treatment to still reflect the effect of nitrogen supply on flower induction. As all plants began to flower earlier in the 'clay' treatment, correlations between flower production and tissue N concentration in the 17th week were not significant. More importantly, the significant correlation between tissue N concentration and (through flower induction) flower abundance within the 'clay+N' treatment indicates that the effect of nitrogen on plant flowering is not only responsible for the delayed flowering in conditions of an increased nitrogen supply, but could be one of the causes for plant-to-plant variability in flowering.

We thus propose here that nutrient supply and biomass allocation to roots and shoots should be incorporated into the age- and size-dependent models for plant reproductive effort. A separation between the induction of flowering and the production of flowers (flower abundance) is also suggested as the most appropriate framework for a causal understanding of the interrelated effect of all these factors.

Finally, the network of plasticity correlations (Fig.8) shows that a relatively low degree of similarity was attained in the plastic response of the different pairs of characters across the four treatments. Only the biomass and the tissue P concentration showed significant (negative) correlations, and flower production was not significantly correlated with any other trait (variable). Important changes in the correlation structure of traits were observed between the 'clay' and the 'clay+N' treatments (except for the relationship between ABG/BGB and flower abundance). Not only the biomass production, tissue N and P concentrations and flower production of *R. drepanensis* were thus significantly plastic, but also the relation between these characters is substantially changing across environment: it is thus also plastic.

A last remark has to be made concerning the relationship between flower abundance and tissue N concentration across treatments. After the discussion above on the effect of nitrogen supply on flower induction, it seems remarkable that no significant correlation was found between these two characters. This is because the negative, linear relationship between tissue N concentration and flower production is broken under conditions of very low nutrient supply, namely the 'sand' treatment (Fig. 10, left). The relationship thus becomes nonlinear when including highly limiting nutrient conditions. This effect is further clarified by comparing the reproductive effort (as flower production per gram of vegetative biomass) with the tissue N concentration (Fig. 10, right). In the 'sand' treatment, the decrease in tissue N concentration with increasing age was coupled with a strong decrease in reproductive effort. These plants were obviously unable to maintain the expenses associated with reproduction under such a poor nitrogen supply. The short, annual life cycle of *R. drepanensis* may explain why the very little plants from the 'sand' treatment still 'tried' to reproduce, although they could not 'afford' it: this was anyway their only chance for surviving the 'approaching' summer drought.

## 5. CONCLUSIONS

In a laboratory experiment, potassium nitrate fertilization resulted in a delayed flowering response in *R. drepanensis*, both under nutrient-rich (clay:sand mixture) and nutrient-poor (sand) sediment conditions. It also resulted in a higher flower abundance in the nutrient-poor sediment. Our results thus suggest that the effect of nitrate fertilization on flower induction and on flower abundance are independent.

Flower induction in *R. drepanensis* is very responsive to water temperature (Santamaría & Hootsmans, in prep.) and to low-nitrogen conditions, and the influence of photoperiod in comparison is small. Flower abundance is further influenced by plant biomass (see also Santamaría & Van Vierssen, in prep.), and the high plant-to-plant variability was mainly related to the biomass allocation in belowground organs (roots and rhizomes). The obligate annual character of *R. drepanensis*, together with its adaptation to the lack of predictability characteristic of its habitat was proposed as the framework explaining the above described patterns of reproductive allocation.

Two consequences of our findings will be stressed here, as they have important implications for the management of Mediterranean temporary wetlands. Firstly, an extended (summer) inundation period is unlikely to result in higher seed production: conditions triggering flower production occur in late spring, and plant exhaustion follows reproduction. Secondly, high nitrogen loads in these wetlands may postpone flowering until it is too late to reproduce, resulting in a fast decline of the vegetation as soon as the seed bank from previous years is exhausted. As high temperatures strongly inhibit flower development (Santamaría & Hootsmans, in prep.), *R. drepanensis* reproduction will be strongly affected by any delay even if the wetland is not drying up in early summer.

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## Chapter 6

### Effect of bicarbonate availability and age on the primary production of *Ruppia drepanensis* Tineo from a Mediterranean brackish marsh.

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**ABSTRACT:** Measurements of oxygen production rate at different light intensities were performed using *Ruppia drepanensis* plant material from a brackish marsh in Doñana National Park (SW Spain). Two different bicarbonate concentrations (0.02 and 3 mM) were obtained by adding  $\text{NaHCO}_3$  to the original water. Higher production rates were found at the highest bicarbonate concentration, but only at high light intensities. As shallow water bodies of the Doñana brackish marsh showed a decrease in bicarbonate concentration coupled with the increase in plant biomass along the growing season, the possibility of a limiting effect of low bicarbonate concentrations on the submerged vegetation development is discussed.

## 1. INTRODUCTION

The importance of inorganic carbon as a major nutrient and potential limiting factor to photosynthesis and growth has been well established. Differences in carbon extraction capacity and bicarbonate use between marine and freshwater macrophytes result in a more frequent occurrence of carbon limitation of photosynthesis among the second group under field conditions (Madsen & Sand-Jensen, 1991). The species *Ruppia drepanensis* Tineo occurs in saline inland lakes and brackish lagoons of Spain, Italy and North Africa. It inhabits shallow temporary waterbodies and follows an annual life cycle (Cirujano, 1986). Although the species of the genus *Ruppia* are often considered as marine macrophytes in ecophysiological works, isolation of the shallow lakes and lagoons where *R. drepanensis* occurs results in dissolved inorganic carbon (DIC) dynamics more similar to the ones of alkaline freshwaters. Along the growing season in the Doñana brackish waterbodies (SW Spain), increase in biomass of *R. drepanensis* populations is coupled with a strong decrease in the available DIC: pH rises from 8 to 10, at which  $\text{CO}_2$  is virtually absent, and bicarbonate alkalinity drops together with an increase in carbonate alkalinity (unpublished data). Consequently, photosynthesis depends almost completely on bicarbonate in these localities, and primary producers have to face a strong decrease in this source of carbon at the end of the season.

By analysing the affinity constant and maximum production of Photosynthesis-Irradiance (PI) curves modelled by the rectangular hyperbola equation (Michaelis-Menten model) under two different bicarbonate concentrations, we try to establish if carbon availability has an effect on the shape of PI curves of *R. drepanensis* under non-saturated light conditions. This experiment was repeated twice during the growing season, as plant age is known to affect photosynthesis (Hootsmans & Vermaat, 1991).

## 2. MATERIAL AND METHODS

Water and plant material for the photosynthesis measurements in the laboratory were collected from a shallow waterbody of the brackish marsh area of the Doñana National Park, before (20.3.92, referred to as "March" measurements) and after (9.5.92, referred to as "May" measurements) seed production. A vegetation dominated by *R. drepanensis* occurred in this locality, together with scarce specimens of *Zannichellia obtusifolia* and *Chara galioides*.

Water was filtered (Whatman GF/C filters) and divided into two fractions: one was kept at the original bicarbonate concentration (0.02 mM), but  $\text{NaHCO}_3$  was added to the second one to reach a bicarbonate concentration of 3.75 mM. Whole plants (including rhizomes and roots) were carefully washed and divided into groups (approx. 4 g fresh weight each), which were put in Winkler bottles overnight in order to deplete the dissolved oxygen concentration in the water. Respiration rates were measured prior to overnight oxygen depletion.

Four different light levels were obtained by shading sunlight with neutral density nets. Photosynthetically Active Radiation (PAR) at 1 cm below the water surface was periodically measured during each experiment by means of a cosine-corrected LICOR sensor. Winkler bottles were submersed in a 15 cm deep water bath filled with tap water in order to control the temperature. Bottles were not incubated in the field locality because of the increased turbidity resulting from the necessary handling activities inside the waterbody.

Oxygen concentration and temperature inside the bottles were recorded by using a WTW oximeter (0.1 mg/l precision). Temperature range was 20-24 °C. Blank bottles showed no significant increase in oxygen concentration because of the repeated but brief opening of the bottles. During the experiment duration, oxygen concentration was measured 3-4 times each 15 to 30 minutes. Linear regression of the oxygen measured concentrations against time was used to calculate the oxygen production (or consumption) rate. After the measurements, plants were removed from the bottles and weighed (dry weight, 70°C 24 h). Three replicates were used at each light level, and the use of different plants and bottles assured the independence of the different datapoints along the PI curve.

PI curves were fitted using the Non-Linear Regression procedure of the Statgraphics statistical package, based on the Marquardt algorithm. Two different models were fitted: the rectangular hyperbola, or Michaelis-Menten (MM) model

$$P = \frac{P_m * I}{K_m + I} - R \quad (1)$$

where: P: gross production, I: light intensity, P<sub>m</sub>: maximum gross production, K<sub>m</sub>: half saturation constant, and R: dark respiration; and one modification of the MM model (hereafter referred to as the "Linear PhotoInhibition" model, LPI) which incorporates the photoinhibitory effect of high light intensities as a negative linear function of I:

$$P = \frac{P_m * I * (1 - \beta * I)}{K_m + I} - R \quad (2)$$

Comparison between the two different models was performed for each datapoints set (treatment) by using the following F statistic:  $F = (RSS_1/df_1)/(RSS_2/df_2)$ , in which RSS stands for residual sum of squares and df for degrees of freedom.

Multiple comparisons among fitted curves (different treatments fitted using the same model) were performed applying comparisonwise error rates (CER) adjusted according to experimentwise error rates (EER) of 0.05, 0.01 and 0.001 and the number of comparisons, and the following F statistic:

$$F = \frac{[RSS_{1,2} - (RSS_1 + RSS_2)] / [df_{1,2} - (df_1 + df_2)]}{(RSS_1 + RSS_2) / (df_1 + df_2)} \quad (3)$$

where  $RSS_{1+2}$  is the RSS of the regression of the two datasets together (Vermaat & Hootsmans, 1991). Multiple comparisons between the parameters of the fitted curves were performed applying the CER calculated as above, and a t test based on the parameter values and parameter standard errors (se) provided by the Non-Linear Regression. The latter could be used as the datapoints were all independent. In both cases, comparisons were performed between the two bicarbonate levels within each of the two plant ages, and between the two plant ages within each of the two bicarbonate levels, thus yielding a total of 4 comparisons.



TABLE 1: Parameter estimates (mean  $\pm$  standard error) from the curve fittings. Maximum Gross Production (Pm) and Dark Respiration (R) are in  $\mu\text{g.O}_2 \text{ g.dw}^{-1} \text{ min}^{-1}$ . Half-Saturation Constant (Km) is in  $\mu\text{E m}^{-2} \text{ s}^{-1}$ . Photoinhibition Constant ( $\beta$ ) is in  $(\mu\text{E m}^{-2} \text{ s}^{-1})^{-1}$ . Affinity Constant ( $\alpha = \text{Pm}/\text{Km}$ ; in  $\mu\text{g.O}_2 \text{ m}^{-2} \text{ s } \mu\text{E}^{-1} \text{ g.afdw}^{-1} \text{ min}^{-1}$ ) calculated from the parameter estimations have also been included for the Michaelis-Menten fitting.

Michaelis-Menten model				
	Pm	Km	R	$\alpha$
Mar,0.02	93 $\pm$ 8	21 $\pm$ 16	49 $\pm$ 6	4.37
Mar,3.75	178 $\pm$ 20	95 $\pm$ 41	65 $\pm$ 13	1.88
May,0.02	48 $\pm$ 4	47 $\pm$ 21	30 $\pm$ 3	1.01
May,3.75	83 $\pm$ 11	210 $\pm$ 99	27 $\pm$ 7	0.40
Linear photoinhibition model				
	Pm	Km	R	$\beta$
Mar,0.02	139 $\pm$ 20	92 $\pm$ 41	49 $\pm$ 5	24*10 <sup>-5</sup> $\pm$ 7*10 <sup>-5</sup>
Mar,3.75	225 $\pm$ 67	161 $\pm$ 110	65 $\pm$ 13	16*10 <sup>-5</sup> $\pm$ 16*10 <sup>-5</sup>
May,0.02	57 $\pm$ 8	86 $\pm$ 41	30 $\pm$ 3	12*10 <sup>-5</sup> $\pm$ 7*10 <sup>-5</sup>
May,3.75	59 $\pm$ 20	78 $\pm$ 87	29 $\pm$ 7	-22*10 <sup>-5</sup> $\pm$ 24*10 <sup>-5</sup>

### 3. RESULTS AND DISCUSSION

Comparisons between the two different models fitted showed non-significant results for all datapoint sets (treatments). Residual Mean of Squares (RSS/df) was lower for the LPI model for the datasets without bicarbonate addition, and it was lower for the MM model for the datasets with bicarbonate addition, although these differences were not significant in any of the comparisons. This is probably due to the high variability found in the replicate measurements at each light intensity. So, although a photoinhibition effect may have been present in the treatments without bicarbonate addition, low number of datapoints (light levels) and probably the variability in plant response did not permit to discriminate it properly.

Consequently, for further comparisons we limit ourselves to the simplest model, i.e. the Michaelis-Menten.

The four comparisons that were made between curves (different treatments) were all significant at an EER of 0.01. Multiple comparisons at an EER of 0.001 yielded significant results for both comparisons "March vs. May" (with and without bicarbonate addition). Thus, it can be concluded that the variables tested (bicarbonate concentration and plant age) had a significant effect on the overall shape of the PI curve. Plant age (before/after seed production) seems to have had a stronger effect, with "March vs. May" comparisons being still significant at an EER of 0.001.

When performing the multiple comparisons between the parameters of the curves, we found significant differences for Pm in all comparisons tested (EER 0.05; 3 of 4 comparisons were also significant at an EER 0.001), no significant differences in any comparison for Km, and significant differences only in both comparisons "March vs. May" (EER 0.05) for the parameter R.

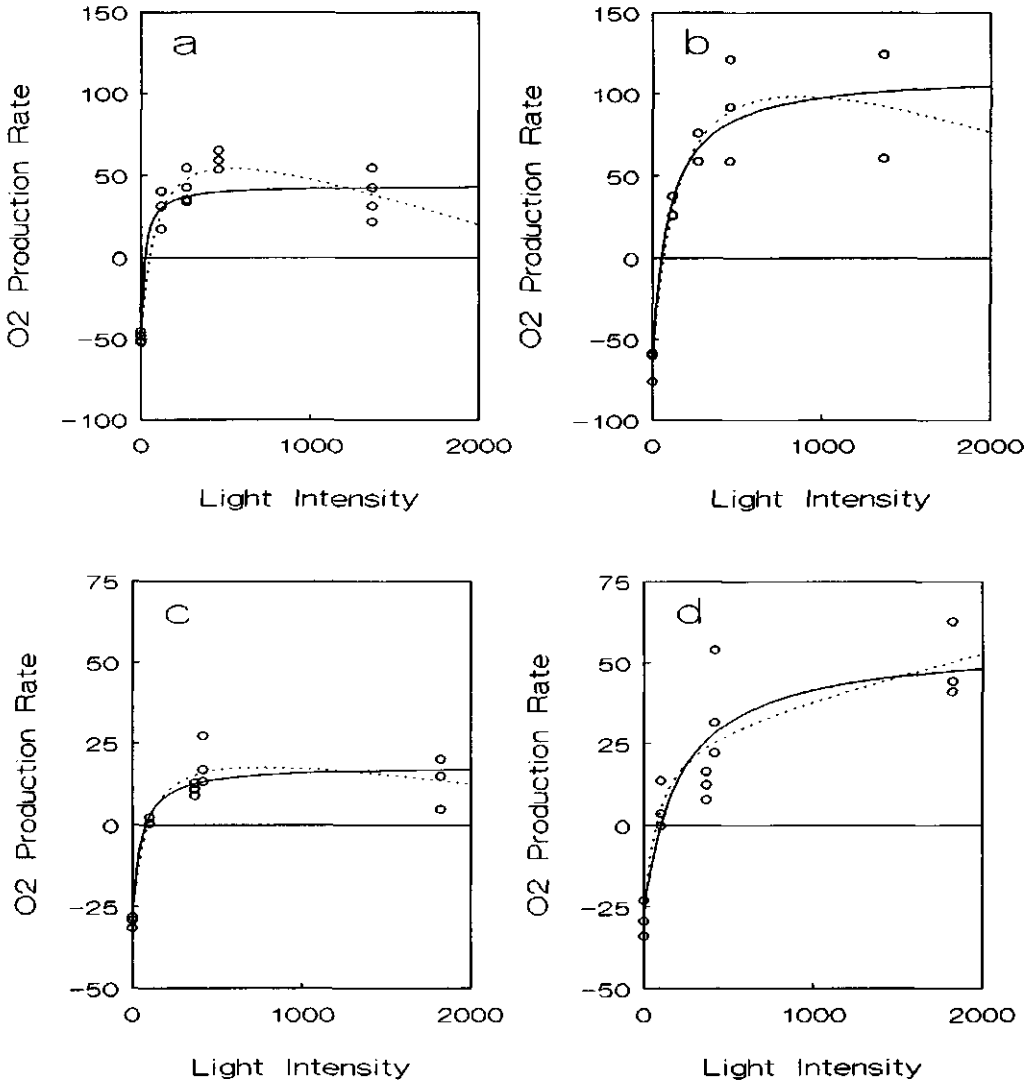


FIG. 1: Photosynthesis-Irradiance curves. Oxygen Production Rates are in  $\mu\text{g O}_2 \text{ g dw}^{-1} \text{ min}^{-1}$ , Light Intensities are in  $\mu\text{E m}^{-2} \text{ s}^{-1}$ . Solid lines represent the Michaelis-Menten equation, broken lines represents the Linear PhotoInhibition equation (see text). a: March, 0.02 mM HCO<sub>3</sub><sup>-</sup>; b: March, 3.75 mM HCO<sub>3</sub><sup>-</sup>; c: May, 0.02 mM HCO<sub>3</sub><sup>-</sup>; d: May, 3.75 mM HCO<sub>3</sub><sup>-</sup>. Note the differences in the Y-axis scale between the graphs a and b and the graphs c and d.

Maximum gross production seems to be the only parameter strongly affected by the two variables under study. The opposite happened for the half saturation constant, which remained unaffected. Although high standard errors of the  $K_m$  estimates could have accounted for this result, an inspection of the values of both  $K_m$  and the affinity constant (calculated by dividing  $P_m$  by  $K_m$ ) reveals that affinity (the initial slope of the PI curve) decreases with increasing bicarbonate content (Table 1). This suggest that carbon limitation

did not occur at low light intensities. Hence, it seems as if the light-limited rate of photosynthesis did not depend on carbon availability and plant age. At saturating light intensity, gross production increased with increasing bicarbonate availability, and decreased with increasing plant age (Table 1 and Fig.1).

The dark respiration parameter was not affected by the bicarbonate concentration, but it decreased significantly with increasing age. This is probably due a decrease in metabolic activity which accompanies the decay of the plant following seed production.

#### 4. CONCLUSIONS

When using the MM model to describe the photosynthetic rate at different light intensities, the light-limited rate of photosynthesis does not seem to depend on carbon availability or plant age. However, at saturating light intensities, gross production increases at increasing DIC concentrations, and decreases after seed production.

Two ecological implications of this should be mentioned. Under low incident-PAR regimes, light is the main factor limiting plant productivity. The retarded vegetation development probably also restricts the drop in bicarbonate concentration and, thus, DIC does not become limiting. On the other hand, under saturating PAR levels, vegetation development improves light conditions (by reducing sediment resuspension; Duarte *et al.*, 1990) and lowers DIC concentration, which then becomes the main limiting factor and restricts photosynthetic activity. Both factors are limiting plant development in an alternative way, an can be considered as important restrictors for population growth in an environment were salinity and temporality have been classically seen as the only factors strongly affecting submerged vegetation dynamics.

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## Chapter 7

# Influence of environmental parameters on the biomass development of *Ruppia drepanensis* populations in Doñana National Park: The importance of conditions affecting the underwater light climate

L. Santamaría, C. Montes<sup>1</sup> & M.J.M. Hootsmans

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**ABSTRACT:** The development of submerged plant populations dominated by *Ruppia drepanensis* Tineo in the brackish marsh of the Doñana National Park (SW Spain) was coupled to seasonal variation in environmental factors for two consecutive years. Plant biomass increased exponentially in early spring (March), with steady biomass yields (up to 100 g afdw m<sup>-2</sup>) together with abundant flowering and fruiting in late spring (April-May). Wind-induced sediment resuspension and periphyton growth strongly influenced the light climate experienced by the submerged vegetation, while a phytoplankton effect was generally negligible. Development of the submerged vegetation coincided with a decrease in water extinction coefficient and in bicarbonate concentration. Thus, where dense macrophyte meadows develop, light climate probably is the limiting factor in the early spring, while temperature and bicarbonate levels are so by the end of the season. Interannual variation was found to be very high, both in abundance and distribution of the submerged vegetation, mainly because of differences in rainfall which influenced the inundation cycle. Grazing by waterfowl can also account for this effect, as in dry years birds concentrate in the few wetlands still containing water.

