Plastizität der Photosynthese der Makroalge *Cladophora glomerata* (Chlorophyta)

Strategien phototrophen Erfolges im Fließgewässer

Dissertation

zur Erlangung des akademischen Grades

doctor rerum naturalium (Dr. rer. nat.)

vorgelegt dem Rat der Biologisch-Pharmazeutischen Fakultät

der Friedrich-Schiller-Universität Jena

von Diplom-Biologe

Ingo Ensminger

geboren am 19. Dezember 1966 in Frankfurt am Main

Gutachter:

1. Prof. W. Braune, Jena
2. Prof. B. A. Whitton, Durham (GB)
3. PD Dr. D. Hanelt, Helgoland

Tag des Rigorosums: 08. November 2000

Tag der öffentlichen Verteidigung: 27. November 2000

INHALTSVERZEICHNIS

ABKÜRZUNGEN

α_I	Anfangssteigung der lichtungesättigten Photosyntheserate
ANOVA	*analysis of variance*
Chl	Chlorophyll
ΔF	Differenz zwischen transienter und maximaler Fluoreszenz bei aktinischer Bestrahlung ($\Delta F = F_m' - F_t$)
$\Delta F/F_m'$	effektive Quantenausbeute bei aktinischer Bestrahlung/"Effektivität der Photosynthese"
DEPS	*de-epoxidation status*, Grad der lichtabhängigen Umsetzung von Violaxanthin zu Anthera- und Zeaxanthin
F_0	Grundfluoreszenz nach Dunkeladaptation
F_0'	minimale Fluoreszenz der lichtadaptierten Probe, gemessen nach 5 s Abschattung und Bestrahlung mit schwachem Rotlicht ($\lambda > 700$ nm)
F_m	maximale Fluoreszenz nach Dunkeladaptation
F_m'	maximale Fluoreszenz bei aktinischer Bestrahlung
F_t	transiente Fluoreszenz bei aktinischer Bestrahlung
F_v	variable Fluoreszenz nach Dunkeladaptation ($F_v = F_m - F_0$)
F_v/F_m	optimale Quantenausbeute nach Dunkeladaptation/"Kapazität der Photosynthese"
HC	*high current*
HL	*high light*
HPLC	*high-performance-liquid-chromatography*
IL	*intermediate light*
LL	*low light*
NPQ	*non-photochemical quenching* (nicht-photochemischer Abbau von Anregungsenergie. Es handelt sich hierbei um Prozesse der Fluoreszenzlöschung, die nicht mit der Änderung des Q_A-Redoxzustandes verbunden sind, z.B. Quenching durch Thylakoidenergetisierung, Photoinhibition und *state-transition*)
NPQ_{rev}	im Schwachlicht (10 - 20 µmol Photonen m^{-2} s^{-1}) revertierbarer Anteil des NPQ
PAR	*photosynthetic active radiation* (photosynthetisch aktive Strahlung)
P_{max}	maximale Photosyntheserate
PSII	Photosystem II
rETR	relative Elektronentransportrate
$rETR_{max}$	maximale Elektronentransportrate
VAZ	Summe der Xanthophyllzykluspigmente Violaxanthin, Antheraxanthin und Zeaxanthin

1 EINLEITUNG

> *Within the underwater environment, light availability is of major importance in determining how much plant growth there is, which kinds of plant predominate and, indeed, which kinds of plants have evolved. It is not the whole story - biotic factors, availability of inorganic carbon and mineral nutrients, temperature, all make their contribution - but it is a large part of that story.*
>
> *J. Kirk 1994*

