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# Experiments with duckweed–moth systems suggest that global warming may reduce rather than promote herbivory

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## SUMMARY

1. Wilf & Labandeira (1999) suggested that increased temperatures because of global warming will cause an increase in herbivory by insects. This conclusion was based on the supposed effect of temperature on herbivores but did not consider an effect of temperature on plant growth.
2. We studied the effect of temperature on grazing pressure by the small China-mark moth (*Cataclysta lemnata* L.) on *Lemna minor* L. in laboratory experiments.
3. Between temperatures of 15 and 24 °C we found a sigmoidal increase in *C. lemnata* grazing rates, and an approximately linear increase in *L. minor* growth rates. Therefore, an increase in temperature did not always result in higher grazing pressure by this insect as the regrowth of *Lemna* changes also.
4. At temperatures below 18.7 °C, *Lemna* benefited more than *Cataclysta* from an increase in temperature, causing a decrease in grazing pressure.
5. In the context of global warming, we conclude that rising temperatures will not necessarily increase grazing pressure by herbivorous insects.

*Keywords*: *Cataclysta*, grazing, herbivory, *Lemna*, temperature

## Introduction

Duckweeds (Lemnaceae) are often abundant in ditches and ponds (Landolt, 1986). Especially when nitrogen and phosphorus concentrations in the water column are high, the surface area can become covered with dense floating mats of duckweed (Lüönd, 1980, 1983; Portielje & Roijackers, 1995). These mats have large impacts on freshwater ecosystems, restricting oxygen supply (Pokorny & Rejmánková, 1983), light availability of algae and submerged macrophytes (Wolek, 1974) and temperature fluxes (Dale & Gillespie, 1976; Landolt, 1986; Goldsborough, 1993). These changed conditions often have a negative effect on the biodiversity of the ecosystem (Janse & van Puijenbroek, 1998). Other free-floating plants such as red water fern (*Azolla filiculoides*), water hyacinth (*Eichhornia crassipes*) and water lettuce (*Pistia stratiotes*) often cause serious problems in tropical and subtropical regions (Mehra *et al*., 1999; Hill, 2003).

Various species of herbivorous insects consume free-floating macrophytes. Several species of weevils (Coleoptera: Curculionidae) are able to consume large amounts of red water fern, water hyacinth and water lettuce (Cilliers, 1991; Hill & Cilliers, 1999; Aguilar *et al*., 2003), while the larvae of the semi-aquatic Small China-mark moth (*Cataclysta lemnata*) are capable of removing large parts of floating cover of Lemnaceae covers (Wesenberg-Lund, 1943). Duckweed is not only used as food source, but also as building material

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for the cases for the larvae and pupae (Petrischak, 2000). The moth lays eggs on the undersides of duckweeds. After approximately 1 week caterpillars hatch and they immediately start building cases to protect their bodies. After a few weeks pupation takes place in floating cases of duckweed fronds.

In the last century the earth's climate has warmed by 0.6 °C (Houghton *et al*., 2001). As temperature is one of the most important factors controlling biological rates (Cossins & Bowler, 1987; Gillooly *et al*., 2002) and especially the development of insects (Saunders, 1982; Huffaker & Gutierrez, 1999; Bale *et al*., 2002), it can be expected that these changes will also influence the grazing pressure of herbivorous insects on floating macrophytes. In general, the diversity of herbivorous insects and their grazing pressure on plant hosts increases with decreasing latitude, suggesting a higher grazing pressure with rising temperatures (Wilf & Labandeira, 1999). As the grazing pressure of modern terrestrial insects generally increases with decreasing latitude, Wilf & Labandeira (1999) hypothesised that at a constant latitude grazing pressure of these insects also increases with rising temperatures. This hypothesis was confirmed in their study of the fossil record in south-western Wyoming. However, the effect of rising temperatures on the grazing pressure of a single aquatic insect species on its plant host has never been thoroughly investigated.

We tested the hypothesis that rising temperature increases the grazing pressure of *C. lemnata* L. on *L. minor* L. by conducting grazing experiments in the laboratory at different temperatures.