## 1. INTRODUCTION

The Doñana National Park is one of the largest National Parks in Europe (approx. 50000 ha.), and constitutes one of the most important breeding localities for European wetland birds, as well as a vital passage and wintering zone along a main migration route (Finlayson & Moser, 1991). It includes about one third of the original floodplain marsh at the estuary of the Guadalquivir river, which constitutes now about 50 % of the surface of the Park (Grillas *et al.*, 1993). The Doñana marsh is subject to annual cycles of inundation and desiccation, and most of its waterbodies have water from late autumn to late spring (Fig. 1). Dependence on rainfall for inundation makes inundation cycles highly unpredictable, as rainfall distribution shows a high interannual variability. A detailed description of the aquatic systems of the marsh may be found in Montes *et al.* (1982).

Relatively dense submerged macrophyte meadows have been reported to occur in the Doñana marsh (Grillas *et al.*, 1993). Duarte *et al.* (1990) identified the aquatic plants as the major primary producers of the marsh, thus providing a basis for the high abundance and diversity of invertebrates, fishes and birds. Five species of submerged angiosperms and two genera of charophytes occur in the marsh, ranging from freshwater species (such as *Myriophyllum alterniflorum* D.C.) to species characteristic of high salinities (such as *Aithenia orientalis* (Tzvelev) García-Murillo & Talavera). The distribution of the different species roughly follows the NW-SE increase in salinity. Several authors (Duarte *et al.*, 1990; Van Vierssen & Den Hartog, unpublished data) hypothesise salinity to govern the spatial distribution of the submerged plant communities, while factors affecting the light climate, seed-bank and nutrient supply would determine the development of the macrophyte meadow. *Ruppia drepanensis* Tineo, a species with a wide salinity tolerance (Cirujano, 1986), is the most abundant submerged macrophyte in the marsh (Grillas *et al.*, 1993).

This study is part of a research project (Santamaría, 1994) focusing on the understanding of the eco-physiology, life-cycle and population dynamics of the most abundant submerged macrophyte, *R. drepanensis*, as a basis for the management of the semiarid temporary wetlands. The research project combined different laboratory experiments with two years of field data collection in the Doñana National Park brackish marsh.

In this paper, we set out spatial and temporal variability on the development of the submerged vegetation of the Doñana National Park. The distribution of the different brackish water communities and its relationship with the water salinity is first analyzed. After this, we concentrate on the factors which may explain the spatial and temporal changes in biomass density of macrophyte meadows dominated by *R. drepanensis*. Special attention was paid to the factors affecting light climate, such as water turbidity and periphyton development, and to the availability of carbon, nitrogen and phosphorus to the plants.

## 2. MATERIALS AND METHODS

### 2.1. Study sites

Four localities were selected for the collection of biweekly samples. The shallow lakes ('lucios'; 0.5 m depth) Lucio del Aro and Lucio de Vetas Altas Chico, together with a channel from the old salt-pans of San Isidoro ('Canal Salinas') were sampled in 1991 (Fig. 2). In 1992, samples were taken again in the Lucio del Aro, and in one of the deeper (up to

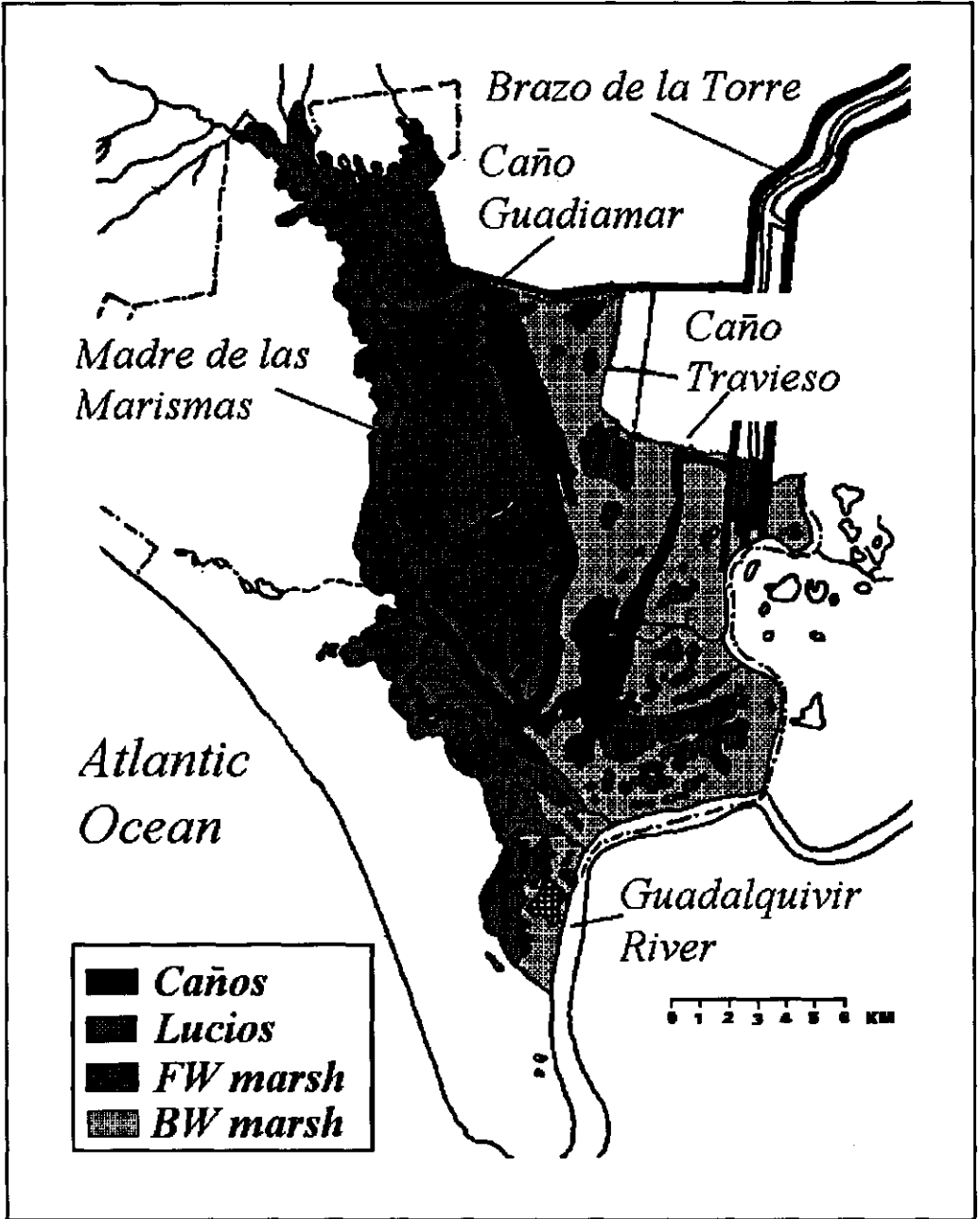


FIG. 1: Scheme of the different hydrological units at the Doñana marsh. Caños: river courses, receiving water from the streams reaching the marsh from the North; mainly freshwater. Lucios: shallow floodplain lakes, most of them depending nowadays on direct rainfall water; mainly brackish water. FW marsh: floodplain marsh ('quebradas'), flooded for a shorter time than the lucios; mainly freshwater. BW marsh: floodplain marsh ('quebradas'), mainly brackish water.

1 m), small artificial lucios ('sacatierras') dug during the construction of a clay wall which encloses the South-Eastern side of the marsh ('Sacatierra del Muro'). *R. drepanensis* was the dominant species in all these localities (more than 90 % of the biomass of the submerged plant community), except in the Canal Salinas, where it co-dominated with *A. orientalis*.

In March-May 1991, a survey was carried out to map the distribution of *R. drepanensis* in the Doñana marsh. However, certain areas of the marsh could not be visited due to internal regulations of the Park, aiming to restrict disturbance to nesting birds. Submerged macrophytes density and species composition were estimated visually and expressed in 5 semi-quantitative categories. At 36 localities of the marsh, samples were taken for taxonomic determination. Electrical conductivity and pH (WTW specific electrodes and meters), as well as the depth were measured. Taxonomic determinations followed Talavera & García (1987) for *R. drepanensis*, García-Murillo & Talavera (1986) for *A. orientalis*, and Talavera *et al.* (1986) for *Zannichellia obtusifolia* Talavera, García-Murillo & Smit. The specimens of *Callitriche* found in our samples were assigned to *Callitriche truncata* Guss.,

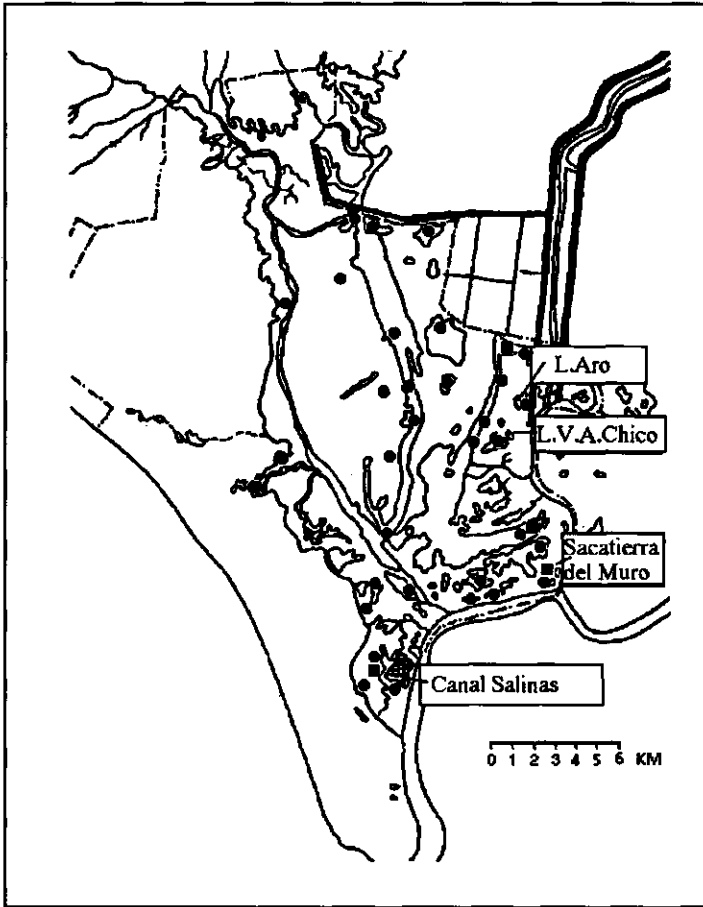


FIG.2: Different sampling stations at the Doñana marsh. Labels: biweekly sampling. Filled squares: biomass sampling in June 1991. Indicated are also the localities in which conductivity and species composition of the submerged plant community were recorded in May 1991 (circles).



the only species present in the Doñana marsh (P. García-Murillo, pers.comm.; Grillas *et al.*, 1993). Charophytes were not determined.

During this survey, samples for the determination of the leaf chlorophyll content of *R. drepanensis* plants were collected in 6 different localities differing in submerged vegetation density and water transparency. In the first week of June 1991, when we estimated that submerged biomass would be maximal, samples were collected in four localities to assess the representativeness of the results from the biweekly sampling for other wetlands of the Doñana brackish marsh. These four localities were chosen to represent a maximum of the observed spatial variability (see Fig.2). Three replicate samples were taken at each locality, using a 40x40 cm<sup>2</sup> sampling square.

## 2.2. Methods

At each locality, a separate area (permanent quadrat) was selected for the collection of macrophyte biomass samples. This area was subdivided into 30 squares of 5x5 m<sup>2</sup> each. Every two weeks, samples were taken in the centre of 3-5 different, randomly selected squares. 10 m minimum distance between all sampling points assured little disturbance of the neighbouring sites due to the sampling activities. Submerged vegetation biomass was sampled using sampling squares of 30x30 (Canal Salinas and Sacatierra del Muro) and 40x40 (Lucio del Aro, Lucio de Vetas Altas Chico) cm<sup>2</sup>.

Both above and belowground biomass were collected by sieving the upper sediment layer through a 1 mm sieve. Biomass of the different species was then discriminated, and individuals of *R. drepanensis* identified and counted by using the scar of the original seed in the rhizome. After subsampling for determination of the leaf chlorophyll content (5-10 leaves) and biomass allocation (belowground fraction, basal shoots, vertical shoots and seeds), the fresh (fw) and dry (dw; 70 °C, 24 h) weight of the samples was determined. *R. drepanensis* biomass was then subdivided into two fractions: one was used for the determination of the tissue N and P concentration, while the rest was used to determine the ash-free dry weight (afdwt; 520 °C, 3 h). A regression of afdwt on dw using the values from the afdwt-subsamples was then used to calculate the total afdwt of each sample.

Leaf chlorophyll determination followed Wintermans & De Mots (1965), modified as suggested by J.E. Vermaat (pers.com.). 5-10 fresh leaves were carefully grinded in 96 % ethanol, and this extract was used for spectrophotometrical determination of chlorophyll-*a* and -*b*. Phaeopigments-*a* and -*b* were also determined after acidification with 0.06 N hydrochloric acid, but were always present in negligible concentrations and hence will not be reported on.

Determination of the tissue N and P content followed Novozamsky *et al.* (1983), and was done using intact plant samples (no separation in above- and belowground fractions). It consisted of digestion of the plant material in a mixture of selenium, hydrogen peroxide, salicylic acid and sulphuric acid, followed by a determination of the total N and total P using an Aquatec® auto-analyzer.

A second separate area was marked at each locality for incubation of microscope glass slides to evaluate periphyton colonization. 12 stations were installed at 10 m intervals, each of them consisting of 4 slides maintained at 10 cm from the bottom by a cork attached to a rope. In the Canal Salinas, two incubation levels were used, at 10 and 20 cm from the bottom. 8 slides from two different, randomly-selected stations were sampled fortnightly, so that the incubation time increased through the season ('accumulative' data). In two stations,

the 8 slides were renewed biweekly, providing us with simultaneous data on the biweekly colonization rates ('colonization' data).

Determination of periphytic light attenuation followed Vermaat & Hootsmans (1994), and was measured as the proportional transmittance reduction relative to transmittance through a clean slide with sunlight and a LICOR LI-192S irradiance sensor (measuring photosynthetically active radiation). Periphyton was previously removed from the lower side of the slides. After measuring the attenuation, periphyton was scraped off the upper-side of the slides to determine its chlorophyll and biomass (dw and afdw) density (same methods as described above).

Water maximum and minimum temperatures were registered fortnightly at one single point in L. Aro and Sacatierra del Muro (they correspond, thus, to the previous two-weeks period). Samples from the water column were taken at three different points of each locality. At these points, water temperature, electrical conductivity, dissolved oxygen concentration and pH were measured using WTW specific electrodes and meters. The extinction coefficient was calculated from the simultaneous measurements of two inter-calibrated LICOR LI-192S irradiance sensors maintained at 10 cm vertical distance. Light extinction was assumed to be homogeneous along the water column (depth < 50 cm). Samples for the determination of alkalinity (titrimetry with 0.02 N H<sub>2</sub>SO<sub>4</sub>, and phenolphthalein and methyl orange as end-point indicators; Greenberg *et al.*, 1992), seston (dw and afdw) and chlorophyll (same method as above) concentration were taken in 1 litre polythene flasks, and analyzed within 12 hours. Samples for nitrate and orthophosphate determination were taken in acid-rinsed 150 ml polythene flasks, and preserved with sulphuric acid until further determination. Ammonia concentration (NH<sub>3</sub>+NH<sub>4</sub><sup>+</sup>) was only measured in 1992, by means of spectrophotometric determinations following the nesslerization method (Greenberg *et al.*, 1992). Dissolved orthophosphate was measured with an Aquatec<sup>®</sup> auto-analyzer, and nitrate was measured according to the sulphanilic method following nitrate reduction in a column of cadmium (Greenberg *et al.*, 1992).

### 3. RESULTS AND DISCUSSION

#### 3.1. Composition and distribution of the brackish water communities in the Doñana marsh

In May 1991, *R. drepanensis* occurred all along the Eastern half of the Doñana marsh (Fig.3), with coverage percentages of 50 to 100 % in half of this area. A few occasional specimens were also found in the fresher, North-Western area of the marsh. The west side of the Caño Guadiamar may be taken as the limit for the brackish, *R. drepanensis*-dominated communities of the marsh, although its connection with the Southern extreme of Caño Travieso results in a decreasing abundance of this species along that Caño.

The relative abundance of different species varied importantly along the brackish marsh. *R. drepanensis* was present in 28 of the 36 stations. While other factors affecting the biomass development of the different species still caused a high variability in macrophyte cover, the present data support our initial hypothesis: salinity is the major factor explaining the changes in species composition of the communities. In Fig.4, we drew the enveloping line for the estimated abundance of each species versus the measured conductivity, and a clear replacement pattern appears between *C. truncata*, *Z. obtusifolia* and *R. drepanensis*. Charophytes seem to be widely distributed along the marsh, dominating at lower conductivities and becoming sub-dominant at conductivities above 30 to 40 mS cm<sup>-1</sup>. This is

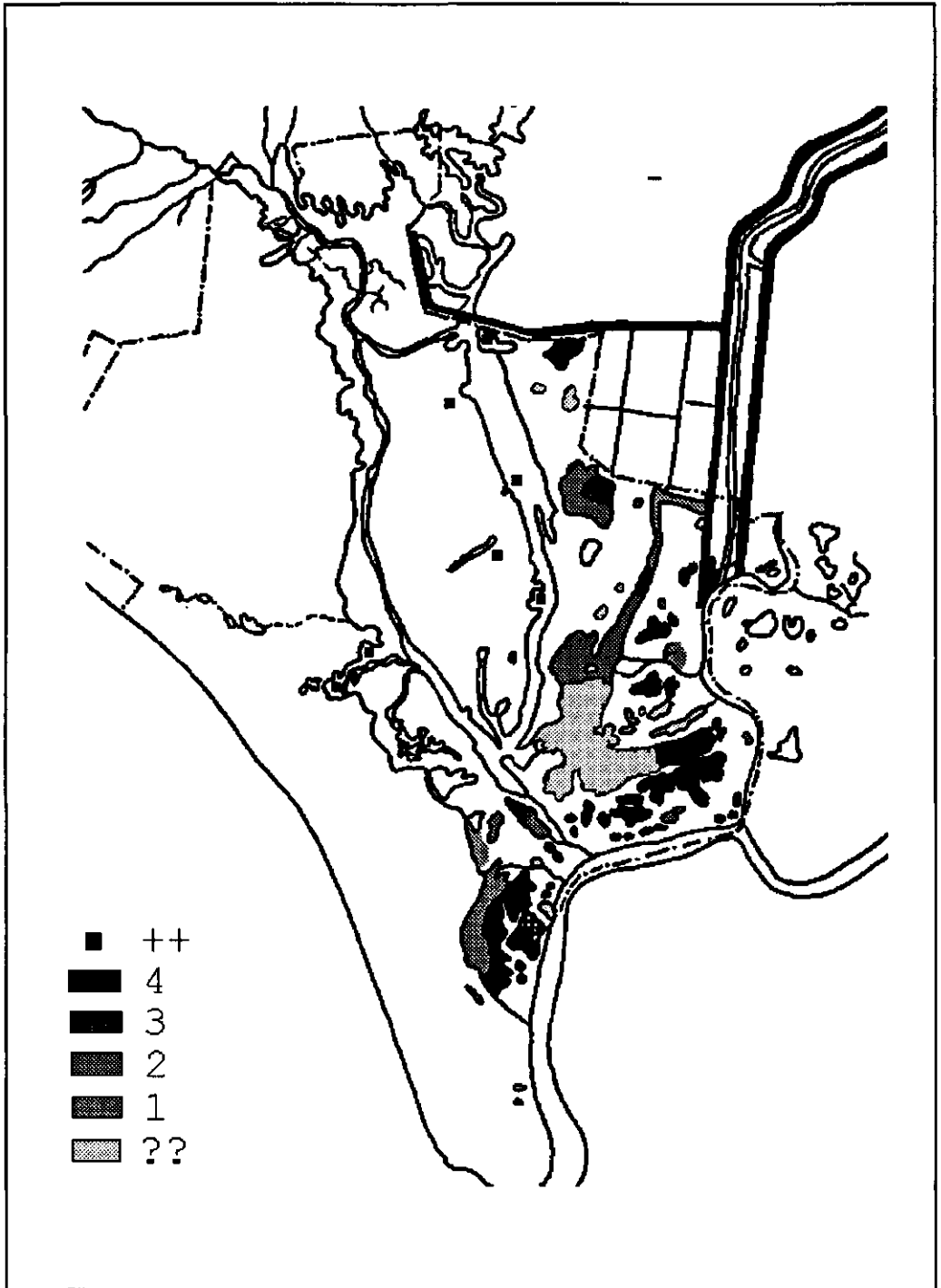


FIG.3: Distribution of *R. drepanensis* in the Doñana marsh (April-May 1991). ++: present. 1: 0-12.5 % cover. 2: 12.5-25 % cover. 3: 25-50% cover. 4: 50-100 % cover. ??: Not visited.