Benthische Algen bilden die Grundlage vieler Nahrungsnetze im Fließgewässer, sie gelten als Senke und Transformierer für Nährstoffe und bilden den Lebensraum für eine Vielzahl von Organismen (Stevenson 1997). Artenzusammensetzung, Biomasse und Produktivität zeigen eine räumliche und zeitliche Heterogenität (Blum 1956), deren Ursprung in der Interaktion biotischer und abiotischer Faktoren liegt. Der alles beherrschende Faktor in diesem Geschehen ist das Auftreten von Störereignissen oder *disturbances* (*patch dynamics concept*, Townsend 1989). Diese jeweils nur kurze Zeit wirksamen Umweltveränderungen (im Gegensatz zu Streß, als langanhaltende Veränderung der Umwelt, Stevenson 1997) entfernen regelmäßig die Organismen aus ihrem Lebensraum. Dadurch wird Raum frei, der im Anschluß an das Störereignis erneut durch Individuen derselben oder einer fremden Art besiedelt werden kann (Townsend 1989). Ein Störereignis setzt die benthische Lebensgemeinschaft gleichsam zurück auf einen Startwert, gefolgt von der Neu- oder besser Wiederentwicklung der Algengemeinschaft (Picket & McDonnel 1989). Diese "Neuentwicklung" der Biozönose erfolgt nach bestimmten Regeln: Das grundsätzliche Mosaik der Umweltbedingungen, definiert durch die *ultimate factors* der Landschaft (Klima, Geologie und Mensch, siehe Biggs 1996), ist festgelegt und vorhersagbar, auch wenn eine solche Vorhersage innerhalb eines gegebenen *patches* oder Fleckens nicht möglich ist (Allan 1995).

Der Erfolg einer Spezies bei der Neubesiedlung hängt zum einen ab von der Verfügbarkeit von Überdauerungsorganen und/oder der erfolgreichen Ausbreitung von Reproduktionseinheiten (*propagules*), zum anderen aber auch von den zur Verfügung stehenden Ressourcen wie Nährstoffen und Licht (Stevenson 1996). Veränderungen der Artenzusammensetzung (Sukzession) könnten nicht stattfinden, würden die verschiedenen Arten mit den zur Verfügung stehenden Ressourcen die gleichen Leistungen vollbringen. Die Dynamik der Biozönose wird an dieser Stelle durch deren unterschiedliche artspezifische Leistungen vorangetrieben. Diese *differential species performance* ist Ausdruck der Ökophysiologie der Arten und ihres Umgangs mit den verfügbaren Ressourcen (Stevenson 1996).

Anhand der dargestellten Zusammenhänge lassen sich Bedingungen formulieren, die erfüllt sein müssen, damit der individuelle Erfolg einer Spezies in diesem durch Störungen geprägten Ökosystem gewährleistet ist:

1. Trotz weitgehender Auslöschung einer Population muß nach Wegfall der Störung eine rasche Wiederbesiedlung oder Regeneration durch Überdauerungsstadien, Überlebende der Störung, Einwanderer oder Reproduktions- und Ausbreitungseinheiten erfolgen können.

2. Der Zeitpunkt des Eintritts einer Störung ist nicht vorhersagbar. Eine Spezies wird deshalb um so erfolgreicher ein Habitat wiederbesiedeln können, je früher ihr eine Einwanderung oder eine Regeneration aus überlebenden Organen gelingt. Die Nutzung zur Verfügung stehender Stoff- und Energieressourcen muß optimiert werden können. Diese Plastizität wichtiger Prozesse stellt eine Schlüsselfunktion dar, die selbst unter zunächst suboptimalen Umweltbedingungen mindestens die Erhaltung vegetativer Stadien erlaubt.

Für benthische Algen folgt aus Punkt 2. vor allem die Forderung nach einer optimalen Nutzung der Ressource Licht, da diese die unabdingbare Voraussetzung für die Existenz phototropher Organismen darstellt (siehe Übersichten Kirk 1994 und Hill 1996). Temperatur, Substrat, Strömung sowie der Gewässerchemismus inklusive des pH und der Nährstoffe stellen natürlich weitere wichtige, die benthischen Algen prägenden, abiotischen Faktoren dar (Whitton 1975). Ebenso spielt unter den biotischen Faktoren sowohl die Konkurrenz innerhalb einer trophischen Ebene um Raum und Ressourcen als auch zwischen den trophischen Ebenen durch Beweidung (*grazing*) eine große Rolle (Hairston, Smith & Slobodkin 1960). Dennoch kommt dem Licht, als *the ultimate substrate* (Barber & Andersson 1992), eine herausragende Rolle zu.
Vom gesamten Spektrum des Sonnenlichts stellt bei Pflanzen der Bereich von 400 - 700 nm den für die photosynthetischen Prozesse wichtigen und als PAR (*photosynthetic active radiation*) bezeichneten Anteil dar (Kirk 1994). Sofern nicht anders vermerkt, erfolgt die Verwendung des Begriffes "Licht" in dieser Arbeit synonym zu PAR. Licht ist zeitlich und räumlich sehr variabel verfügbar. In unbeschatteten Abschnitten der Ilm in Thüringen konnten im Juni im Gewässer nahe der Sohle über 2000 µmol Photonen m^{-2} s^{-1} gemessen werden (Foerster, Ensminger, Hagen & Braune submitted), während in beschatteten Abschnitten die Photonenflußdichte lediglich 25 bis 50 µmol Photonen m^{-2} s^{-1} betrug (Ensminger, unveröffentlicht).
Adaptation, die evolutive Anpassung an ein bestimmtes Lichtklima (Falkowski & LaRoche 1991), ermöglicht es einzelnen Spezies, bestimmte Habitate aufgrund der Eigenschaften ihres