## Materials and methods

### *Experiments*

In the laboratory, a population of *C. lemnata*, collected for the field near Wageningen (The Netherlands), was bred for several generations in 20-L aquaria under controlled conditions (temperature 25 ± 1 °C, day–night cycle 14–10 h, light intensity of 180 μmol $m^{-2}$ $s^{-1}$). Larvae of *C. lemnata* were fed with *L. minor*, grown in the laboratory at 25 °C and a constant irradiation of 180 μmol $m^{-2}$ $s^{-1}$. Both *L. minor* and *C. lemnata* larvae were cultivated on the medium described by Szabo, Roijackers & Scheffer (2003).

To determine the consumption rate and biomass increase in larvae of *C. lemnata* a laboratory experiment was conducted in climate chambers at five different water temperatures (15, 19, 24, 28 and 33 °C). In the climate chambers, a day–night cycle of 14–10 h and a light-intensity of 85 μmol $m^{-2}$ $s^{-1}$ was maintained.

The dry weights (g DW; 24 h at 60 °C) of 10 randomly-selected third stage larvae (according to Petrischak, 2000) and their cases were determined at the start of the experiment. The remaining larvae (approximately 500) were randomly divided between the five different temperature regimes in 20-L aquaria. After an acclimatisation period of 24 h at each temperature, 14 round enclosures (surface area of 25 $cm^2$) were filled with 0.300 g (0.021 g dry weight) of *L. minor*, covering about 75% of the surface area. The enclosures were placed in 2-L aquaria with acclimatised medium. Ten randomly selected larvae were then moved to 10 of the enclosures, while the remaining four enclosures were used as controls to determine the growth rate of *L. minor* at the different temperatures. After 2 days the dry weight of *L. minor*, the larvae and the cases was determined again.

### *Statistical analysis*

Data sets smaller than 50 samples were tested for normality using the Shapiro-Wilk test; larger datasets were analysed with the Kolmogorov–Smirnov test. Normally distributed datasets ($P \geq 0.05$) were tested using one-way analysis of variance (ANOVA) and further analysed with either Tukey's HSD *post hoc* test (equal variances; $P \geq 0.05$) or Tamhane's T2 *post hoc* test (variances not equal; $P < 0.05$). For the *post hoc* tests a significance level of 0.05 was used. When data sets were not normally distributed ($P < 0.05$), they were analysed using Kruskal–Wallis and Mann–Whitney *U*-tests, for the latter using a significance level of 0.005 (0.05/10).

### *Modelling*

Increase in the larval biomass as well as in the dry weight of the cases was estimated by calculating the difference between the mean values at the start of the experiment and the mean values at the end of the experiment.

The consumption rate of the larvae in the experiment was assessed using the following simple equation

(eqn 1). As the time scale in the experiments was relatively short (2 days), we assumed that the consumption rate of the larvae (C in g DW day$^{-1}$) was approximately constant. Furthermore, we assumed that *L. minor* (*Y* in g DW) grew exponentially during the period of the experiment:

$$\frac{dY}{dt} = \mu Y - C \quad (1)$$

This model can be solved as:

$$Y_1 = \frac{C}{\mu} + \left(Y_0 - \frac{C}{\mu}\right)e^{\mu t} \quad (2)$$

in which $Y_0$ is the initial amount of *L. minor* (g DW), μ is the relative growth rate per day and *t* time in days. The relative growth rate (μ) at the different temperatures was estimated by calculating the mean growth rates for the controls, where the consumption rate *C* was zero.

The fraction of the consumed *L. minor* used for the cases was obtained by dividing the mean weight increase in the cases (g DW day$^{-1}$) by the mean total consumption of the larvae. Efficiency of the larvae was calculated by dividing the mean biomass increase in the larvae by the mean total consumption rate (*C*) of *Lemna* minus the mean weight increase in the cases.

Using regression, the effect of temperature on the consumption rate and the larval biomass increase were modelled, using two different functions.