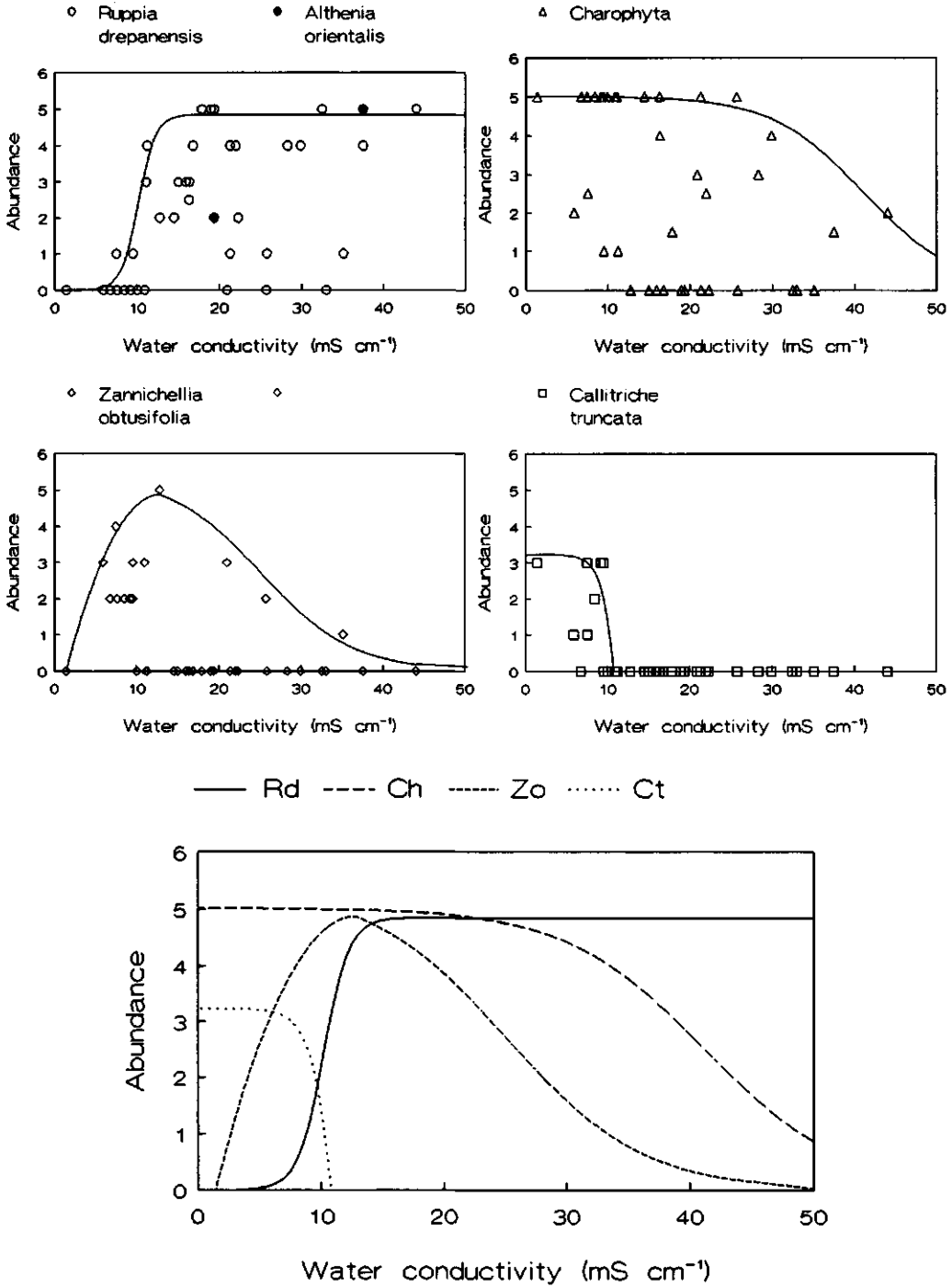


FIG.4: Relationship between water electrical conductivity and the abundance of different species of submerged macrophytes estimated along a survey of 36 different waterbodies from the Doñana brackish marsh.

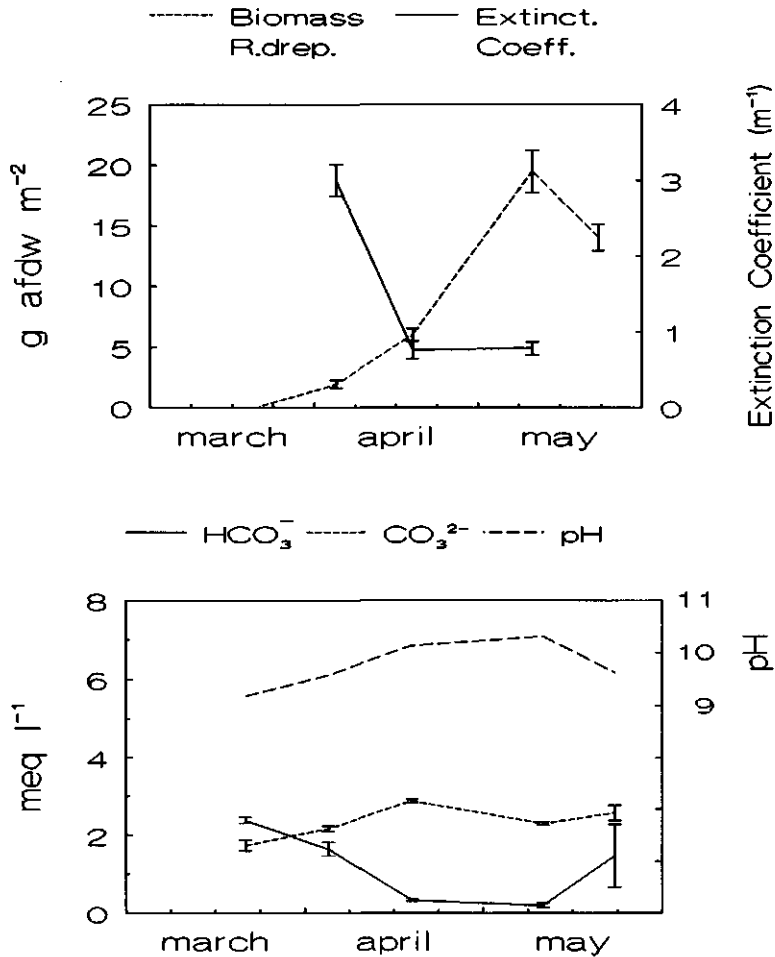


FIG.5: Biomass yield, extinction coefficient, pH, and bicarbonate and carbonate concentration from L. de Vetas Altas Chico, 1991.  $n=3$ ; biomass yield  $n=5$ .

in agreement with Grillas *et al.* (1993), who found a similar replacement pattern along the salinity gradient, and mentioned *R. drepanensis* and *Z. obtusifolia* to be the dominant angiosperm species in the Doñana marsh. Similarly, Van Vierssen & Den Hartog (unpublished data) described the *Zannichellietum peltatae* (Potametea: Calitricho-Batrachion) and *Rupprietum drepanensis* Brullo & Furnari 1970 (Ruppieteae: Ruppion) as two separate associations characteristic of the Mediterranean temporary brackish waters. Moreover, they also proposed salinity as the major factor determining the species composition of the syntaxa in Southern Europe, while the importance of other factors would be superior in Northern Europe. Finally, Duarte *et al.* (1990) proposed a conductivity of 10 mS cm<sup>-1</sup> (equivalent to 6 g l<sup>-1</sup> salinity) to discriminate the occurrence of brackish water macrophytes in the Doñana marsh. In our survey, *C. truncata* became absent and *R. drepanensis* became quickly

## Sacatierra del Muro - 1992

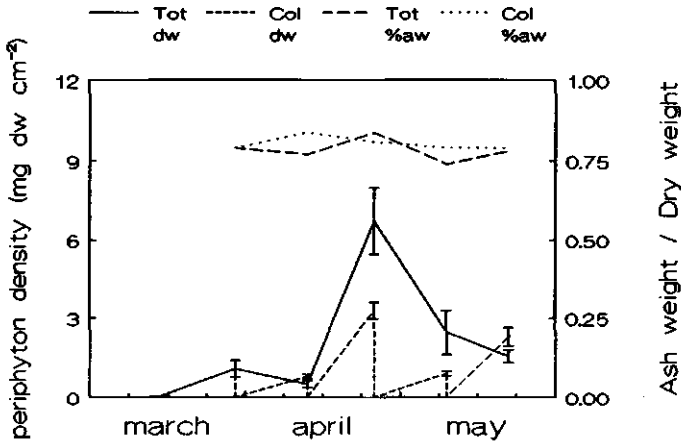


FIG.6: Periphyton density in Sacatierra del Muro. Tot: accumulative measurements. Col: biweekly colonization measurements. %aw: ash fraction (ash weight / dry weight).  $n=3-4$ .

dominant around this conductivity, while *Z. obtusifolia* reached in this transition its maximum abundance.

In most brackish wetlands, a shore-centre zonation was observed, with *Ranunculus baudotii* Godron in the most external fringe, *Z. obtusifolia* and some scarce specimens of *C. truncata* in the shallow band next to the shoreline (5-10 cm depth), and *R. drepanensis* (occasionally accompanied by *A. orientalis*) in the centre. Shoreline abundance of *R. baudotii* was attributed by Van Vierssen & Verhoeven (1983) and Grillas *et al.* (1993) to the combined effect of the limitations imposed by its anemophilous pollination and the advantage derived by its capability to form land-forms when the wetland dries. Abundance of *Z. obtusifolia* and *C. truncata* in shallow areas of wetlands of relatively high salinity may be related with the ability of these short-lived species to use the first part of the growing season, when salinities are lower, to complete their life cycle (Van Vierssen & Van Wijk, 1982). High water turbidity apparently precludes their early development in deeper places (see following sections), where *R. drepanensis* becomes the dominant species later in the season.

### 3.2. The life-cycle of *R. drepanensis*

The results of the observations during the springs of 1991 and 1992 in 4 different localities of the marsh will be summarised below. The graphs shown in this section combine and summarise the observations at different localities in the two subsequent years. A detailed account of all these observations may be found in Santamaría (1994; Appendix).

At the beginning of the growing season, the germinated seedlings began their growth while the wetlands were at their maximum water depth (30 to 50 cm), had a high water turbidity (extinction coefficient  $\geq 3$ ), relatively low pH values (around 8) and a high bicarbonate concentration (3 to 5 meq l<sup>-1</sup>). Turbidity was mainly caused by wind-induced

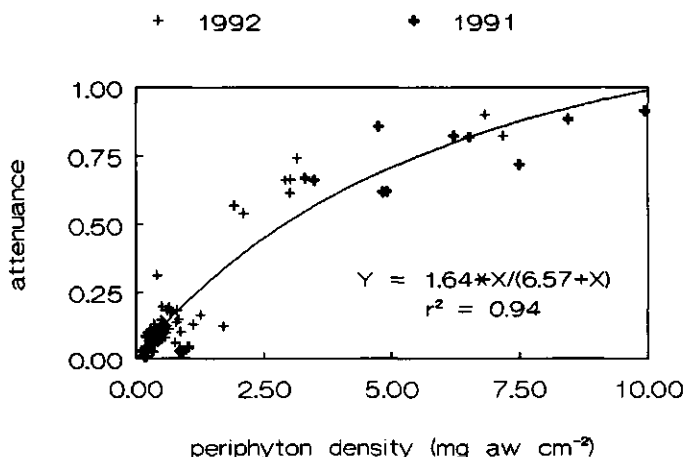


FIG. 7: Relationship between periphyton density and attenuation. All data from different localities are pooled. A similar relationship was found for the periphytic afdw ( $Y = 1.59 * X / (1.61 + X)$ ,  $r^2 = 0.89$ ).

resuspension of the loose sediments (similarly to that reported in Duarte *et al.*, 1990), with seston values up to 15 mg aw l<sup>-1</sup>. This was the situation following the spring rains (end of March) in the shallower wetlands of the brackish marsh. In the smaller, deeper 'sacatierras' (which collect the rainfall water earlier due also to the slight NW-SE inclination of the marsh), and in the channels of the Salinas de San Isidoro, a full development of the submerged vegetation was already reached by then.

Parallel with submerged vegetation development, a fast decrease in water turbidity (up to an extinction coefficient  $\approx 1$  m<sup>-1</sup>), an increase in the pH (up to 10.5) and a drop in bicarbonate concentration (to below 0.5 meq l<sup>-1</sup>; Fig. 5) was then observed.

Biomass yield of *R. drepanensis* reached peak values of 10 to 20 g afdw m<sup>-2</sup>, except in the 'sacatierras' from the SE area of the marsh, where it could reach 90 g afdw m<sup>-2</sup>. The biomass samples taken in May 1991 at 3 additional localities where charophytes and *A. orientalis* were relatively abundant and the data from the mixed population of *R. drepanensis* and *A. orientalis* in the Canal Salinas suggest a maximum yield of 10 to 40 g afdw m<sup>-2</sup> for the brackish macrophyte communities of the non-artificial wetlands of the marsh. Values reported by Duarte *et al.* (1990; 591 g fw m<sup>-2</sup>) and Grillas *et al.* (1993; 18.5 g dw m<sup>-2</sup>) were comparable.

The two localities with dense *R. drepanensis* meadows showed abundant periphyton growth, with colonization rates up to 3 mg dw cm<sup>-2</sup> wk<sup>-1</sup> and biomass yields up to 11 mg dw cm<sup>-2</sup> (Fig. 6). This thick periphyton layer had a high ash content (70 to 80 %), and intercepted a substantial fraction of the incident light (70-80 % for periphyton densities above 5 mg aw cm<sup>-2</sup>; Fig. 7). Nevertheless, detachment of the periphyton at the end of the inundation cycle reduced again the periphyton density to values below 3 mg dw cm<sup>-2</sup>. In the shallower wetlands of the brackish marsh, where vegetation development occurs relatively late, periphyton biomass was always below 1 mg dw cm<sup>-2</sup>, and attenuated only 5 to 20 % of the incident light.

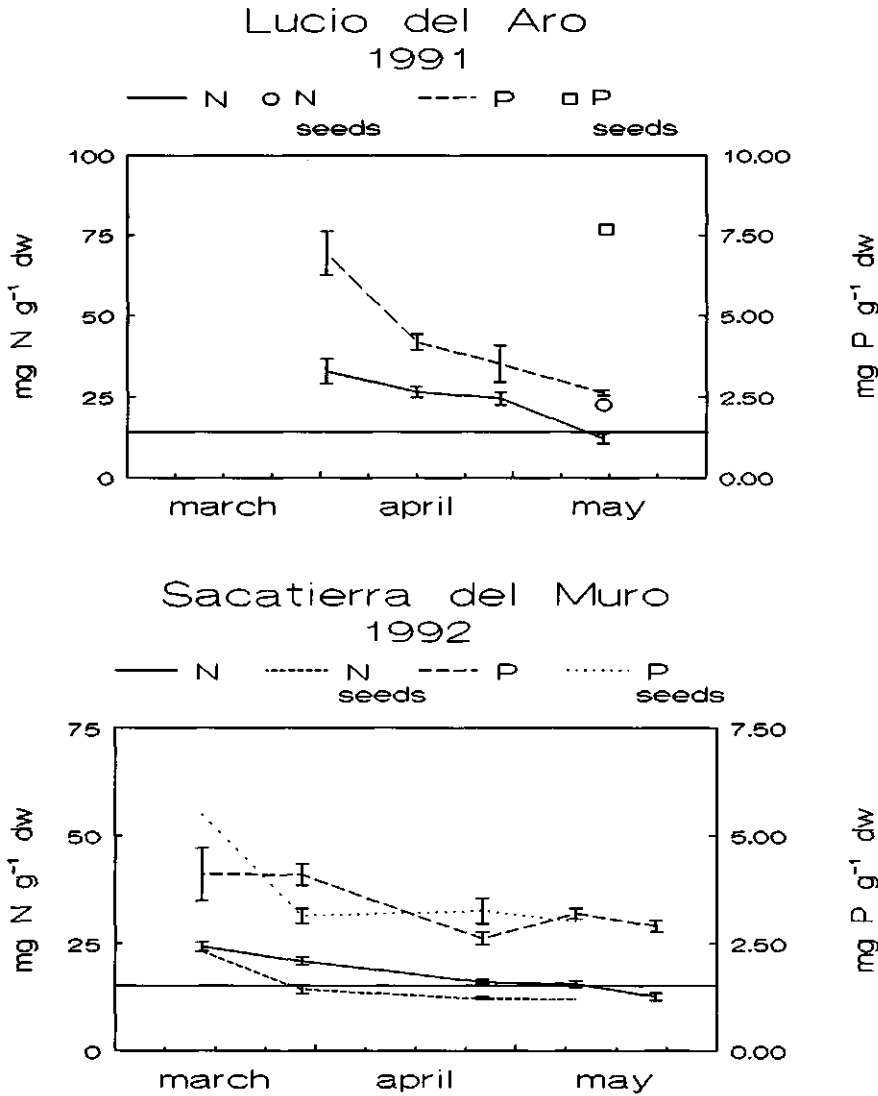


FIG. 8: Seasonal development of the tissue nitrogen and phosphorus content in *R. drepanensis* plants and seeds from the Doñana brackish marsh.  $n=3-5$ .

Leaf chlorophyll content of *R. drepanensis* remained close to  $1 \text{ mg Chl-}a \text{ g}^{-1} \text{ fw}$  during most of the growing season, and chlorophyll-*b* accounted for 30-40 % of the total chlorophyll. Nevertheless, the sampling in May 1991 at 6 different localities differing in water turbidity and plant density revealed that chlorophyll content was significantly higher (up to  $3 \text{ mg Chl-}a \text{ g}^{-1} \text{ fw}$ ) in the localities with poorer light climate, while the chlorophyll-*b* fraction remained the same. This is in agreement with the laboratory findings of Santamaría & Van Vierssen (1994).