Photosyntheseapparates erfolgreich zu besiedeln. Akklimation, die physiologische Anpassung an das Lichtklima, erlaubt einer Spezies innerhalb der im Zuge der Evolution entwickelten Grenzen den Photosyntheseapparat bestimmten Umweltveränderungen anzupassen (Falkowski & LaRoche 1991). Neben einer Maximierung der Photosyntheseraten gehört hierzu auch die Ausbildung effektiver Lichtschutzmechanismen, um eine Beeinträchtigung der Photosynthese bei übersättigenden Lichtintensitäten zu vermeiden (Barber & Andersson 1992).
Übersättigende Lichtintensitäten treten dann auf, wenn der Photosyntheseapparat unter starken Anregungsdruck gerät. Dies ist in einer dynamischen Umwelt der Fall bei

1. hohen Raten der photosynthetisch aktiven Strahlung und hohen Temperaturen (es kann dann zur Hemmung der Donor-Seite durch Schädigung der wasserspaltenden Enzyme kommen, im weiteren zu Schäden durch die Zunahme der Oxygenasefunktion der RUBISCO und die Bildung reaktiven Sauerstoffs, siehe Übersicht Hall & Rao 1995) oder

2. mittleren Raten der photosynthetisch aktiven Strahlung und tiefen Temperaturen (durch Herabsetzung der biochemischen Reaktionen und verminderte Raten der CO_2-Fixierung, siehe Übersicht von Huner, Öquist & Sarhan 1998).

Starker Anregungsdruck kann zu einer lichtabhängigen Erniedrigung der photosynthetischen Effizienz und damit zu Photoinhibition führen (Krause 1988; Osmond 1994). Hiervon ist primär Photosystem II (PSII) betroffen (Barber & Andersson 1992). Die biophysikalischen und biochemischen Prozesse, die zur Senkung der Effizienz des PSII führen, sind abhängig von der Intensität und der Dauer der Bestrahlung (Russel et al. 1995). Nach ihrer Erholungskinetik wird die dynamische von der chronischen Photoinhibition unterschieden (Osmond 1994). Dynamische Photoinhibition ist innerhalb von Sekunden bis Minuten reversibel und beruht vor allem auf nicht-photochemischer Löschung der Anregungsenergie, die zur Ableitung überschüssiger Energie in Form von Wärme führt (Weiß & Berry 1987). Von der dynamischen Photoinhibition ist allein die photosynthetische Effizienz betroffen, nicht die photochemische Kapazität des PSII (Öquist, Chow & Anderson 1992). Der Xanthophyllzyklus, bei dem in höheren Pflanzen und Grünalgen Violaxanthin pH-abhängig de-epoxidiert und in Anthera- und Zeaxanthin umgewandelt wird (Demmig-Adams, Gilmore & Adams III 1996; Casper-Lindley & Björkman 1998), ist der entscheidende Mechanismus, der mit dieser thermischen Löschung von Anregungsenergie zusammenhängt. Chronische Photoinhibition kann Erholungskinetiken von mehreren Stunden aufweisen, die von der Geschwindigkeit des Austausches bzw. der Neusynthese degradierten D1-Proteins abhängt (Barber & Andersson 1992).