For describing the effect of temperature on the consumption rate (*C*), the sigmoid-shaped Hill-function was used (Hill, 1910):

$$C(T) = \frac{C_{max} T^n}{T_h^n + T^n} \quad (3)$$

where $C_{max}$ is maximum consumption rate (g DW day$^{-1}$), *T* is the rearing temperature (°C), $T_h$ is the temperature where 50% of the maximum consumption is reached (°C) and *n* is an empirical constant determining the shape of the curve.

To describe the temperature effect on the larval biomass increase, the Brière-1-function (Brière *et al.*, 1999) was used. While this function is most often used for describing the temperature-dependent development rate of insects (day$^{-1}$), it is here used to describe the temperature-dependent biomass increase (*B*) (g DW day$^{-1}$):

$$B(T) = aT(T - T_0)(T_L - T)^{1/2} \quad (4)$$

Here *T* is the rearing temperature (°C), $T_0$ is the low temperature development threshold (°C), $T_L$ is the lethal temperature threshold (°C) and *a* is an empirical (scaling) constant.

In order to explore changes in grazing pressure with temperature, the sensitivity of both the larval consumption rate and the growth rate of *L. minor* to changes in temperature were determined.

Sensitivity of the larval consumption rate is described by the first derivative of eqn 3, scaled to percentages of the maximum performance. Based on Landolt & Kandeler (1987) the growth capacity of *L. minor* can be described by a linear equation in a wide range of temperatures (from the minimum temperature, 5 °C, to the optimum temperature, 26 °C). The slope of this equation is the sensitivity of *L. minor* to changing temperatures.

## Results

### *Experimental results*

The growth rate of *L. minor* (Fig. 1) was highest at 24 °C. ANOVA showed significant differences among the temperatures ($F_{4,15} = 10.96$, $P < 0.001$). Tukey's *post hoc* test demonstrated that the growth rate of *L. minor* differed significantly for the two lowest temperatures in comparison with 24 °C.

Differences between the temperatures were significant for *C. lemnata* larval biomass increase (Kruskal–Wallis test: $\chi^2 = 39.809$, d.f. = 4, $P < 0.001$). Mann–

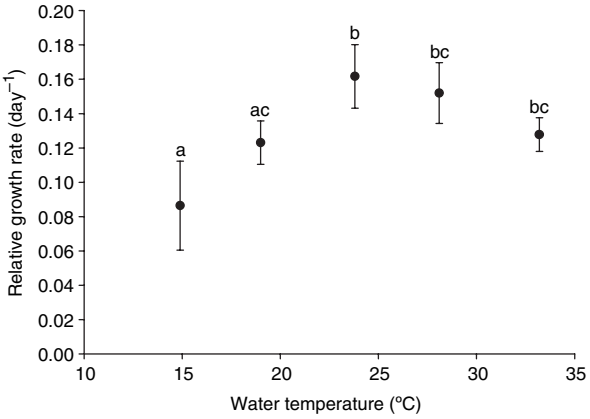


**Fig. 1** Effect of temperature on the relative growth rate of *L. minor*. Error bars ±1 SD. Different letters indicate significant differences ($P < 0.05$).