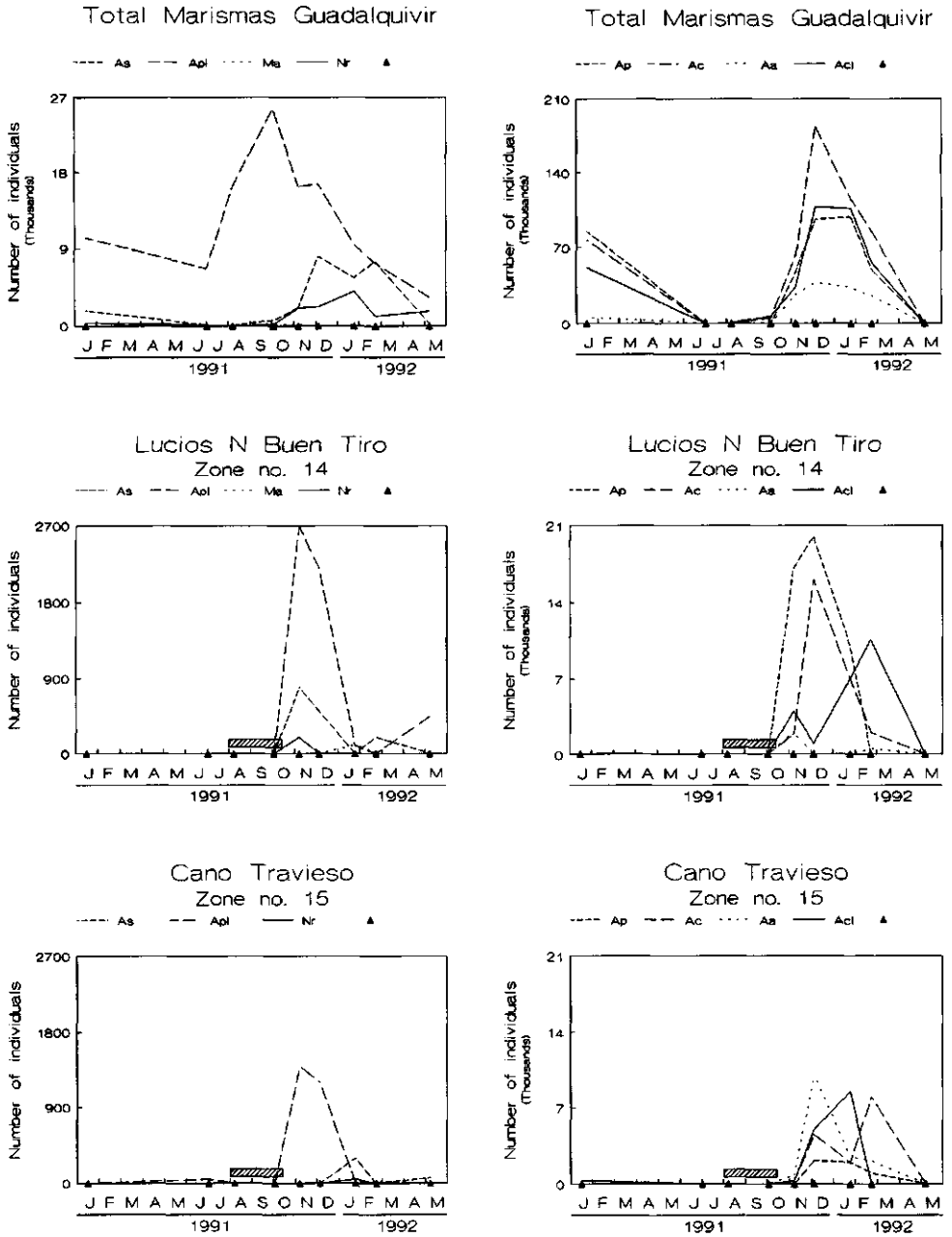


FIG.9: Number of individuals of different waterfowl species in the total area (note 10 times higher Y-scale) and in different sectors of the floodplain marsh in the estuary of Guadalquivir River (Marismas del Guadalquivir). Data courtesy of the Doñana Biological Station (EBD). As: *Anas strepera*. Apl: *Anas platyrhynchos*. Ma: *Marmaronetta angustirostris*. Nr: *Netta rufina*. Ap: *Anas penelope*. Ac: *Anas crecca*. Aa: *Anas acuta*. Acl: *Anas clypeata*. Hatched area: dry period. Triangles indicate the dates in which censuses were done.

Flowering and seed production were rapidly followed by plant deterioration, with decreasing leaf chlorophyll content, total biomass and above- to belowground biomass ratio. Seston and bicarbonate concentration concurrently increased (up to 50-200 mg aw l<sup>-1</sup> and 2-3 meq l<sup>-1</sup>, respectively), and in one locality (Sacatierra del Muro) the extinction coefficient rose again to values close to 3. Also dissolved orthophosphate and chlorophyll concentrations in the water column, which had remained low during most of the growing season (0-35 µg P l<sup>-1</sup> and 0-2 mg Chl-*a* l<sup>-1</sup>, respectively), increased concurrently with plant deterioration by the end of May (up to 60-100 µg P l<sup>-1</sup> and 5-7 mg Chl-*a* l<sup>-1</sup>).

Both nitrogen and phosphorus content in *R. drepanensis* tissue decreased with time following the increase in biomass yield (Fig. 8). While phosphorus remained in all localities but the Lucio de Vetos Altas Chico well above the threshold values from Gerloff & Kromholz (1966; 1.3 mg P g<sup>-1</sup> dw), nitrogen content approached the threshold (13 mg N g<sup>-1</sup> dw) when plant density was high (i.e., in May). We might thus expect nitrogen to be the limiting nutrient (if any) for *R. drepanensis* in the Doñana marsh, as also suggested by the N:P ratio found in the sediment of the 4 localities sampled (1.4 to 3.6, versus a 10:1 relationship in the plant tissue).

In the Canal Salinas, tissue nitrogen and phosphorus content of *A. orientalis* was similar to that of *R. drepanensis*. Tissue nitrogen content of the seeds of *R. drepanensis* was generally the same as the vegetative tissue content, but it increased at the end of the season (except in the Sacatierra del Muro).

Poor seed production in the shallower wetlands (less than 0.5 g afdw m<sup>-2</sup> in the Lucio del Aro, about 1 g afdw m<sup>-2</sup> in the Lucio de Vetos Altas Chico) in 1991 was followed by a very scarce vegetation development in 1992. Seed yield was, on the contrary, very high in the Canal Salinas (up to 6 g afdw m<sup>-2</sup>) and in the Sacatierra del Muro (up to 4 g afdw m<sup>-2</sup>). Average density of *R. drepanensis* reported by Grillas *et al.* (1993) for the seed bank of the Doñana marsh was 2.8 g dw m<sup>-2</sup>.

Still, an increase in *R. drepanensis* biomass density coupled with a certain decrease of the water turbidity began to occur in the Lucio del Aro in March 1992. Subsequently, biomass density decreased to zero and turbidity increased again (with extinction coefficients from 5 to 10). Rhizome fragmentation and broken leaf tips, together with the abundance of coots (*Fulica atra*), red-crested pochards (*Netta rufina*) and mallards (*Anas platyrhynchos*) in the Lucio (we observed 500 coots, 40 pochards and 45 mallards on 10.4.92, while in 1991 only a few avocets, *Recurvirostra avosseta*, and black-winged stilts, *Himantopus himantopus*, were present) suggested that intense waterfowl grazing may be responsible for the decrease and disappearance of the (already scarce) vegetation from the Lucio in 1992.

Data from aerial waterfowl censuses supplied by the Doñana Biological Station were consistent with this hypothesis. While in January 1991 no individuals have been counted in the sectors 14 and 15, abundant counts of gadwall (*Anas strepera*), red-crested pochard (*Netta rufina*), wigeon (*Anas penelope*), teal (*Anas crecca*), pintail (*Anas acuta*) and shoveler (*Anas clypeata*) are reported for January and February 1992 (Fig. 9). As overall counts for all these species were similar in January 1991 and January 1992 for the overall floodplain marsh of the Guadalquivir estuary, changed waterfowl distribution inside the marsh probably is responsible for the differences in local abundance.

These data point out that waterfowl grazing can be an important factor affecting the dynamics of the vegetation in the marsh. Interannual changes in foraging localities may depend both on changes in the inundation of the marsh and on disturbance-induced variation in submerged vegetation abundance (for example, very little vegetation may be expected to grow in Lucio del Aro in 1993). It stresses the importance of maintaining the spatial

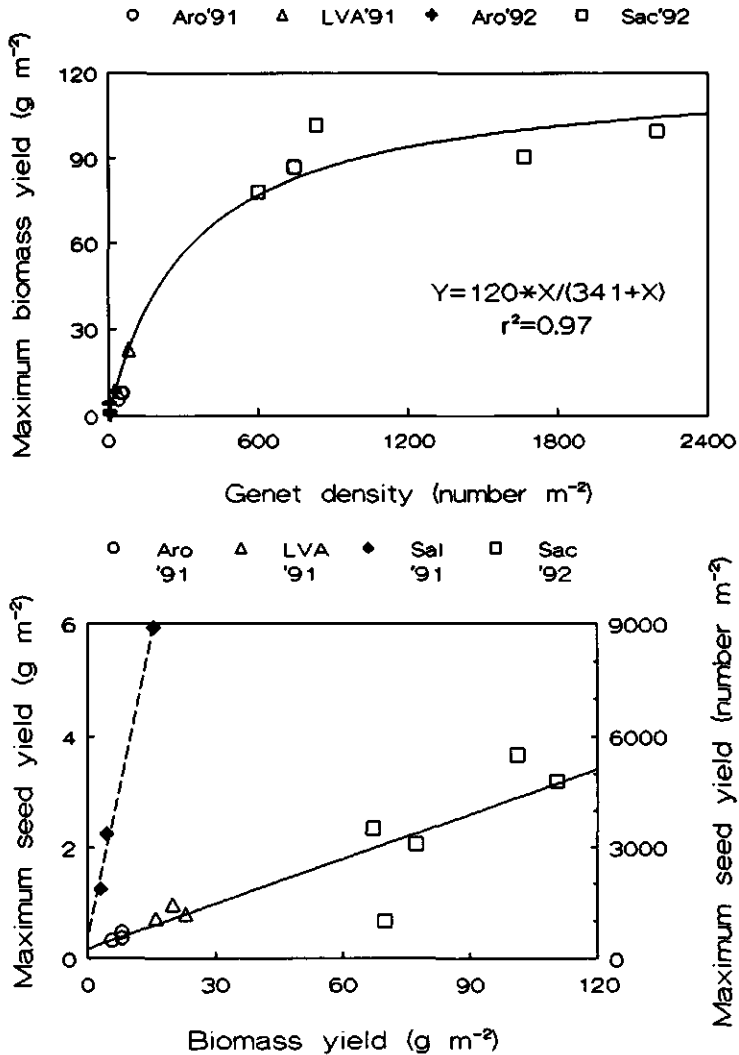


FIG.10: Relationship between genet density and biomass yield (above) and between biomass yield and seed production (below; data from Canal Salinas were fitted separately).

variability in the marsh as having a conservational value in itself.

Other data from spring 1992 in the Lucio del Aro may exemplify the changes caused by the absence of submerged vegetation. Contrary to 1991, the concentration of dissolved phosphorus ( $40 \mu\text{g P l}^{-1}$ ) and bicarbonate (3 to 5 meq l<sup>-1</sup>) remained high, and suspended chlorophyll showed relatively high values during April (20 to 35 mg Chl-*a* l<sup>-1</sup>, concentrations characteristic for eutrophic waters according to Ryding & Rast, 1989). Seston values remained high (close to 100 mg aw l<sup>-1</sup>), and so did the water extinction coefficient (5-10 m<sup>-1</sup>). The positive feed-back between submerged vegetation growth and decreased water turbidity (through a decreased wind-induced sediment resuspension; Duarte *et al.*, 1990), and the negative relationship between macrophytes and algal growth (through a reduction in the

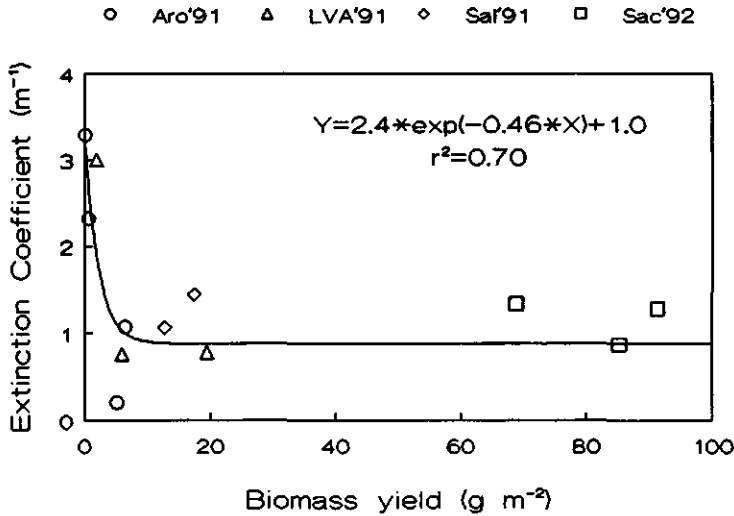


FIG. 11: Relationship between the development of the submerged vegetation (here as biomass density in g afdw m<sup>2</sup>) and the water turbidity (extinction coefficient, in m<sup>-1</sup>). Each datapoint is the average of 3-5 measurements.

phosphorus available in the water column) are supported by these observations. The first relationship will be considered in more detail in the following section.

### 3.3. Quantification of key factors controlling submerged vegetation development

On the basis of the results presented above, we propose (1) the seed bank density, and (2) the ability of vegetation to reduce water turbidity through vegetative growth, as the two key factors triggering the switch between two organizational states: a macrophyte-dominated, 'clear' state and a 'turbid' state which, in the absence of macrophytes, results in phytoplankton dominance. In this section, we will attempt to present a quantification of these main relationships. Still, strict causality becomes a loose concept when the evolution of a system is considered. The relationships presented below should better be considered as the interactive result of the development of a system where internal variables are interconnected by different feed-backs.

The positive feed-back between seed-bank and vegetation density was already suggested by Grillas *et al.* (1993), who found positive correlations between the biomass density of different aquatic angiosperms and their abundance in the seed bank for different localities of the Doñana marsh. On the contrary, charophyte oospores were abundant all along the marsh, but this did not result in high biomass in all localities.

This positive feed-back implies two different relationships: a positive effect of plant (or seedling) density on the total biomass yield, and a positive relationship between the vegetative biomass yield and the seed yield. Both relationships are described for *R. drepanensis* in Fig. 10. Maximum biomass yield was hyperbolically related to genet density, maximum yield being reached at densities above 600 genets m<sup>-2</sup>. Maximum seed yield

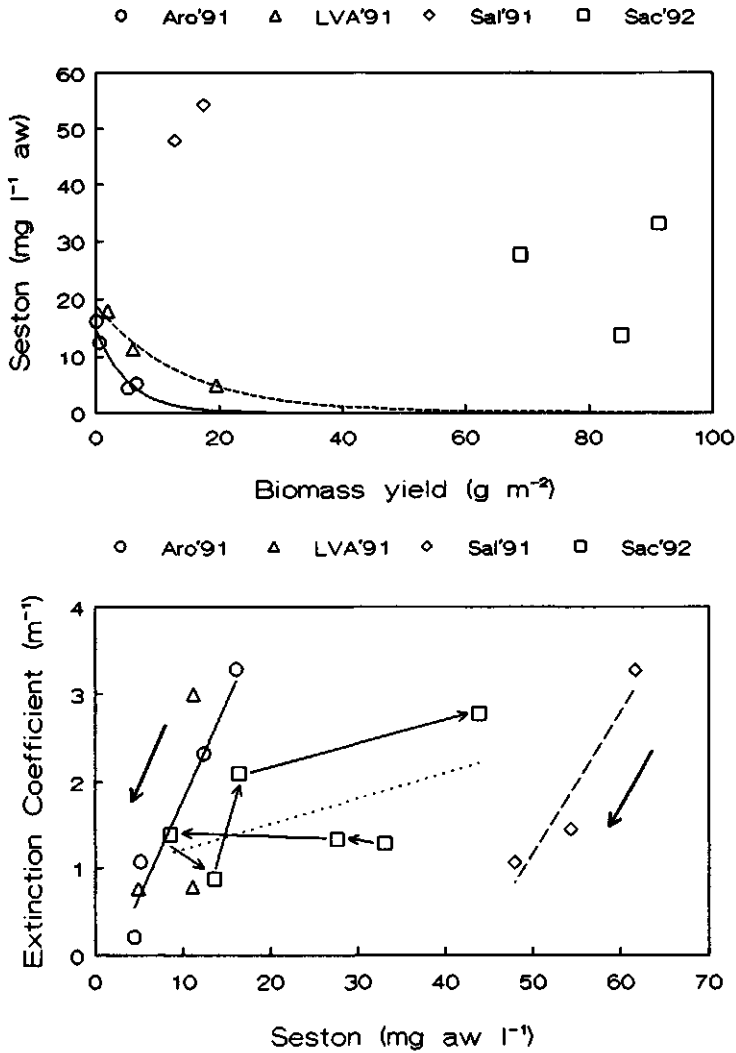


FIG. 12: Relationship between the density of the submerged vegetation and the seston (above), and between the seston and the light extinction coefficient in the water column (below; arrows indicate the time sequence through the growing season). Each datapoint is the average of 3-5 measurements.

depended linearly on biomass yield, with a recorded maximum of about 6000 seeds m<sup>-2</sup>.

An important outlier was found in the Canal Salinas, where *R. drepanensis* co-dominated with *A. orientalis*, and the relationship between *R. drepanensis* biomass and seed yields is much higher (maximum yield: 9000 seeds m<sup>-2</sup>). The coexistence of two species apparently resulted in an improved seed yield for *R. drepanensis*.

The hyperbolic relationship between genet density and biomass yield is attributable to a self-thinning effect at high genet densities (Silvertown, 1982). A double logarithmic plot of individual plant biomass on genet density showed a linear relationship with a slope equal

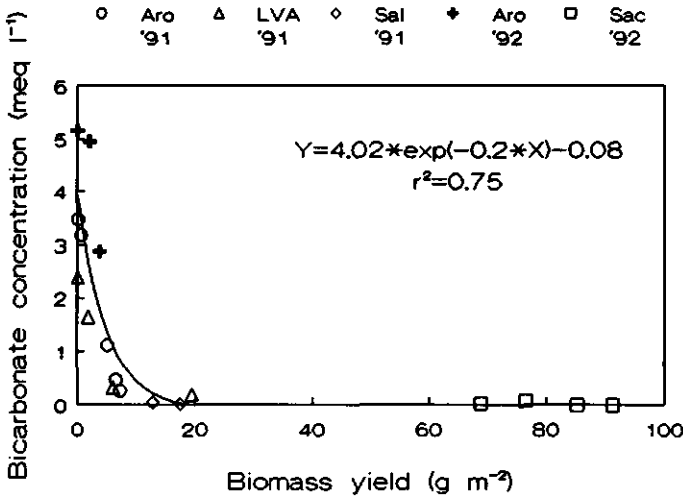


FIG. 13: Relationship between the biomass density of the submerged vegetation and the bicarbonate concentration in the water column. Each datapoint is the average of 3-5 measurements.

to -0.89 and intercept equal to 1.71. General slope values of -1.49 and -1.25 have been respectively proposed for the weight-density relationship of terrestrial and freshwater macrophytes (Duarte & Kalff, 1987). Slope values close to -1 have been proposed for sparse populations ceasing to grow when the carrying capacity of the habitat is reached, without the intervention of previous mortality (Silvertown, 1982); and for dense plant populations growing under a restricted light climate (Kays & Harper, 1974). Considering that dense *R. drepanensis* populations where maximal biomass was measured grew in shallow wetlands with transparent water, the low slope values are probably attributable to the lack of density-dependent mortality previous to habitat saturation. This is in agreement with the lack of a density-dependent decrease in fecundity at these higher densities (Fig. 10; see also Silvertown, 1982). The life-cycle of *R. drepanensis* is thus based on a fast growth until habitat carrying capacity is reached, immediately followed by an intense seed production.

The water extinction coefficient decreased exponentially with biomass density, and a minimum was quickly reached at relatively low biomass values (around  $10 \text{ g m}^{-2}$ ; Fig. 11). Duarte *et al.* (1990) suggested that increased water transparency in spring was due to the effect of submerged macrophytes on bottom stabilization, and thus the reduction of wind-induced sediment resuspension. This seems to be the case in Lucio del Aro and Lucio de Vetas Altas Chico, where a negative exponential relationship was also found between seston and biomass density (Fig. 12). Nevertheless, seston concentration was much higher than expected in the two localities with well-developed, dense macrophyte meadows (Sacatierra del Muro and Canal Salinas), while the water transparency was still high.

We hypothesise that the relationship between seston and water extinction coefficient was different for those two wetlands (Fig. 12). As seston organic fraction was similar for all localities (15 to 25 %), and sediment composition is quite homogeneous all along the brackish marsh, we hypothesise here a vegetation-mediated effect on seston optical properties. In localities where a dense macrophyte meadow covers the sediment, seston originates mainly from the wind-induced resuspension of particles already settled on the

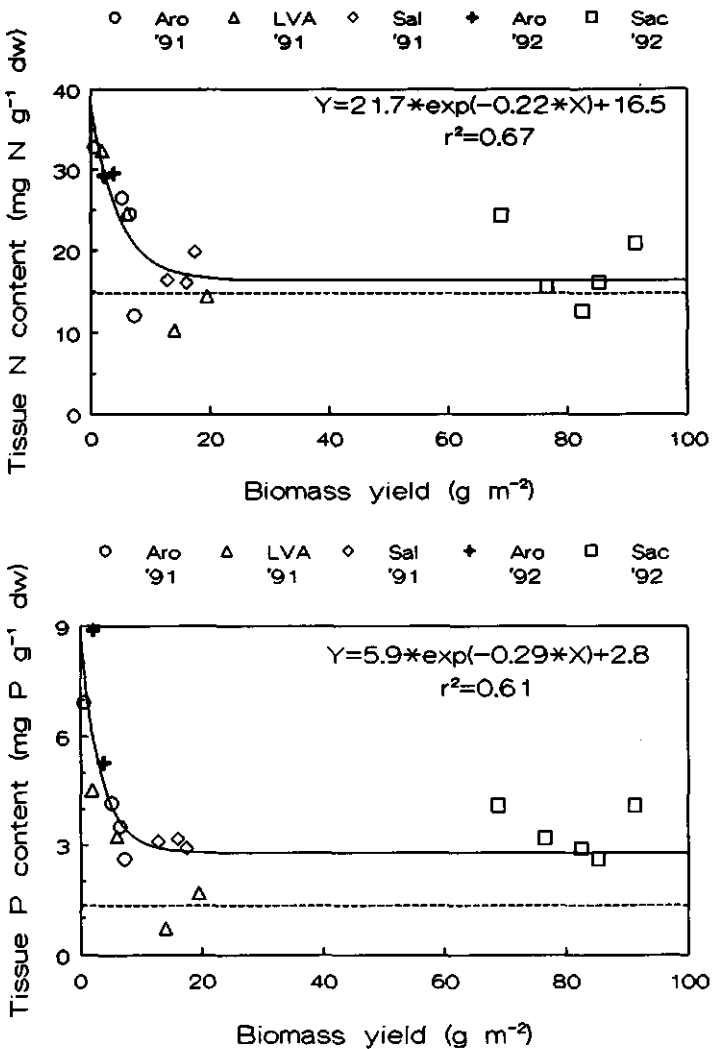


FIG.14: Relationship between the biomass density of the submerged vegetation and the concentration of total nitrogen (above) and total phosphorus (below) in the plant's tissue. Each datapoint is the average of 3-5 measurements. Broken lines indicate Gerloff & Krombholz's (1966) threshold values for the onset of nitrogen and phosphorus limitation.

macrophyte leaves (pers.obs.). These periphytic particles have a larger size than the loose sediment particles resuspended in vegetation-denuded areas or at the beginning of the season. In consequence, the relationship between seston concentration and water turbidity changes, and high concentrations of seston do not result any longer in a high water turbidity.