Eine zweite Strategie, die auf eine Verminderung potentieller Schädigung durch übersättigende Lichtintensitäten abzielt, besteht in der Reduzierung der Lichtsammelkapazität durch Reduzierung des Chlorophyllgehaltes, insbesondere des in den Antennen der Grünalgen lokalisierten Chlorophyll b (Huner, Öquist & Sarhan 1998).

Die Ilm (Kapitel 7.1, Fig. 1) ist für die Untersuchung benthischer Algen und ihrer Umwelt in vielerlei Hinsicht ein interessantes Fließgewässer. Der Fluß entspringt am Nordhang des Thüringer Waldes in einer Höhe von 500 m ü. NN und mündet nach einer Fließstrecke von 137 km bei Großheringen in 115 m u. NN in die Saale. Das entstehende Gefälle ist mit 3,16 % beträchtlich und führt zu einem stark turbulenten Fließen der Ilm (L. Krey 1995). Das Einzugsgebiet beträgt rund 1035 km^2. Teile des Ilmtales und seiner Talflanken stehen heute unter Naturschutz oder gehören zum Landschaftsschutzgebiet mittleres Ilmtal. Die Niederschläge liegen zwischen 1200 mm/a im Oberlauf und bis zu 550 mm/a im Unterlauf. Damit wird das Abflußverhalten im wesentlichen durch das Niederschlagsregime im Oberlauf in den Randlagen des Thüringer Waldes geprägt.
Die Verteilung der Niederschläge weist ein Minimum im April sowie Maxima im Frühsommer und ein kleineres Maximum im November auf (Sommerregentyp) (Krey 1995). Der Abfluß zeigt hohe Werte in den Schneeschmelzemonaten, vor allem wenn die Schneeschmelze von "warmen" Regenfällen ausgelöst wird. Weniger regelmäßig kommt es niederschlagsbedingt zu Sommerhochwassern. Die Tagesabflußmenge beträgt im Metarhithral bei Gräfinau-Angstedt ca. 227.300 m^3, im Jahr also ca. 83 Mio. m^3. Bei extremen Hochwasserereignissen, wie im April 1994 mit ca. 100 $m^3 s^{-1}$, beträgt die Tagesabflußmenge über 8,6 Mio. m^3 und damit 10 % des Jahresabflusses (Krey 1995). Hochwasser sind wichtige Quellen natürlicher Störungen in Fließgewässern, in deren Verlauf es zu Abrasion und zur Umwälzung des Substrats und damit zu einer drastischen Beeinflussung der benthischen Organismen kommt (Power 1987). Niedrigwasser und damit verbundener Rückgang der Strömungsgeschwindigkeit (siehe Ensminger, Hagen & Braune submitted) und Temperaturstress (Allan 1995) stellen in gleichem Maß eine Störung dar. Die mittlere Häufigkeit dieser hydrographisch bedingten Störungen in der Ilm beträgt 40 Tage (Mona Vetter, persönliche Mitteilung) und ist damit ungewöhnlich hoch (Biggs, Smith & Duncan 1999).
Die aktuelle biologische Gewässergüte der Ilm wird mit Klasse II (mäßig belastet) angegeben, im Oberlauf bis Ilmenau mit I - II (gering belastet). 1993 wurde die Ilm noch als überwiegend kritisch belastet (Güteklasse II - III) und im Mittellauf unterhalb von Tannroda sogar als sehr stark verschmutzt (Güteklasse III) eingestuft (Thüringer Landesanstalt für Umwelt 1998). Für die Verbesserung der Wasserqualität wird im Oberlauf der Bau der Kläranlage Ilmenau, im

Mittellauf die Verbesserung der Abwasserbehandlung von Langewiesen, der Kläranlagenbau in Kranichfeld und Blankenhain, die Stillegung der Papierfabrik Tannroda und die Entlastung durch die Kläranlage Bad Berka verantwortlich gemacht. Im Unterlauf fand außerdem ein Belastungsrückgang durch die Veränderungen industrieller Einleitungen statt. Fig. 2 in Kapitel 7.1 veranschaulicht die Verbesserung der Wasserqualität anhand der für die Eutrophierung der Gewässer verantwortlichen Frachten an löslichem ortho-PO_4^{3-}-Phosphor und NO_3^--Stickstoff im Zeitraum 1990 bis 1998.