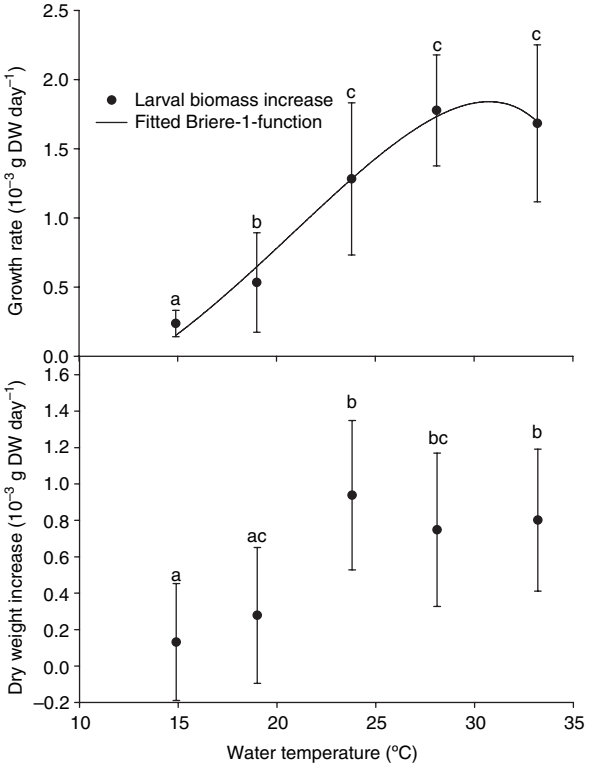


**Fig. 2** Effect of temperature on the growth rate of *C. lemnata* larvae (top graph) and on the weight increase in the *C. lemnata* larval cases (bottom graph). Error bars ±1 SD. $R^2$ of the fitted Brière-1-function: 0.62. Fitted parameters: $a = 1.465\ 10^{-6}$; $T_0 = 13.4$ °C; $T_L = 36.3$ °C; optimum at 28.7 °C. Different letters indicate significant differences ($P < 0.05$).

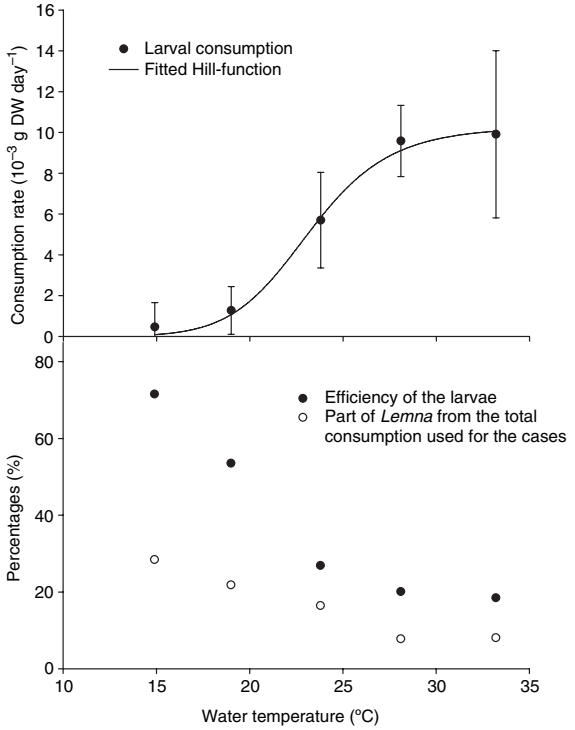


**Fig. 3** Effect of temperature on the consumption rate of *C. lemnata* larvae (top graph) and on the efficiency of *C. lemnata* larvae and the fraction of total consumption of *L. minor* used for the cases (bottom graph). Error bars ±1 SD. $R^2$ of the fitted Hill-function: 0.67. Fitted parameters: $C_{max} = 0.0103$; $T_h = 23.2$; $n = 10{,}76$. Different letters indicate significant differences ($P < 0.05$).

Whitney *U*-tests demonstrated that the two lowest temperatures differed significantly from the three highest temperatures as well as from each other (Fig. 2, top).

The increase in weight of larval cases was higher for the three highest temperatures compared with 15 and 19 °C (Fig. 2, bottom). ANOVA indicated significant differences between the temperatures ($F_{4,55} = 9.96$, $P < 0.001$) and Tukey's *post hoc* test separated 15 and 19 °C from 24 and 33 °C.

ANOVA showed that the estimated consumption rate differed significantly between the temperatures ($F_{4,65} = 32.03$, $P < 0.001$). Tamhane's *post hoc* test divided the data into three groups, with the two lowest temperatures in the first group, 24 °C as a standalone and the two highest temperatures in the latter group. The particularly high variance at 33 °C suggests that tolerance of individual larvae to this temperature varied substantially (Fig. 3, top).

The fraction of *L. minor* used for the cases dropped rapidly with rising temperatures, from 28% at 15 °C to approximately 8% at 28 and 33 °C (Fig. 3, bottom). At 15 °C the efficiency of the larvae proved to be extremely high (Fig. 5), with over 71% of the ingested *L. minor* was converted into body weight. However, at 28 and 33 °C the efficiency dropped to 18–20%, indicating an increase in metabolic rate of the larvae with rising temperatures.