Periphytic algae and bacteria are unlikely to fully account for this effect, because the organic fraction of the seston did not change among localities. But intense plant photosynthesis and the increase in water pH from 8 to 10 through the growing season may

have resulted in a profuse marl deposition in the periphyton layer (Allen, 1971; Sand-Jensen, 1977; Gons, 1982). It is significant that biomass densities of 10 g afdw m<sup>-2</sup> are also a threshold value for bicarbonate depletion (attributable to the intense macrophyte metabolism; see Fig.13). Thus, agglomeration of seston particles mediated by marl deposition on the leaves of *R. drepanensis* may complement sediment stabilization in explaining the positive relationship found between water transparency and submerged vegetation density.

Finally, it seems clear that the conditions that characterise the clear-water state result in a switch from light-limited to nutrient-limited vegetation growth. Increased biomass density correlates with an exponential drop in water bicarbonate concentration (the only form of carbon available for photosynthesis at pH 8 to 10), and in tissue nitrogen and phosphorus content (Fig.14). While the first results in lower photosynthetic rates (Santamaría *et al.*, in press), the maintenance of tissue nitrogen contents above the threshold of 13 mg N g<sup>-1</sup> dw at high biomass densities has probably an increasing metabolic cost, as concluded in Santamaría *et al.* (1994). The restricted nutrient supply, together with the rise in water temperatures (see also Santamaría & Hootsmans, 1994), drive to an abundant flowering and seed set and the subsequent deterioration of the vegetative apparatus. This should be understood as an integral part of the dynamics of the macrophyte-dominated state. Man-induced changes in the direction of higher nutrient loads, external water inputs in late spring, or an extended inundation cycle in summer will thus have their impact on the normal sequence of the macrophyte's life-cycle, and may be detrimental for the submerged vegetation of the marsh.

#### 4. CONCLUSIONS

We have made it plausible that the development of the *R. drepanensis* vegetations at the Doñana brackish marsh is mainly controlled by two factors: the seed bank density, and the reduction of water turbidity by macrophyte growth. The interplay of these two factors determine the switch between a clear, macrophyte-dominated state and a turbid, algae-dominated state in early spring. Disturbances such as an intense grazing by waterfowl (and possibly also the trampling by cattle; Duarte *et al.*, 1990) may contribute to push the system into the 'turbid' state, especially in wetlands with relatively sparse macrophyte meadows.

Periphyton accumulation may be considerable in abundantly vegetated wetlands, attenuating up to 80 % of the incident light to the leaf surface. Easy dislodgement, however, may limit the periods of heavy incrustation. Also, since water transparency is high, periphyton shading must be quite considerable to really limit photosynthesis in these shallow wetlands. Nevertheless, the reduction in light and carbon supply to the leaves (Sand-Jensen, 1977, 1983) may interplay with the low bicarbonate concentrations and the low nitrogen and phosphorus levels in determining the deterioration of the vegetative apparatus of the plants following seed set.

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## Chapter 8

### General conclusions: a model for the life-cycle of submerged macrophytes in Mediterranean temporary wetlands

L. Santamaría

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## 1. INTRODUCTION

In this final chapter, I will attempt a synthesis of the previous. First, a summary is given of the main results. When possible, cross-links between field and laboratory outcomes are made. Subsequently, I will consider how all these results fit in the initial conceptual model. Finally, implications of these findings for the management of Mediterranean temporary wetlands will be discussed.

## 2. THE ECO-PHYSIOLOGY OF *R. drepanensis*: LABORATORY STUDIES

### 2.1. Light climate

*Ruppia drepanensis* Tineo showed a rather limited capacity for morphological acclimation to restricted light regimes (see Chapter 2). Its capacity for photosynthetic acclimation, however, was considerable. Both light harvesting efficiency and light saturated photosynthesis were higher for the plants grown at lower irradiance levels. Still, growth rates decreased with decreasing irradiance, probably due to the higher metabolic expenses of building and maintaining a more efficient light harvesting system.

Plants grown under a shorter photosynthetic period showed a higher rate of light-saturated photosynthesis, but a less efficient light harvesting system. Still, the reduced respiratory needs resulted in an overall increase in net photosynthesis. The reduced metabolic needs obtained at the cost of a lower light harvesting efficiency also resulted in a higher PQ, and growth rate was thus maximal for the dim-treatments (long photoperiod, short photosynthetic period).

Interaction of low irradiances and shorter photosynthetic period, hypothesised here to be associated with an increased water turbidity, resulted in a strong reduction in seed production. The induction of flowering was significantly postponed under short photoperiods (10 versus 16 h day<sup>-1</sup>) and low irradiances ( $\leq 145 \mu\text{E m}^{-2} \text{s}^{-1}$ ), and flower production was severely restricted under low irradiances. Moreover, seed production was strongly reduced in plants grown under a short photosynthetic period, and it was virtually null at irradiances equal or below  $110 \mu\text{E m}^{-2} \text{s}^{-1}$ . Hence, in spite of the high plasticity shown by *R. drepanensis* in its photosynthetic acclimation to low light regimes, the sensitivity of seed production to sustained low light regimes may easily result in a strong population decline after summer drought.

These results fitted well with the conclusions from our field research (see Chapter 7). Reduction in water turbidity due to sediment stabilization was found to follow when a certain threshold in macrophyte biomass density was reached (see below). Morphological acclimation resulting in shoot elongation and thus in the positioning of leaves closer to the water surface would be, in this context, a less efficient strategy. Hence, photosynthetic acclimation to low light regimes is necessary to develop a certain biomass density (even at a high metabolic cost) in the turbid early-spring water. Under prolonged low light periods ( $\leq 8 \text{ E m}^{-2} \text{ day}^{-1}$ ), *R. drepanensis* is probably unable to support the demand of seed production. But once a certain biomass density is achieved, the improved light regime will allow for a fast growth and an abundant flowering and seed production.

## 2.2. Temperature

The growth of *R. drepanensis* was optimal at 20 °C and lower at 14 and 30 °C (see Chapter 3). The lower growth rates at 30 °C were attributed to an increased respiratory rate, while the decreased growth rates at 14 °C probably resulted from the poorer irradiance-saturated photosynthesis at such temperature. Moreover, temperature and photoperiod interacted in their effect on biomass yield, short photoperiod resulting in significantly lower biomass only at low temperatures.

*R. drepanensis* showed again a relatively high capacity for photosynthetic acclimation, in this case to high temperatures (30 °C). This acclimation was achieved by widening the temperature response curve to reach higher net photosynthetic rates at high temperatures (as compared with those displayed by plants grown at 20 °C). However, acclimation was not enough to maintain a growth rate similar to those of plants grown at 20 °C.

Temperature proved to be a factor of major importance in influencing *R. drepanensis* sexual reproduction. Flower induction was inhibited at 14 °C, and flower development was also inhibited at 30 °C. No seeds were thus produced by the plants grown at 14 and 30 °C. Photoperiod, irradiance and probably plant size may also affect flowering time and abundance, once the temperature range is adequate for flower induction and development.

## 2.3. Nitrogen effects: nitrate fertilization and ammonia toxicity

Fertilization with ammonium nitrate in the root zone resulted in a slower growth of the plants, together with an increase in dark respiration (see Chapter 4). The net rate of photosynthesis remained unaffected. We attributed this effect to ammonia toxicity. By uncoupling ATP formation during the photophosphorylation process, ammonia reduces carbon fixation and hence plant growth without affecting photosynthetic oxygen production. Total ammonia ( $\text{NH}_3 + \text{NH}_4^+$ ) concentration in the porewater ranged from 0.5 to 3.5 mg N l<sup>-1</sup> for the non-fertilized treatment (11 g N m<sup>-2</sup> in the sediment), from 5 to 40 mg N l<sup>-1</sup> for the low-dose fertilization (sediment+fertilizer: 22 g N m<sup>-2</sup>) and was higher than 15 mg N l<sup>-1</sup> for the high-dose fertilization (sediment+fertilizer: 44 g N m<sup>-2</sup>).

Although the nitrogen levels used in the experiment were within the range of those measured in the sediment of the Doñana brackish marsh waterbodies, porewater ammonia concentrations measured in Doñana in 1992 were always below 0.1 mg N l<sup>-1</sup>. Hence, at present ammonia toxicity is not likely to affect *R. drepanensis* populations in the Doñana brackish marsh.

Fertilization with potassium nitrate resulted in a delayed flowering response, both under nutrient-rich (clay:sand mixture) and nutrient-poor (pure sand) sediment conditions (see Chapter 5). It also resulted in a higher flower abundance in the nutrient-poor sediment. High plant-to-plant variability within the treatments was related to the biomass allocation in belowground organs (roots and rhizomes), which we hypothesised to be also associated with nutrient uptake. Resource allocation seems the most appropriate framework to integrate the combined influences of nutrient supply, biomass allocation to roots and shoots, plant size and possibly age on the plant reproductive effort. Moreover, our results suggested that the effects of (at least) some of these factors on flower induction and on flower abundance are independent.

#### 2.4. Carbon supply and bicarbonate use

In the course of the growing season in the Doñana brackish waterbodies, the increase in biomass of *R. drepanensis* populations is coupled with a strong decrease in the available dissolved inorganic carbon (DIC): pH rises from 8 to 10, at which  $\text{CO}_2$  is virtually absent, and bicarbonate concentration drops whilst carbonate concentration increases (see Chapter 7). Consequently, photosynthesis depends largely on bicarbonate in these localities, and primary producers have to face a strong decrease in this source of carbon at the end of the season. Photosynthesis-irradiance (PI) curves measured using water collected in field with and without bicarbonate addition (resulting concentrations: 3.75 and 0.02 mM  $\text{HCO}_3^-$ , respectively) showed that the light harvesting efficiency did not depend on carbon availability, while the light-saturated photosynthesis increased at increased DIC concentration (from 93 to 178  $\mu\text{g O}_2 \text{g}^{-1} \text{dw min}^{-1}$ ; see Chapter 6).

Under reduced light regimes such as occurring in the Doñana wetlands at the beginning of the season, light is probably the main factor limiting plant productivity. The retarded vegetation development probably restricts the drop in bicarbonate concentration, and thus DIC does not become limiting. But when the biomass density is high enough to stabilize the sediment and water turbidity decreases, incident irradiance may become saturating. DIC will subsequently drop and become the main limiting factor for photosynthetic production. It is in agreement with our ideas about resource allocation to suggest that these conditions should also lead to plant reproduction, and subsequently to plant senescence.

#### 2.5. Ageing and senescence: the effect of seed production

PI curves were measured in the field before and after seed production. Increased plant age resulted in a decrease in light-saturated photosynthesis, while light harvesting efficiency was not affected (see Chapter 6). Combining results from different laboratory experiments (Chapters 2 and 4), it was concluded that an age effect on photosynthesis was only evident after the initiation of reproduction: whilst apparent quantum yield ( $\alpha$ ), maximum gross production ( $P_m$ ) and net photosynthesis did not change significantly with plant age before flowering and seed production, all of them decreased significantly following reproduction (see Chapter 2).

It was hypothesised (Chapter 1) that in this annual species the vegetative tissue will senesce after seed-setting. Senescence will direct all the plant effort to reproduction, allowing also the translocation of nutrients from the vegetative tissues to the seeds (Silvertown, 1982). As the high demand generated by seed production results in a rapid deterioration of the vegetative apparatus, it seems unlikely that an extended inundation period following seed production would result in a much higher offspring for the *R. drepanensis* meadows. Especially in addition to the high summer temperatures, which have been found to be inhibitory for flower development.

#### 2.6. Extrapolation from photosynthesis to growth

During two of our laboratory experiments, photosynthetic performance of the cultured plants could be compared with measured rates of biomass increase (see Chapters 2 and 3). The

difference between instantaneous growth rates calculated from the two was variable, depending on the treatment (growth conditions). While both rate types closely agreed for the plants grown at certain conditions (16 h daylength and  $285 \mu\text{E m}^{-2} \text{s}^{-1}$  irradiance for the experiment in which light climate was varied;  $20^\circ\text{C}$  for the experiment in which temperature was varied), departures from these culturing conditions resulted in twofold differences between the biomass- and the photosynthesis-estimated growth rate estimates.

We have attributed these differences to the influence of growing conditions on the factor used to convert photosynthetic oxygen exchange into cell growth, e.g. on the photosynthesis and respiratory quotients (PQ and RQ;  $\text{O}_2/\text{C}$  ratio on molar basis). For example, PQ has been reported to vary seasonally (Best & Dassen, 1987) and to be affected by plant chemical composition (Lambers & Richter, 1990), and RQ may change for plants grown at different temperatures (Berry & Björkman, 1980).

Accordingly, our photosynthetic calculations of the relative growth rates based on a  $\text{PQ}=1$  overestimated the biomass production at low irradiances ( $110 \mu\text{E m}^{-2} \text{s}^{-1}$ ), and underestimated it both at short photosynthetic periods (10 h) and at high temperatures ( $30^\circ\text{C}$ ), all by a factor 2.

Our care at regarding the use of measured photosynthetic rates to extrapolate the growth dynamics of plant populations must be stressed here. Neglecting the differences in PQ caused not only by differences in nutrient availability, but also by the continuous process of plant ageing, light acclimation and temperature acclimation may bias the derivation of estimated growth rates. Until now, most effort in aquatic ecology has been spent in obtaining accurate estimates of plant and community metabolism (photosynthetic and respiratory rates). An evaluation of the suspected variation in PQ and RQ may prove useful and will certainly improve the accuracy of the models calculating biomass from photosynthetic and respiratory data.

### 3. FACTORS AFFECTING THE DEVELOPMENT OF THE *R. drepanensis* POPULATIONS IN THE DOÑANA MARSH

From the analysis of two years of field data (see Chapter 7), we concluded that two factors controlled the development of the *Ruppia* beds:

- (1) the seed bank density, and
- (2) the reduction in water turbidity due to sediment stabilization, once the bed is dense enough ( $\geq 10 \text{ g afdw m}^{-2}$ ).

The interplay of these two factors seems to determine the switch between a clear, macrophyte-dominated state and a turbid, algae-dominated state in early spring. Biological disturbances affecting sediment stability and macrophyte biomass density (such as intense grazing by waterfowl, trampling by cattle or, in freshwater areas, the grazing by American crayfish, *Procambarus clarkii* (Girard), and the bioturbation by carp, *Cyprinus carpio* L.) may contribute to push the system into the 'turbid' state, especially in wetlands with a low density of macrophytes.

We also concluded that the conditions that characterise the clear-water state result in a switch from light-limited to nutrient-limited vegetation growth. Increased biomass density correlates with an exponential drop in water bicarbonate concentration and in tissue nitrogen and phosphorus content. Low bicarbonate concentrations result in lower photosynthetic rates, as explained above. The low tissue contents of nitrogen and phosphorus suggest limitation by either or both (cf. Gerloff & Krombholz, 1966; Thursby, 1984). Moreover, the

simultaneous deficiency in carbon, nitrogen and phosphorus greatly increases the metabolic cost of their respective uptake, which should have consequences for growth (Best & Mantai, 1978; Terry, 1982). I postulate that these limitations, together with the increased water temperature, trigger the developmental sequence of flowering, seed production and senescence. This should be understood as an integral part of the dynamics of the macrophyte-dominated state, and an excess of nutrients may thus have a more 'limiting' effect than their 'deficiency' for next-year's development of the macrophyte meadows.

Periphyton light attenuation has little significance. Although periphyton accumulation may be considerable in abundantly vegetated wetlands, attenuating up to 80 % of the incident light, rapid dislodgement limited the periods of heavy encrustation to only one to three weeks. Since water transparency is normally high in these wetlands, periphyton shading must be quite considerable to limit photosynthesis. Nevertheless, the reduction in light and carbon supply to the leaves may interplay with the low bicarbonate concentrations and the low N and P levels in triggering the senescence of the vegetative apparatus following seed set.

#### 4. CONSEQUENCES FOR OUR BASIC MODEL HYPOTHESIS

The importance of the seed bank was confirmed by our field observations, as well as those of Grillas *et al.* (1993). The interaction between light climate and submerged vegetation development seems to play a key role at the beginning of the growing season, and determines the subsequent development of the system.

Fig.1 summarises the influence of seed-bank and climatic variability on the switch between the macrophyte-dominated, clear state and the algae-dominated, turbid state. As both loops are stabilized by positive feed-backs, the yearly switch between one or another should be very sensitive to small changes in the initial conditions. This may well explain both the high interannual variability reported for these systems, and the capacity of *R. drepanensis* to recolonize wetlands which showed no vegetation the previous years. In this model, both the climatic and the biological disturbances (represented by 'grazing' and 'trampling' in Fig.1) have an essentially similar role: they may drive the system from the 'clear' to the 'turbid' stable states.

The conceptual model presented in Fig.1 is mainly based on the field observations, together with the data provided by Van Vierssen & Martino (unpublished data) on the germination of *R. drepanensis*. However, several of our experimental data are already of importance. Firstly, the strong capacity of *R. drepanensis* for photosynthetic acclimation to low irradiances and short photosynthetic periods (see Chapter 2) clearly indicates the importance of this process for its whole life-cycle. Indeed, its capacity to quickly develop a dense-enough meadow might determine the yearly survival of the whole population. Secondly, the low reliance of *R. drepanensis* on seasonal variables (e.g., the photoperiod; see Chapter 2) to trigger the onset of reproduction seems to indicate that it is more important for its survival to closely track the hydrological dynamics (anticipated by the temperature stimulus; see Chapter 3).