Wie viele der mitteleuropäischen Fließgewässer wurde auch die Ilm in den vergangenen Jahrzehnten stark anthropogen belastet, wobei das Maximum der Belastung noch zu DDR-Zeiten erreicht wurde. Nach der Vereinigung der beiden deutschen Staaten 1990 wurden viele Industriebetriebe in der Region stillgelegt. Durch diese Deindustrialisierung sowie die geschilderte Verbesserung der Wasseraufbereitung kam es zu einem Anstieg der biologischen Wasserqualität (Peter Müller, Staatliches Umweltamt Erfurt, persönliche Mitteilung). Im Zeitraum 1992 bis 1993 durchgeführte Untersuchungen der benthischen Biomasse ergaben noch besorgniserregende Befunde. Die, wenn auch geringe, Nährstoffbelastung der Ilm führte zur Massenproduktion der fädigen Grünalge *Cladophora glomerata* (L.) Kütz. (Schönborn 1996). Neben der Verdrängung von Arten der autochthonen Fauna und Flora wurde das Problem des Abbaus der entstandenen Biomasse diskutiert. Die negativen Auswirkungen durch die massiven *Cladophora*-Bestände (*ecosystem reorganisation*) führten zur Forderung nach Management- und *restoration*-Maßnahmen für *Cladophora*-dominierte Fließgewässer (Schönborn 1996).

Bis in die Gegenwart erwies sich *C. glomerata* bei steigender Wassergüte und deutlich geringerem Auftreten weiterhin als auffälliges Florenelement der Ilm. Damit stellt sich die Frage, welche Eigenschaften zu ihrem Erfolg in diesem Gewässer beitragen. Begünstigt allein die erhöhte Fracht der Nährstoffe das Wachstum von *C. glomerata*? Kommt sie besonders gut mit den variablen Umweltbedingungen zurecht? Nach Townsend (1989) und Picket & McDonnell (1989) kann man schlußfolgern, daß der Erfolg von *C. glomerata* gegenüber anderen Makroalgen in der durch Störungen geprägten Ilm erklärbar sein muß durch a) Eigenschaften des Lebenszyklus bei der Besiedlung von Habitaten und b) die Fähigkeit, sich den Veränderungen der räumlichen und zeitlichen Mosaike der Fließgewässerhabitate anzupassen. Da die Photosynthese den grundlegenden Lebensprozess der Primärproduzenten darstellt, ist es einleuchtend, die Anpassung von *C. glomerata* an räumliche und zeitliche Veränderungen der Umwelt anhand von Parametern der photosynthetischen Leistung zu bewerten.

Ziel der vorliegenden Arbeit war die Untersuchung von Strategien, die den Erfolg von *C. glomerata* in der Ilm erklären. Die daraus folgenden Hauptfragestellungen waren:

1. Wie gestaltet sich der saisonale Verlauf der Dominanz von *C. glomerata* in einem Fließgewässer mit natürlichen Sommer- und Winterhochwassern? Welche Umweltfaktoren stehen in Beziehung zur beobachteten Besiedlungsstruktur und -dynamik? (Kapitel 7.1)

2. Welche Veränderungen der Photosyntheseaktivität und -pigmente finden im Jahresverlauf und in Habitaten unterschiedlicher Verfügbarkeit der photosynthetisch aktiven Strahlung statt? Welche Faktoren beeinflussen die untersuchten Parameter der Photosynthese? (Kapitel 7.2)

3. Zeigen die Populationen offener und beschatteter Habitate diurnale und saisonale Unterschiede der Photosynthese? Welche regulatorischen Prozesse finden statt, um eine Überlastung des Photosyntheseapparates und eine anhaltende Reduktion der Effektivität der Photosynthese durch Photoinhibition zu vermeiden? (Kapitel 7.3)

4. Welchen (wechselseitigen) Einfluß haben Strömungsgeschwindigkeit und Lichtverhältnisse auf Photosyntheseaktivität, Pigmentzusammensetzung und Wachstum von *C. glomerata* in verschiedenen Habitaten? (Kapitel 7.4)