### *Modelling*

The Brière-1-function described the relation between larval growth and temperature quite well (Fig. 2, top). The function fitted the minimum temperature threshold ($T_0$) at 13.4 °C and the lethal temperature threshold ($T_L$) at 36.3 °C. Maximum performance of the larvae was estimated at 28.7 °C. The fitted Hill-function predicted a maximum consumption rate ($C_{max}$) of 0.0103 g DW day$^{-1}$ (Fig. 3, top). At 23.2 °C

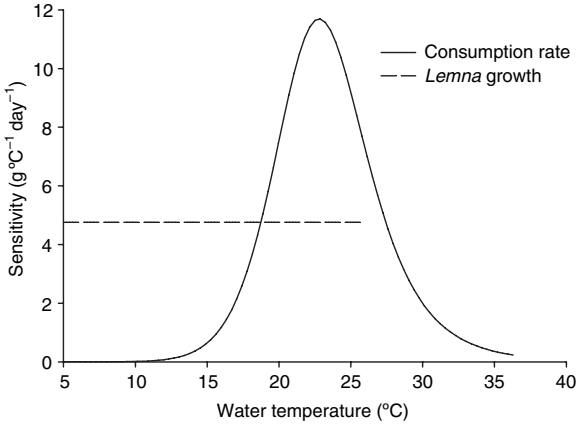


**Fig. 4** Sensitivity of *C. lemnata* larvae and *L. minor* to rising temperatures, as described by the scaled derivatives of the *L. minor* growth rate and larval consumption capacity. Intersection of the two functions at 18.7 °C.

the consumption rate of the larvae was at 50% of the maximum performance ($T_h$).

The functions plotted in Fig. 4 show the sensitivities of the *L. minor* growth rate and *C. lemnata* consumption rate to changes in temperature. According to Landolt & Kandeler (1987), the slope of the linear equation of *L. minor* was 4.76 $°C^{-1}$ $day^{-1}$, which is comparable with the value we obtained from our own data (4.67 $°C^{-1}$ $day^{-1}$). The intersection of the two functions is at 18.7 °C. This means that for temperatures higher than 18.7 °C, the advantage of an increase in temperature is higher for the *C. lemnata* larvae than for *L. minor*.

## Discussion

Based on a palaeontological survey of two climatologically different eras, Wilf & Labandeira (1999) concluded that grazing pressure by insects on their plant hosts generally increases with rising temperatures induced by global warming or decreasing latitude. We showed that the consumption rate of the *C. lemnata* larvae indeed increased with rising temperatures. However, as the growth of *L. minor* increased also, we showed that at lower temperatures (below 18.7 °C) the plant host benefited more from an increase in temperature than the herbivorous insect. Hence our results suggested that at least for *L. minor* and *C. lemnata*, the general conclusion of Wilf & Labandeira (1999) does not hold. Whether grazing pressure increases or decreases seems to be dependent on the conditions, as the plant host and the herbivorous insect may benefit differently from rising temperatures.

As for most other species of Lemnaceae the growth rate also increases linearly with temperature (Landolt & Kandeler, 1987), this phenomenon will not be restricted to grazing on *L. minor*. In Europe, the distribution of *C. lemnata* extends across the continent from Scandinavia and Russia to Spain and Italy (Illies, 1978), and covers the isotherm of 19 °C. Therefore, rising temperatures will not necessarily mean that grazing pressure of *C. lemnata* on Lemnaceae will increase. Predictions by climate scenarios of warming vary per region from 0.1 to 0.4 °C by decade (McCarthy *et al.*, 2001) and for summer periods from 0.08 to 0.6 °C per decade. Thus changes in grazing pressure of *C. lemnata* on Lemnaceae will differ per region, depending upon the prevailing temperatures and on how much these temperatures will rise in the future.