The important role plaid by the submerged vegetation in the functioning of the whole system may contribute to explain the early dichotomy between the 'clear' and the 'turbid' state. Our experimental and field data suggest that both systems present a high internal stability, resulting from multiple feed-backs (Fig.2). In the macrophyte-dominated wetlands, the fast increase in biomass density of *R. drepanensis* is coupled with a decrease in



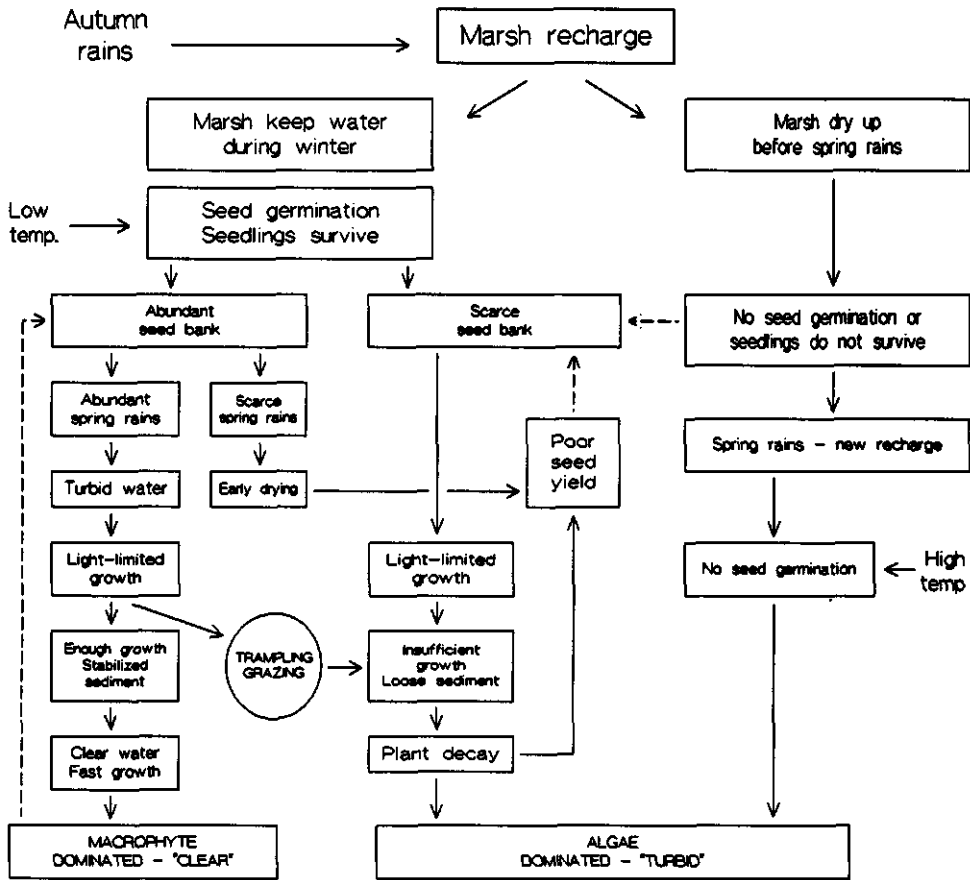


FIG.1: A conceptual model explaining the influence of seed-bank and climatic variability on the switch between the macrophyte-dominated, clear state and the algae-dominated, turbid state for the submerged ecosystems of the Doñana marsh. Arrows indicate the sequence of events, each one influencing next one. Broken arrows indicate interannual relationships (effects manifested in the next year).

bicarbonate concentration and tissue N and P content, and results in a relatively high biomass yield (see Chapter 8). Low N supply and the increasing temperatures of late spring result in an early flowering (see Chapters 3 and 5), which together with the high biomass yield (see Chapters 2 and 8) result in a high seed yield. The expenses of this high seed yield are then concomitant with a strong limitation of vegetative growth due to the low supply of C, N and P (and occasionally to periphyton shading). This causes plant senescence, which is followed by the retranslocation of nutrients from the vegetative apparatus to the seeds.

Insufficient plant growth and the subsequent prevalence of a high water turbidity, whether caused by the coincidence of an scarce seed-bank, an irregular hydrological cycle and/or biological disturbances (see Chapter 8), is here hypothesised to result in the

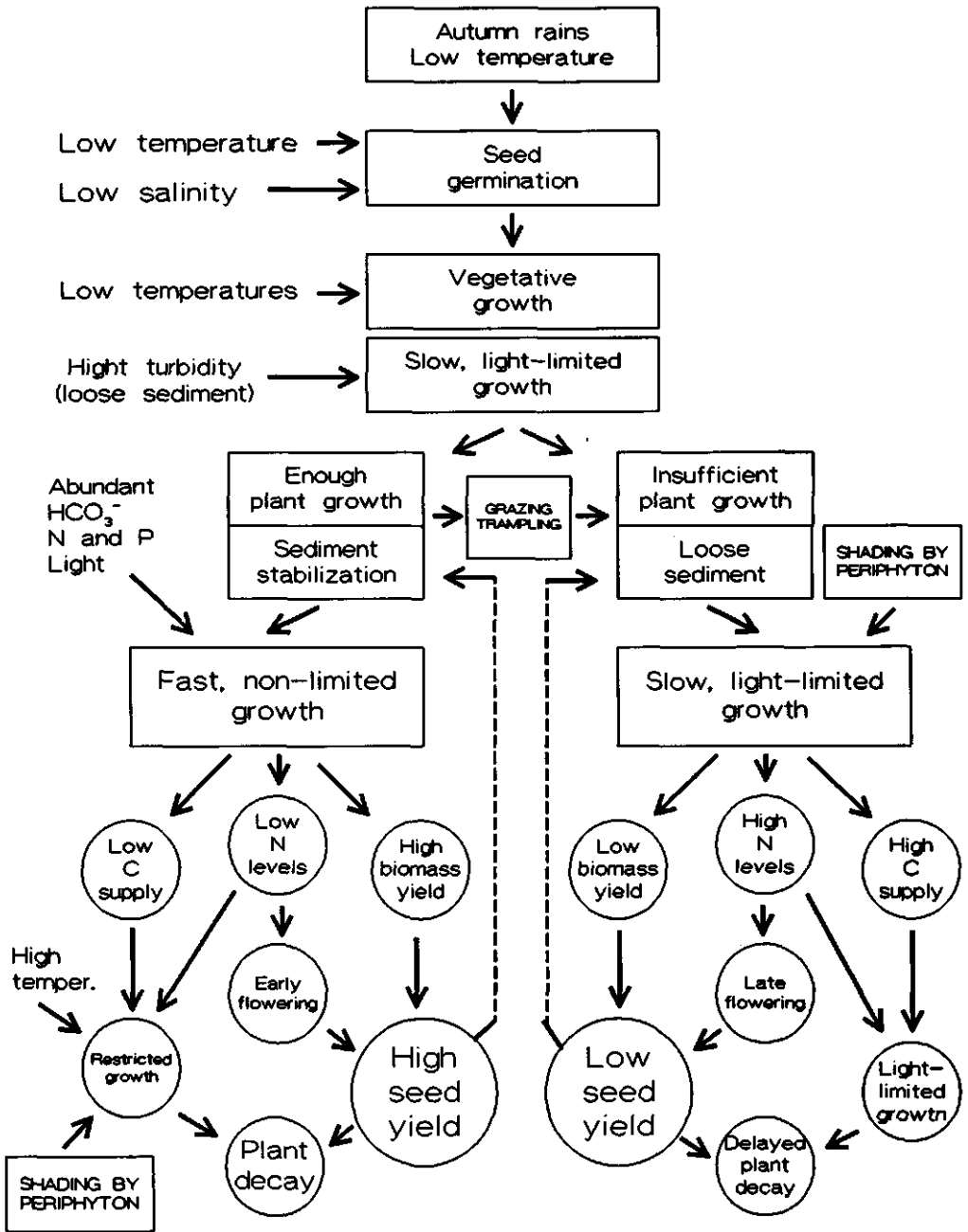


FIG.2: Several feed-backs stabilizing the two alternative stable states proposed in our conceptual model for the submerged ecosystems of the Doñana marsh (circles; squares indicate the processes previous to the dichotomy between both states). Arrows indicate the sequence of events, each one influencing the next one. Broken arrows indicate interannual relationships (effects manifested in the next year).

maintenance of light-limited plant growth. Low biomass yield follows, in spite of the photosynthetic acclimation displayed by *R. drepanensis*, due to the high metabolic costs of the acclimation processes (see Chapter 2). As both carbon concentration and tissue nitrogen content remain high, growth is not further limited. But high N levels subsequently result in a delayed flowering (see Chapter 5). Hence, the late spring temperature rise is not enough to induce flowering and reproduction, and its negative effect on the light harvesting system (see Chapter 2) increases the effect of the low light levels. Late flowering and the low biomass levels result in a poor seed yield. The limited growth and the high C, N and P postpone plant senescence. But as summer temperatures are inhibitory for flower development (see Chapter 3), even if an extended inundation cycle follows, seed yield will be poor.

Three important implications of this model will be stressed here. Firstly, I will insist in the key role plaid by the seed-bank. In a certain sense, an abundant seed bank appears to be a better indicator of the 'good health' of the macrophyte meadow than a high biomass yield. This especially applies at the end of the season, when the decay of the vegetation which accompanies an abundant seed yield results indeed in a future stabilization of the macrophyte-dominated state.

Secondly, both the long-term survival of the seed bank and the recolonization from seeds from neighbouring wetlands seem to be factors of major importance to explain the recovery of the vegetation after unfavourable years, especially taking in account the internal stability of the 'turbid' state. This is, in my view, a promising topic for further research.

Thirdly, while this model is outlined for one single species, the maintenance of a high diversity in the seed-bank may be an important mechanism for the maintenance of the macrophyte-dominated state. Differences in germination optima may result in the early dominance of different species in different years. Still, as all of them contribute to the reduction of water turbidity, positive mutualism may be postulated to be important for the maintenance of the submerged vegetation. The dominance of one species may be interpreted as negative for the accompanying ones. But I might argue that, if the dominant species would not have contributed to increase the water transparency in early spring, the accompanying species would also be absent. Hence, the role of plant diversity in increasing the stability of the submerged vegetation meadows is also suggested here as an important topic for further research.

## 5. IMPLICATIONS FOR THE MANAGEMENT OF THE DOÑANA MARSH

Three of the main variables described in our conceptual model, namely water turbidity, nutrient availability and biotic disturbances, may be affected by the management of the Doñana marsh area. In the following paragraphs, I will briefly discuss recommendations in this respect.

Firstly, water turbidity in early spring plays a key role in determining the temporal (interannual) and spatial variability in submerged vegetation abundance. The submerged macrophytes seem to be especially sensitive to prolonged periods of high water turbidity. It is presently under consideration to restore the flow of water entering the park through the Caño Guadiamar and Caño Travieso, using two pumping stations to take water from the Brazo de la Torre (see map in Chapter 1). The turbidity of the inflowing water may be critical for the submerged vegetation, especially in late spring. From a trophic point of view, a persistent flow of turbid water could be more damaging for the faunal communities of the park than a year short of water.

On the other hand, maintenance of the water levels between the peaks of rainfall occurring in autumn and late winter is also a key factor for the survival of the submerged macrophyte's seedlings. In very dry years, or when winter rains come unusually late, pumping may be beneficial for the submerged vegetation (and thus for the faunal communities feeding on them). The impact of the inflowing turbid water may then be less important, especially taking in consideration that in winter high turbidities are commonly found in the marsh.

Secondly, *R. drepanensis* postpones its flowering following an increase in nitrogen supply. Considering the short time in which the vegetation of the marsh has to complete its life-cycle, and the inhibitory effect of high temperatures on flower development (and thus on seed production), the effect of high nitrogen loads may be critical. Again, great care is recommended if the marsh will be flooded with water coming from an agricultural area. In addition to the described effect on plant reproduction, high nutrient loads have the effects known from eutrophication (periphyton shading, algal growth and macrophyte decline; see Phillips *et al.*, 1978 and De Nie, 1987). The production and faunal diversity of the whole marsh will consequently decrease.

Finally, disturbance caused by cattle trampling, bioturbation by carp, grazing by American crayfish and intensive waterfowl grazing may be significant. In unfavourable years, herbivorous waterfowl will be highly dependent on the few wetlands having aquatic vegetation. Interannual variability makes it difficult to anticipate which wetlands will have abundant vegetation in a certain year. Hence, maintaining the diversity of wetlands in the marsh in an optimal state seems to be a wise strategy to assure its sustainability as waterfowl refuge. In this sense, all other disturbances (cattle trampling, bioturbation by carp, and grazing by crayfish) may be regarded as competing with the bird fauna for the resources offered by the marsh.

Several non-herbivorous bird species may benefit from the abundance of carp and American crayfish. Stimulation of these will result in shifts in the bird fauna, and thus the diversity of the faunal communities living in the Park will change. To anticipate the consequences of any management decision, much more information than presently available is needed, especially concerning the interaction of submerged vegetation, carp and crayfish, and their impact on the trophic chain of the marsh. This seems an area particularly worthwhile to dedicate future research efforts.

## 6. ACKNOWLEDGEMENTS

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# Appendix: Summary of the field data collected in the Doñana brackish marsh

Localities and sampling periods are coded as follows: Aro: Lucio del Aro. LVA: Lucio de Vetas Altas. CSal: Canal Salinas de San Isidoro. Sacat: Sacatierra del Muro. '91: March-June 1991. '92: March-June 1992. A map with the different localities is shown in Fig.3, Chapter 7 (page 198).

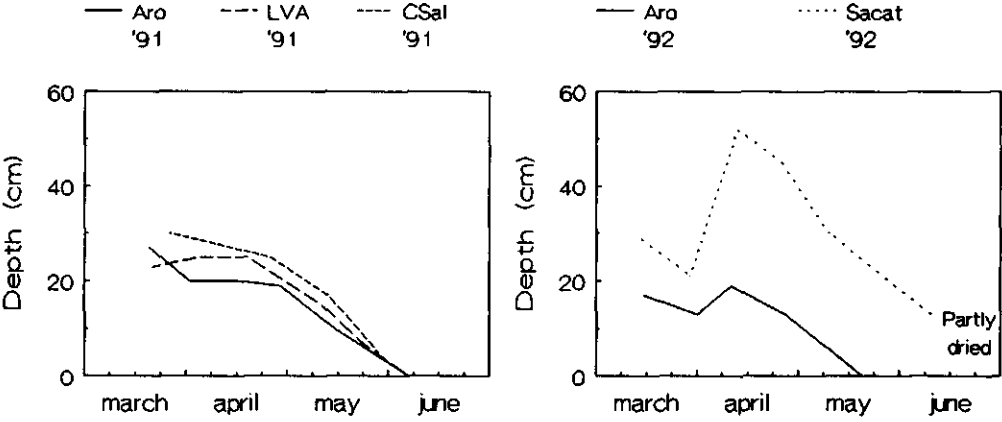


FIG.1: Depth of the area selected for biomass sampling.

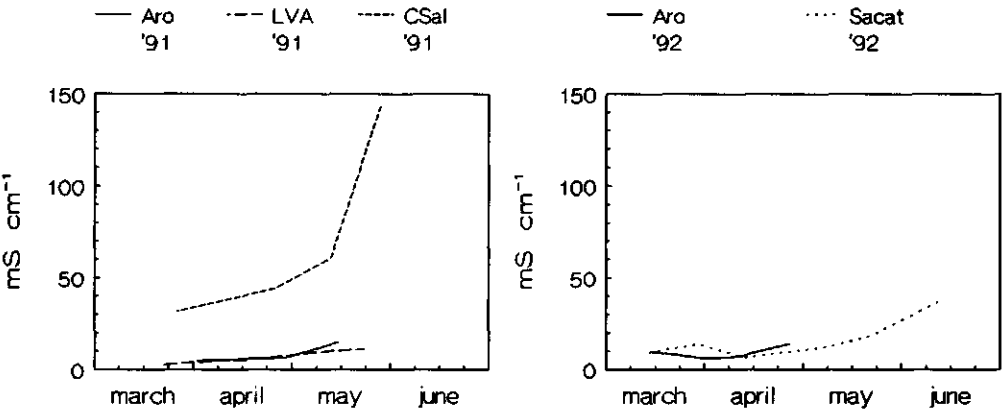


FIG.2: Electrical conductivity of the water.

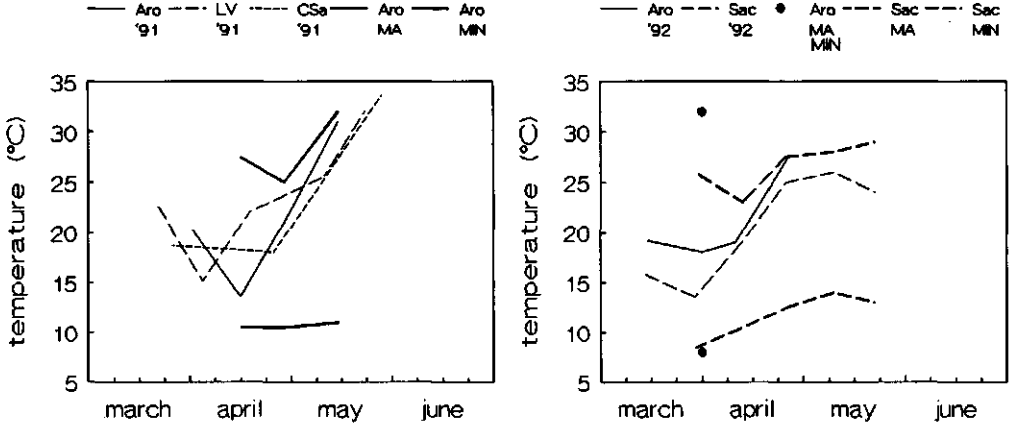


FIG.3: Water temperature. Temperature was always measured between 12:00 and 13:00. MA and MIN refer to the maximum and minimum temperature in the previous two-weeks period.

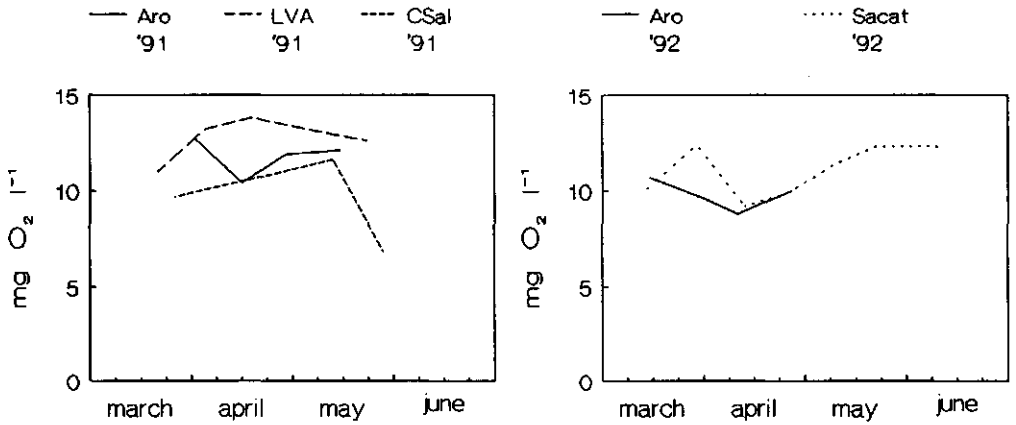


FIG.4: Dissolved oxygen concentration in the water column.

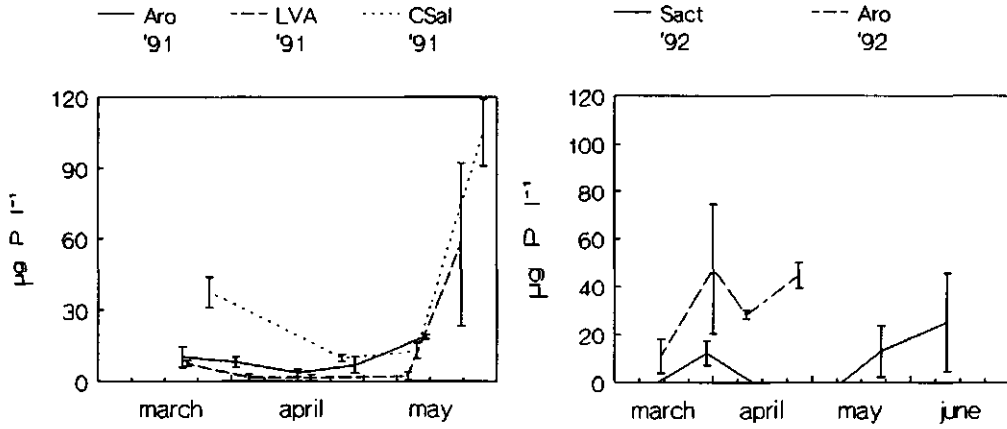


FIG.5: Dissolved orthophosphate concentration in the water column.

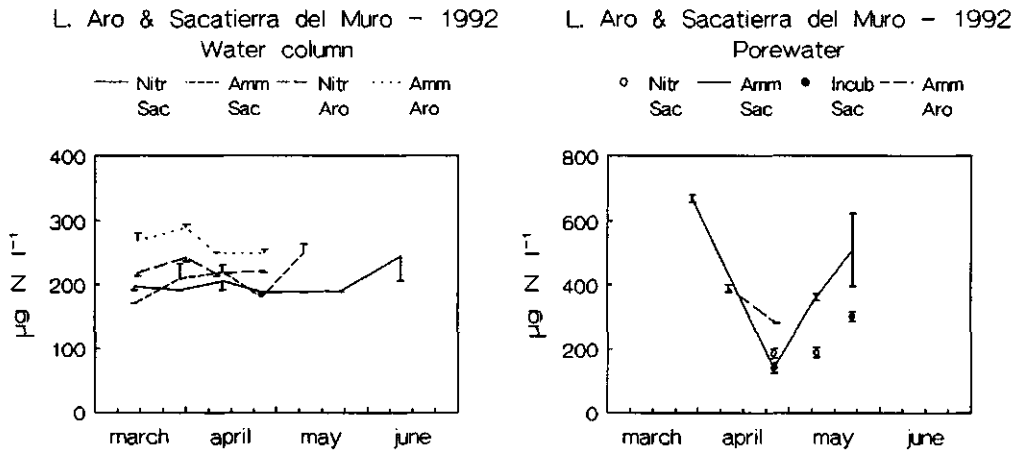


FIG.6: Dissolved nitrate and ammonia ( $\text{NH}_3 + \text{NH}_4^+$ ) concentration in the water column and porewater. Incub: ammonia content of the water after 2-weeks incubation of 20 ml sediment in 100 ml flasks filled with water from the Sacatierra (field conditions).



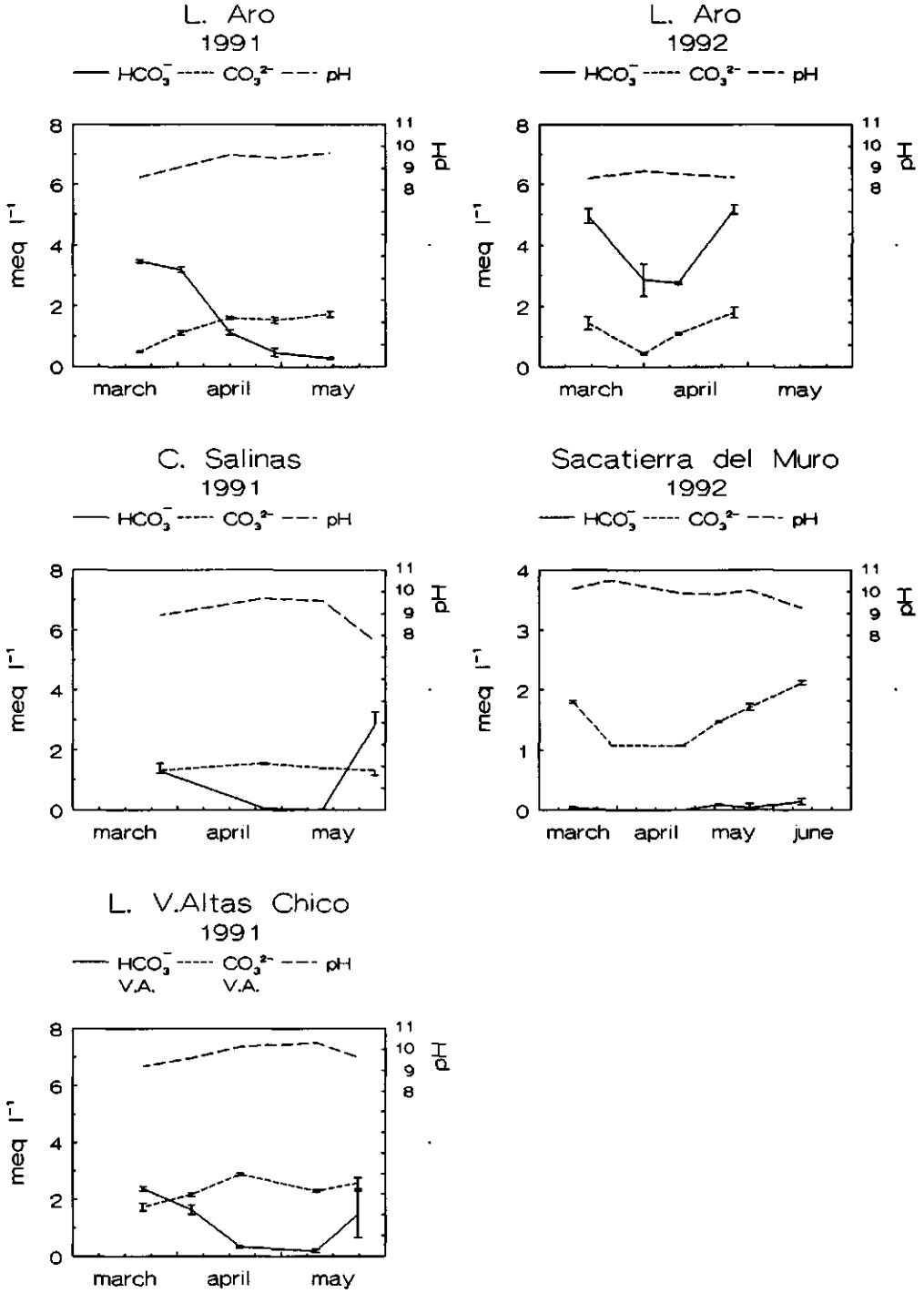


FIG.7: pH and concentration of bicarbonate and carbonate ions in the water column.

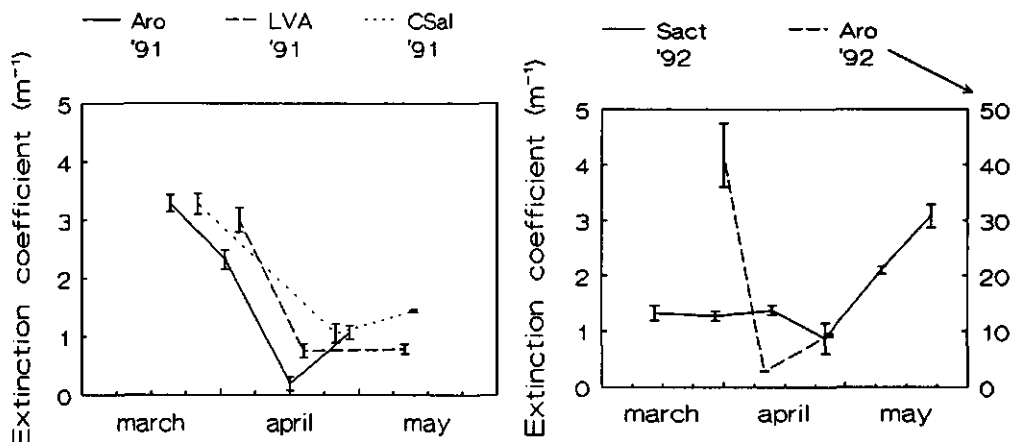


FIG.8: Light extinction coefficient of the water column.

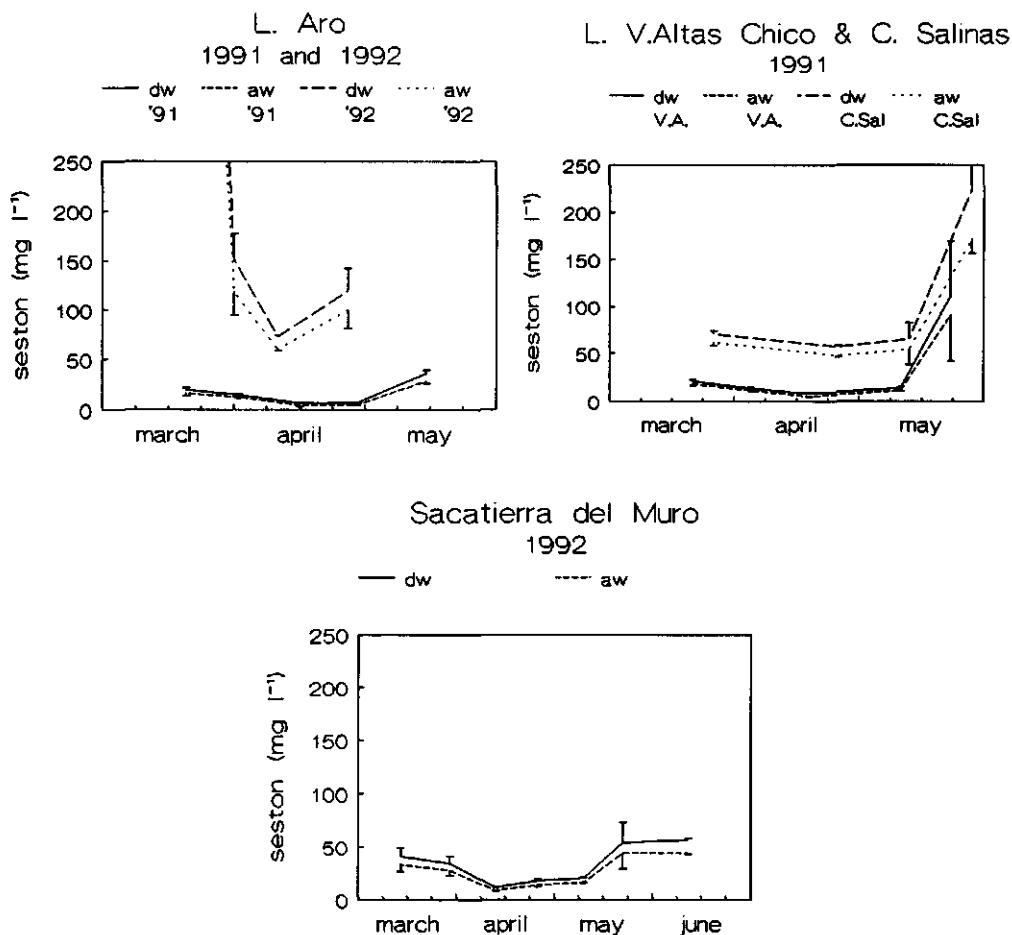


Fig.9: Concentration of seston particles (dw: dry weight; aw: ash weight) in the water column.

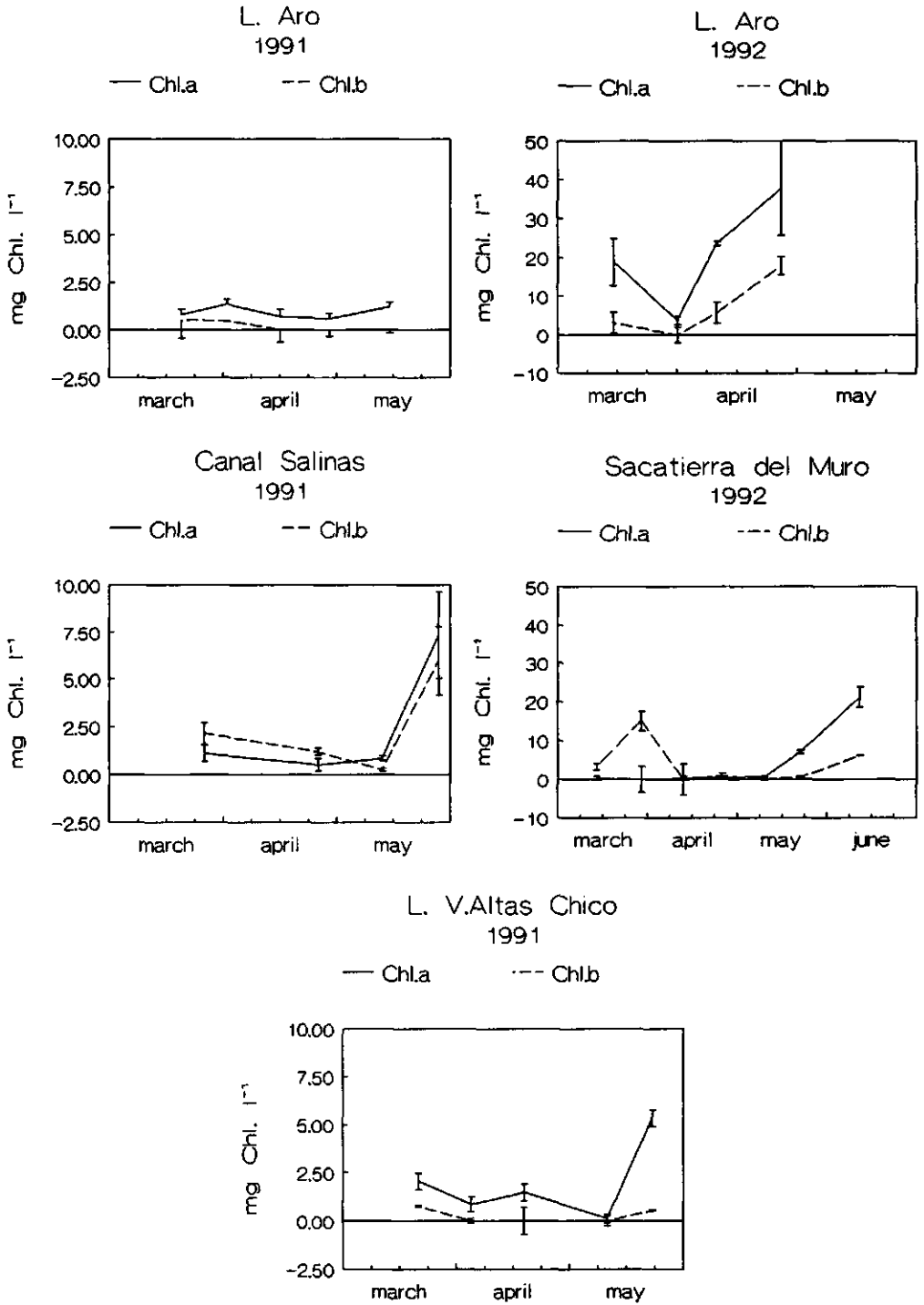


FIG.10: Chlorophyll concentration in the water column.

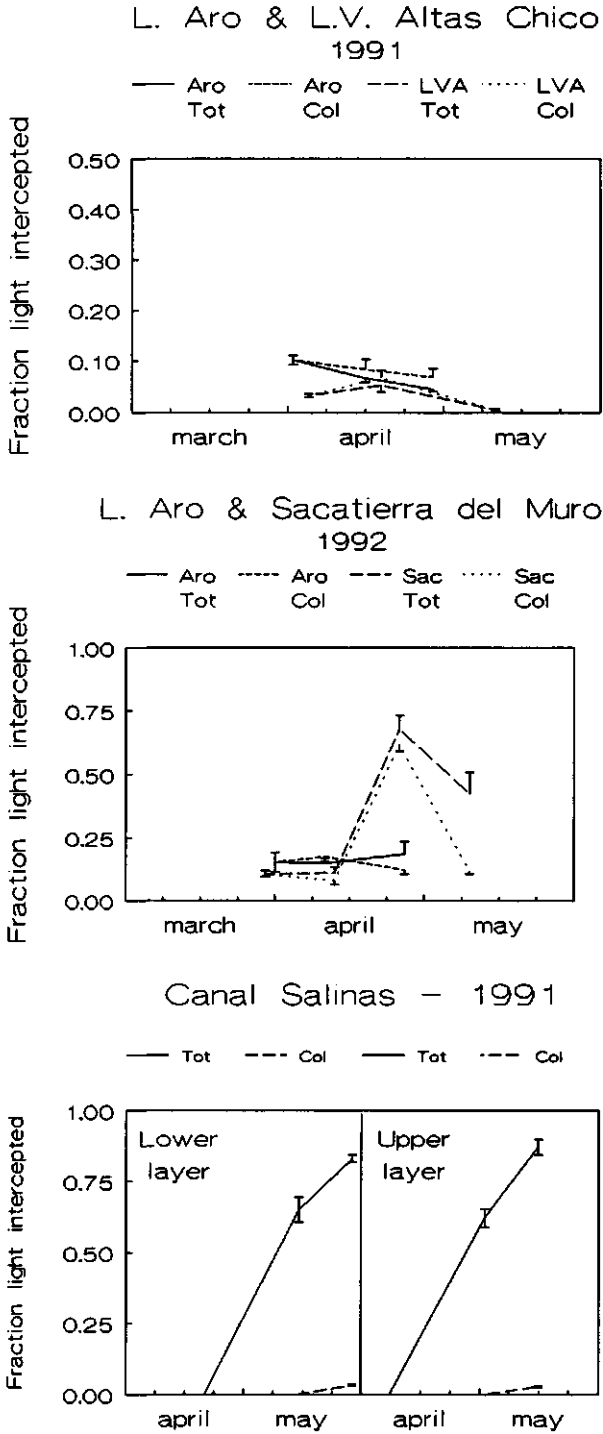
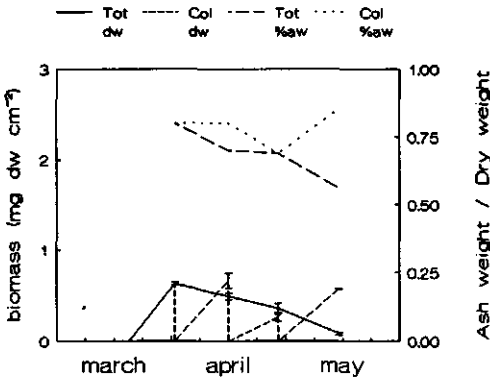
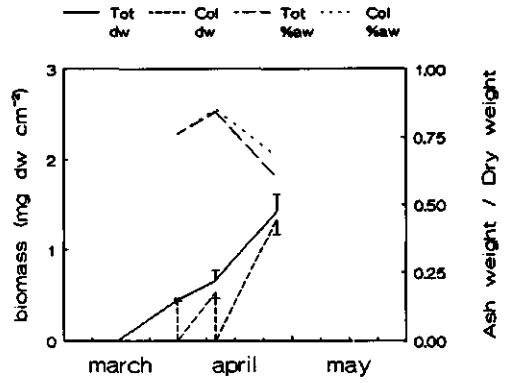


FIG.11: Attenuance caused by periphyton samples developed on microscope slides suspended horizontally in the water column. Tot: accumulative data. Col: biweekly colonization data. Lower layer (also when not specified): 10 cm from the bottom. Upper layer: 20 cm from the bottom.

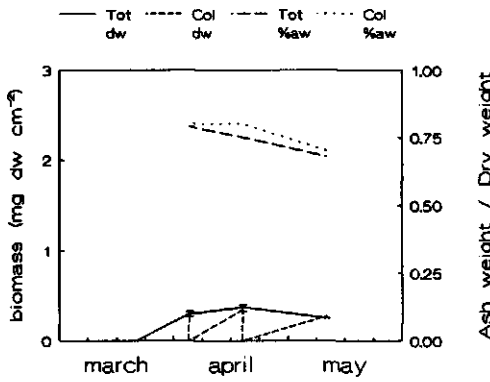
L. Aro - 1991



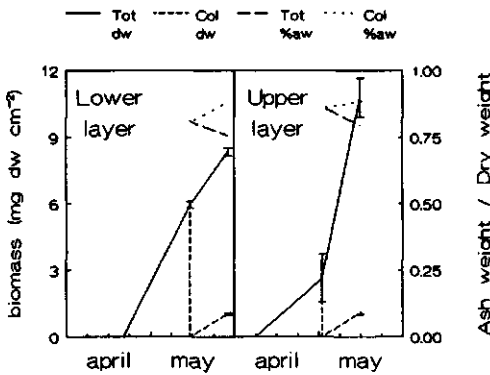
L. Aro - 1992



L. V. Altas Chico - 1991



Canal Salinas - 1991



Sacatierra del Muro - 1992

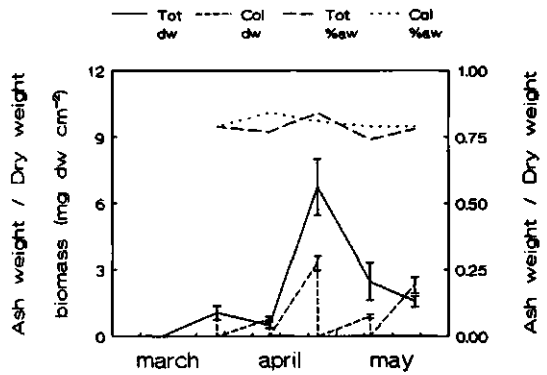
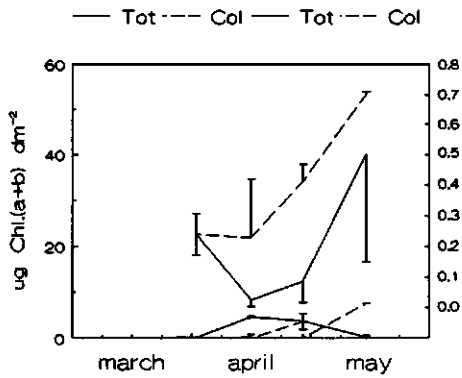
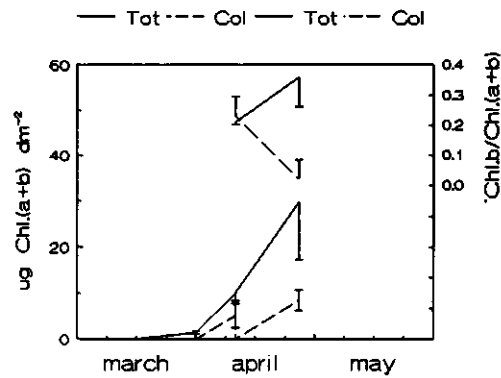


FIG.12: Periphyton biomass developed on microscope slides suspended horizontally in the water column. Tot: accumulative data. Col: biweekly colonization data. Lower layer (also when not specified): 10 cm from the bottom. Upper layer: 20 cm from the bottom.