Our study showed that *L. minor* is able to develop at much lower temperatures than *C. lemnata*, indicating that *L. minor* can start its development earlier and extend it further into the season. The same is true when comparing other Lemnaceae to the temperature threshold of *C. lemnata*. Rejmánková (1973) reported growth of *L. minor* in the field from April to November, whereas *C. lemnata* was active from May till September (Petrischak, 2000). From an evolutionary perspective, the suggestion that development of *C. lemnata* starts later in the season compared with most Lemnaceae is very plausible. By starting their development later, their primary food source will most probably not be limiting. Possibly, rising temperatures because of global warming will also affect this balance. The rising temperatures will cause larvae of *C. lemnata* to start developing earlier in the season, but the same will be true for most *Lemna* species. How this balance will change with rising temperatures is currently hard to predict.

In our experiments temperature was kept constant, but in the field it fluctuates strongly, especially within mats of Lemnaceae. Vertical temperature gradients are observed within the mats (Dale & Gillespie, 1976; Goldsborough, 1993). This will affect both the growth of Lemnaceae and the development of the larvae of *C. lemnata*. While temperature is the main factor controlling growth of Lemnaceae in the field (Rejmánková, 1973), it is not the only factor. Nutrient supply (Lüönd, 1980, 1983; Portielje & Roijackers, 1995), light-conditions (Wolek, 1974), currents (Landolt, 1986) and competition with other macrophytes

(Landolt, 1986; Scheffer *et al*., 2003) all influence the growth rate of Lemnaceae in the field.

In summary, we showed that rising temperatures will not necessarily cause an increase in grazing pressure, as predicted by Wilf & Labandeira (1999). The impact of grazing is not only dependent on the grazing rate of the herbivorous insect, but also on the growth rate of the plants, and both can be affected differently by temperature. In the case of the interaction between Lemnacea and *C. lemnata*, this implies that at low latitudes the effect of global warming is the opposite of the effect at higher latitudes.

## References


Aguilar, J.A., Camarena O.M., Center T.D. & Bojórquez G. (2003) Biological control of water hyacinth in Sinaloa, Mexico with the weevils *Neochetina eichhorniae* and *N. bruchi*. *Biocontrol*, **48**, 595–608.

Bale J.S., Masters G.J., Hodkinson I.D. *et al.* (2002) Herbivory in global climate changes research: direct effects of rising temperature on insects herbivores. *Global Change Biology*, **8**, 1–16.

Brière J.F., Pracros P., Roux A.Y. & le Pierre J.S. (1999) A novel rate model of temperature-dependent development for arthropods. *Environmental Entomology*, **28**, 22–29.

Cilliers C.J. (1991) Biological control of water lettuce, *Pistia stratiotes* (Araceae), in South Africa. *Agriculture, Ecosystems and Environment*, **37**, 225–229.

Cossins A.R. & Bowler K. (1987) *Temperature Biology of Animals*. Chapman and Hall, London.

Dale H.M. & Gillespie T. (1976) Influence of floating vascular plants on diurnal fluctuations of temperature near water surface in early spring. *Hydrobiologia*, **49**, 245–256.

Gillooly J.F., Charnov E.L., West G.B., Savage V.M. & Brown J.H. (2002) Effect of size and temperature on developmental time. *Nature*, **417**, 70–73.

Goldsborough L.G. (1993) Diatom ecology in the phyllosphere of the common duckweed (*Lemna minor* L.). *Hydrobiologia*, **269/270**, 463–471.

Hill A. (1910) The possible effects of the aggregation of the molecules of haemoglobin on its dissociation curves. *Journal of Physiology*, **40**, 4–7.

Hill M.P. (2003) The impact and control of alien aquatic vegetation in South African aquatic ecosystems. *African Journal of Aquatic Science*, **28**, 19–24.

Hill M.P. & Cilliers C.J. (1999) *Azolla filiculoides* Lamarck (Pteridophyta: Azollaceae), its status in South Africa and control. *Hydrobiologia*, **415**, 203–206.

Houghton J.T., Ding Y., Griggs D.J., Noguer M., Van der Linden P.J., Dai X., Maskell K. & Johnson C.A. (2001) *Climate Change 2001: The Scientific Basis. Third Assessment Report of the Intergovernmental Panel on Climate Change. IPCC*, Cambridge University Press, Cambridge.