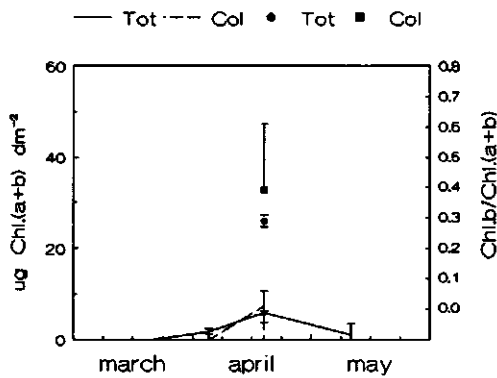
## L.Aro - 1991



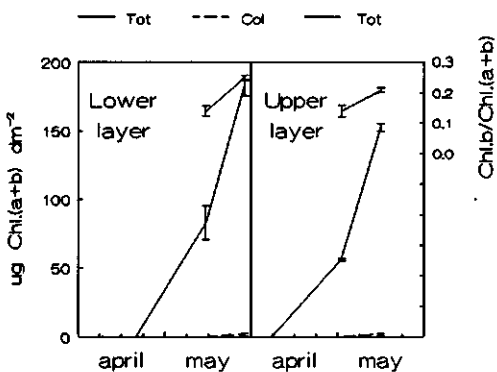
## L.Aro - 1992



## L.V. Altas Chico - 1991



## Canal Salinas - 1991



## Sacatierra del Muro - 1992

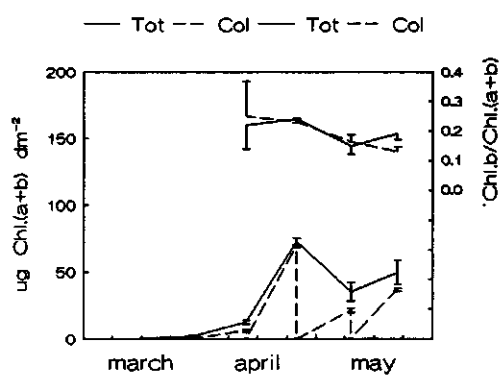


FIG. 13: Chlorophyll concentration in periphyton samples developing on microscope slides. Tot: accumulative data. Col: biweekly colonization data. Lower layer (also when not specified): 10 cm from the bottom. Upper layer: 20 cm from the bottom.



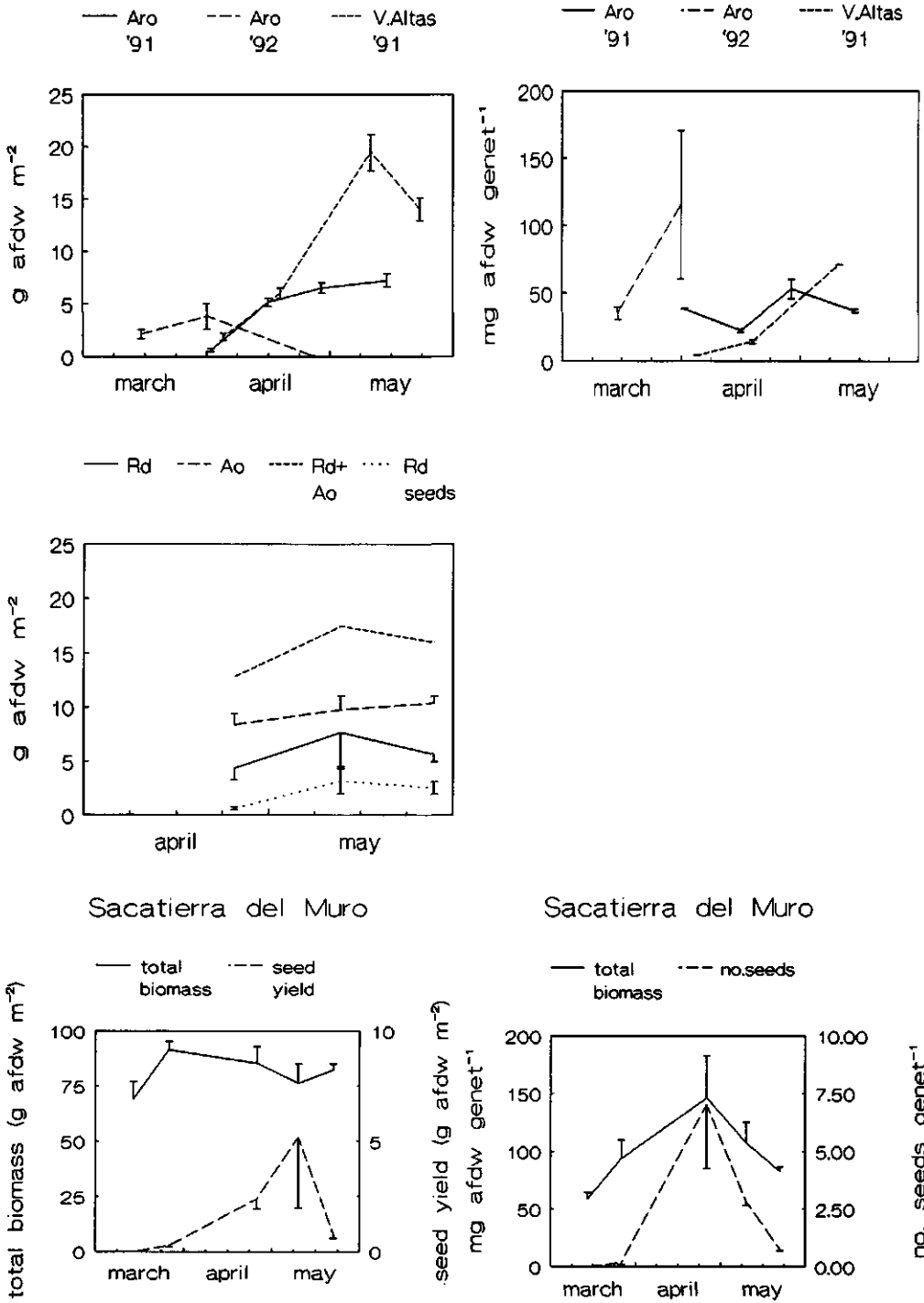
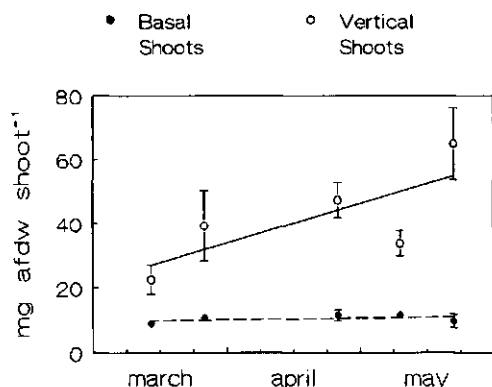


FIG.15: Biomass development of *R. drepanensis* populations.



## Sacatierra del Muro



## Sacatierra del Muro

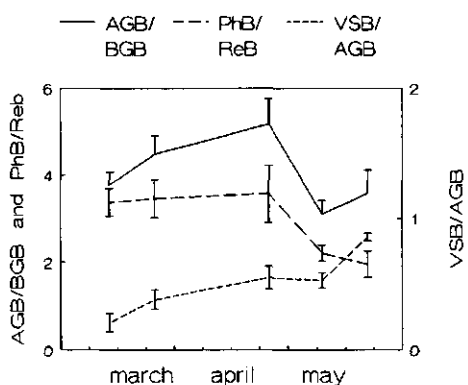
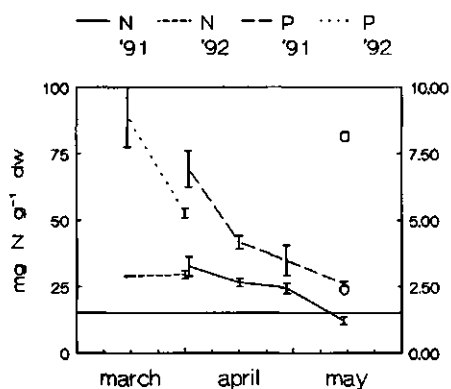
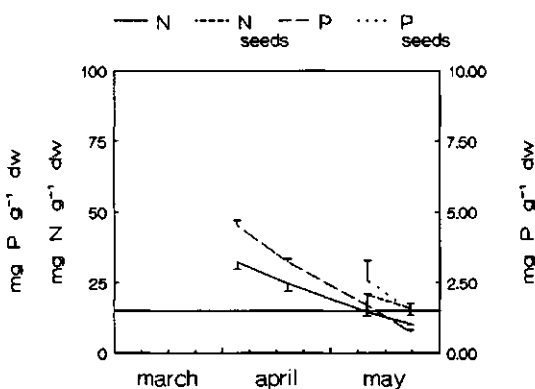


FIG.16: Development of several morphometrical variables in *R. drepanensis* plants. AGB/BGB: above- to belowground biomass ratio. PhB/ReB: 'photosynthetic' to 'respiratory' biomass ratio. VSB/AGB: fraction of the aboveground biomass allocated in vertical shoots.

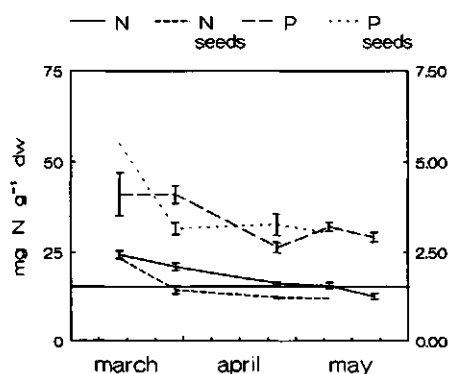
## L. Aro - 1991 and 1992



## L. V. Altas Chico - 1991



## Sacatierra del Muro - 1992



## Canal Salinas - 1991

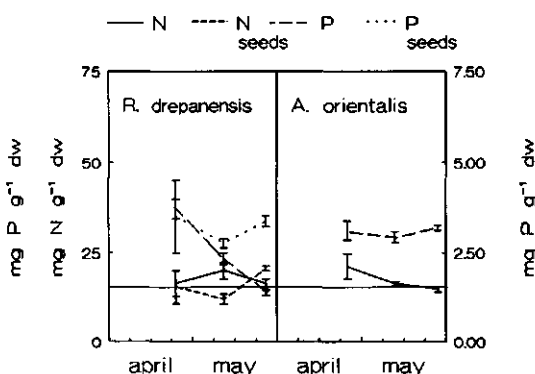


FIG.17: Nitrogen and phosphorus content in the tissue of *R. drepanensis* and *A. orientalis*.

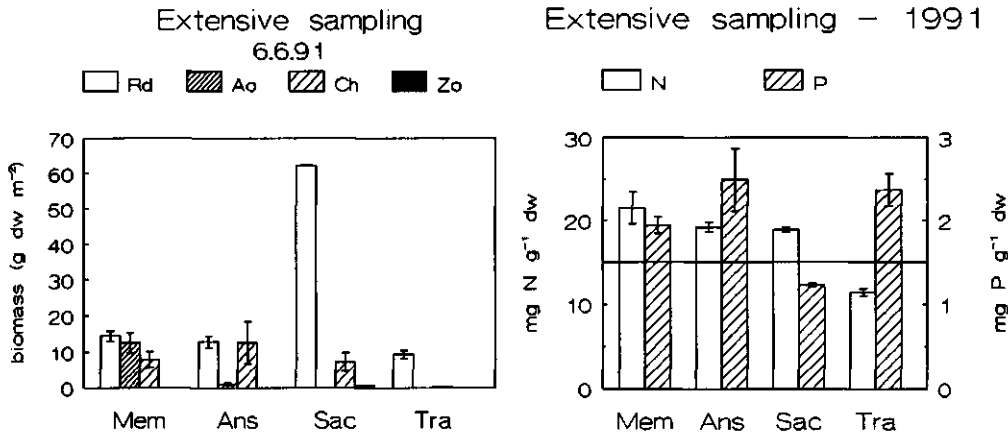


FIG.18: Community structure (as total biomass of the different species; left) and tissue nutrient content of *R. drepanensis* (right) sampled at different stations in the Doñana marsh at the end of the growing season (May-June 1991; filled squares in the map displayed in pg. 195). Mem: Lucio del Membrillo. Ans: Lucio de los Ansares. Sac: Sacatierra del Muro. Tra: Caño Travieso.

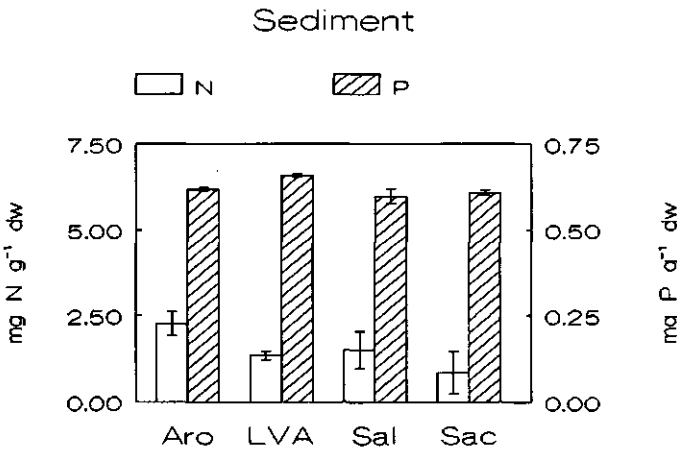
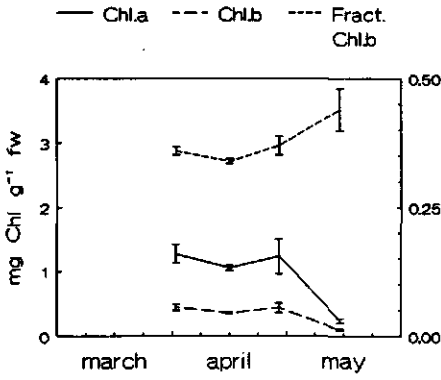
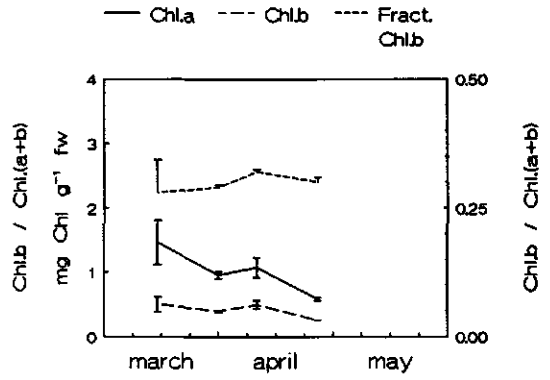


FIG.19: Total nitrogen and phosphorus concentration in the sediment (after digestion with selenium-peroxide-sulphuric acid).

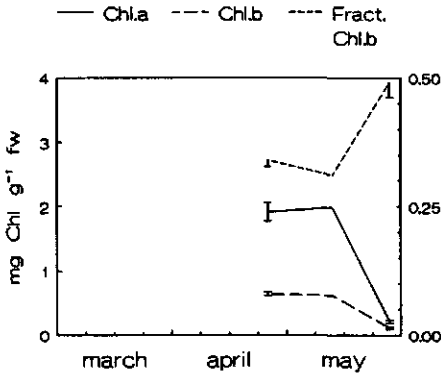
L. Aro - 1991



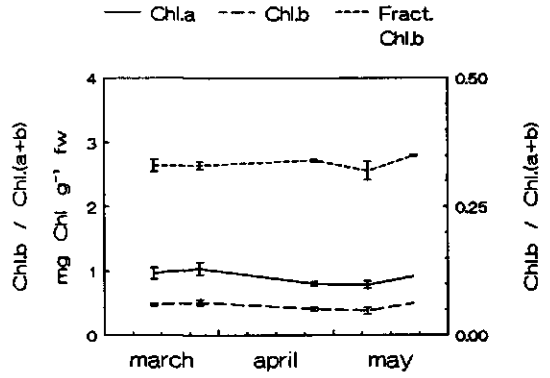
L. Aro - 1992



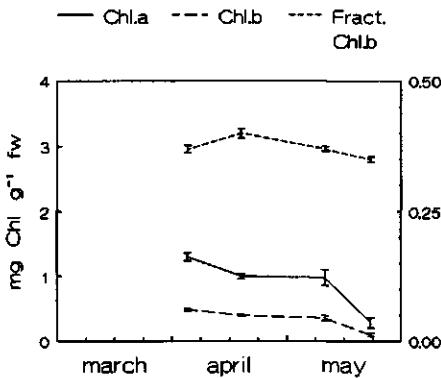
Canal Salinas  
1991



Sacatierra del Muro



L. V. Altas Chico  
1991



Extensive sampling  
26.5.91

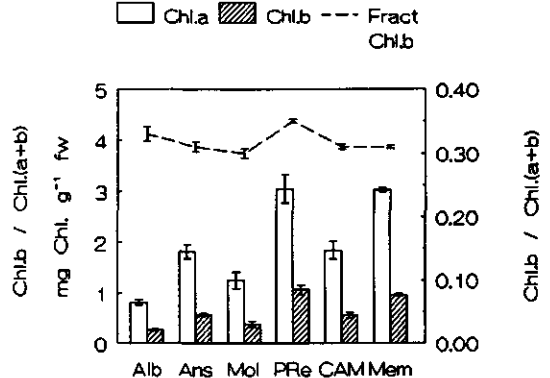


FIG.20: Leaf chlorophyll content of *R. drepanensis*. Lower right graph: localities sampled in May 1991 (note different Y-axis scale). Alb: Lucio del Albardón. Ans: Lucio de los Ansares. Mol: Lucio del Molinillo. PRe: Lucio de los Patos Reales. CAM: Canal de Aguas Minimas. Mem: Lucio del Membrillo.

## Curriculum vitae

Luis Enrique Santamaría Galdón was born 8 November 1966 in Madrid, Spain. In 1984 he received the Secondary School diploma with honours in the 'Joaquín Turina' Lyceum from Madrid, and he began to study biological sciences at the Universidad Complutense de Madrid.

In 1987 he completed the first cycle of the studies, and began his specialization in Environmental Biology in the Universidad Autónoma de Madrid (UAM). In 1989 he obtained his MSc degree. During 1988 and 1989 he worked as research trainee in the Department of Ecology, Faculty of Sciences, UAM, participating in the projects 'Salinity tolerance of three Ostracode species from the Iberian saline lakes', 'Ecology of the wetlands of Aragón' and 'Limnological bases for the restoration of the Horna old gypsum-pit', under the supervision of Prof. dr. C. Montes. The last project was awarded in 1990 with the 2nd Price of Environment by the County of Madrid.

In October-December 1989, he worked in the International Institute for Infrastructural, Hydraulic and Environmental Engineering (IHE, Delft), with a fellowship of the Dutch Cultural Cooperation Programme. The work focussed on a literature review of the ecology of Mediterranean brackish water macrophytes, and was supervised by Prof. dr. W. van Vierssen. In 1990, he began his PhD research at IHE, Delft, under the supervision of Prof. dr. W. van Vierssen and Dr. M.J.M. Hootsmans. While performing his research at IHE Delft, he participated in the academic activities of the Postgraduate Course in Environmental Science and Technology as assistant lecturer. In October-November 1992, we took part in the Postgraduate Diploma Course in Advanced Environmental Ecotechnology (IHE Delft and Wageningen Agricultural University).

In the springs of 1991 and 1992, he carried out two campaigns of field data collection in the Doñana National Park. The field work was performed within the framework of the project 'Ecological bases for an integrated management of the American swamp crayfish (*Procambarus clarkii* Girard) in the Doñana marsh', directed by Prof. dr. C. Montes (Dpt. Ecology, UAM) and funded by the Spanish Institute for Nature Conservation (ICONA).

From October 1994, he is working as scientific researcher for the IHE Delft, assigned to the EU project 'Biological management of irrigation channel problems in irrigated semi-arid agriculture'. The research includes 3 months of field data collection in Bahía Blanca, Argentina.

*Publications*

Santiago, J.M. & Santamaría, L., 1989. Daimiel sobrevive con aguas prestadas. Pasado, presente y futuro de las Tablas de Daimiel. Quercus.

Santamaría, L., Balsa, J., Bidondo, B., Baltanás, A. & Montes, C., 1992. Salinity tolerance of three Ostracode species (Crustacea: Ostracoda) from the Iberian saline lakes. *Hydrobiologia*, 246: 89-98.

Santamaría, L., Dias, C. & Hootsmans, M.J.M., 1993. The influence of ammonia on the growth and photosynthesis of *Ruppia drepanensis* Tineo from the Doñana National Park (SW Spain). *Hydrobiologia*, 275/276: 219-231.

Santamaría, L., Hootsmans, M.J.M., Montes, C. & Vierssen, W. van, in press. Effect of bicarbonate availability on the primary production of *Ruppia drepanensis* Tineo from a Mediterranean brackish marsh. *Verh. Internat. Verein. Limnol.*



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\*) 2 included as of October '95