Huffaker C.B. & Gutierrez A.P. (1999) *Ecological Entomology*, 2nd edn. Wiley, New York.

Illies J. (1978) *Limnofauna Europaea: eine Zusammenstellung aller die europäischen Binnengewässer bewohnenden mehrzelligen Tierarten mit Angaben über ihre Verbreitung und Ökologie*. Fisher, Stuttgart.

Janse J.H. & van Puijenbroek P.J.T.M. (1998) Effects of eutrophication in drainage ditches. *Environmental Pollution*, **102** (Suppl. 1), 547–552.

Landolt E. (1986) The family of Lemnaceae – a monographic study (vol. 1). *Veröffentlichung der Geobotanischen Institut ETH, Stiftung Rübel, Zürich*, **95**, 1–566.

Landolt E. & Kandeler R. (1987) The family of Lemnaceae – a monographic study (vol. 2). *Veröffentlichung der Geobotanischen Institut ETH, Stiftung Rübel, Zürich*, **95**, 1–638.

Lüönd A. (1980) Effects of nitrogen and phosphorus on the growth of some Lemnaceae. *Veröffentlichung der Geobotanischen Institut ETH, Stiftung Rübel, Zürich*, **70**, 118–141.

Lüönd A. (1983) Das Wachstum von Wasserlinsen (Lemnaceae) in Abhängigkeit des Nährstoffangebots, insbesondere Phosphor und Stickstoff. *Veröffentlichung der Geobotanischen Institut ETH, Stiftung Rübel, Zürich*, **80**, 1–111.

McCarthy J.J., Canziani O.F., Leary N.A., Dokken D.J. & White K.S. (2001) *Climate Change 2001: Impacts, Adaptation and Vulnerability. Third Assessment Report of the Intergovernmental Panel on Climate Change*. IPCC, Cambridge University Press, Cambridge.

Mehra A., Farago M.E., Banerjee D.K. & Cordes K.B. (1999) The water hyacinth: an environmental friend or pest? A review. *Resource and Environmental Biotechnology*, **2**, 255–281.

Petrischak H. (2000) Untersuchungen zur Lebensweise des Wasserschmetterlings *Cataclysta lemnata* L. 1758 in einem schleswig-holsteinischen Kleingewasser. *Faunistisch-Ökologische Mitteilungen*, **8**, 61–99.

Pokorny J. & Rejmánková E. (1983) Oxygen regime in a fish pond with duckweeds (Lemnaceae) and *Ceratophyllum*. *Aquatic Botany*, **17**, 125–137.

Portielje R. & Roijackers R.M.M. (1995) Primary succession of aquatic macrophytes in experimental ditches in relation to nutrient input. *Aquatic Botany*, **50**, 127–140.

Rejmánková E. (1973) Seasonal changes in the growth rate of duckweed community (*Lemnetum gibbae*). *Folia Geobotanica Phytotaxa*, **8**, 1–13.

Saunders D.S. (1982) *Insect Clocks*, 2nd edn. Pergamon, Oxford.

Scheffer M., Szabo S., Gragnani A., van Nes E.H., Rinaldi S., Kautsky N., Norberg J., Roijackers R.M.M. & Franken R.J.M. (2003) Floating plant dominance as a stable state. *Proceedings of the National Academy of Science of the USA*, **100**, 4040–4045.

Szabo S., Roijackers R. & Scheffer M. (2003) A simple method for analysing the effects of algae on the growth of *Lemna* and preventing algal growth in duckweed bioassays. *Archiv für Hydrobiologie*, **157**, 567–575.

Wesenberg-Lund C. (1943) *Biologie der Süsswasserinsekten*. Springer, Berlin, Wien.

Wilf P. & Labandeira C. (1999) Response of plant-insect associations to Paleocene-Eocene warming. *Science*, **284**, 2153–2156.

Wolek J. (1974) A preliminary investigation in interactions (competition, allelopathy) between some species of *Lemna*, *Spirodela* and Wolffia. *Veröffentlichung der Geobotanischen Institut ETH, Stiftung Rübel, Zürich*, **42**, 140–162.

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