5. Welche Unterschiede gibt es zwischen *C. glomerata* und anderen (hier am Beispiel der Xanthophycee *Vaucheria* sp.) in der Ilm vorkommenden Makroalgen hinsichtlich der Photosyntheseeigenschaften? Kann die Verteilung der Arten auf Unterschiede in der Photosyntheseleistung zurückgeführt werden? (Kapitel 7.5)

2 BEMERKUNGEN ZU MATERIAL UND METHODEN

2.1 Der Untersuchungsorganismus

Cladophora glomerata (Linnaeus) Kützing ist eine häufige und weltweit verbreitete Makroalge des Süßwassers (Blum 1956, Whitton 1970, Dodds & Gudder 1992). Sie gehört der Gruppe der siphonocladal organisierten Chlorophyceen an (Abbildungen 1 und 2, Bildtafel). Die Thalli sind stark und unregelmäßig verzweigt (van den Hoek 1963). Der mit einem Rhizoid am Substrat anhaftende Thallus bildet Rasen oder angewachsene flutende Büschel, kann aber zuweilen losgerissen und dann freischwimmend sein (Heering 1921).

Von *C. glomerata* ist nur asexuelle Reproduktion durch begeißelte Zoosporen bekannt (Heering 1921, van den Hoek 1963, Whitton 1970), unter ungünstigen Bedingungen werden vor allem im Spätherbst Akineten gebildet (Heering 1921, Rosemarin 1985).

C. glomerata kommt vorwiegend in gut durchlüftetem Wasser sowie größeren und kleineren Flußläufen vor (Heering 1921). Das Wachstum wird durch pH-Werte über 7, viel Licht und hohe Nährstoffgehalte gefördert (Whitton 1970). Die oft beobachtete massenhafte Entwicklung der Alge scheint in erster Linie ein Problem der Gewässereutrophierung zu sein, wie zahlreiche Arbeiten belegen (Sand-Jensen et al. 1989, Dodds 1991, Schönborn 1996). Allerdings schrieb Heering (1921), zu einem Zeitpunkt als das Problem der Gewässereutrophierung noch nicht die heutige Dimension besaß, über das Frühjahrswachstum von *C. glomerata*: "...durch die starke Vermehrung wird sie überdies an den Standorten bald zur vorherrschenden Art". Liebmann (1962) benutzte *C. glomerata* sogar als Leitorganismus zur Indikation kaum verunreinigten, oligosaproben Wassers. Vor diesem Hintergrund sollte für die Ausbildung dominanter Bestände von *C. glomerata* daher nicht nur eine Zunahme der Nährstoffkonzentrationen verantwortlich gemacht werden. Es bietet sich vielmehr an, eine komplexe Ursachenanalyse anzustellen.

Über die Hauptentwicklungszeit der Populationen werden unterschiedliche Angaben gemacht. Heering (1921) gibt die Monate Juli bis November an, im Winter sei *C. glomerata* weniger auffällig und im Frühjahr zeichne sie sich durch beginnende Entwicklung und lebhaft grün gefärbter Triebe aus. Die Bildung eines Wachstumsmaximum im späten Frühjahr und das anschließende Zusammenbrechen der seneszenten Populationen im Sommer wurde von Neil & Jackson (1982) sowie Power (1992) beobachtet. Gelegentlich tritt zusätzlich im Spätherbst eine eingeschränkte Neuausbildung ein (Power 1992).

Für den Zusammenbruch der Populationen in vielen Flüssen und Seen in der Mitte des Sommers werden hohe Temperaturen verantwortlich gemacht. Wong, Clark, Kirby & Kosciuw (1978) geben beispielsweise die kritische Tageshöchsttemperatur des Wassers mit 23,5 °C an.

Morphologisch zeigt die Gattung *Cladophora* eine bemerkenswerte Vielfalt, was zur Beschreibung vieler Arten geführt hat. Letztlich geht die Formenvielfalt aber wohl auf den Einfluß sehr unterschiedlicher Wachstumsbedingungen zurück. Die meisten der verwendeten Unterscheidungskriterien beruhen auf einer Kombination sich überschneidender Eigenschaften (Heering 1921, Söderström, 1963, van den Hoek 1963). Verwendete Merkmale, etwa die Länge der Zellen oder Anzahl und Winkel der Verzweigungen, sind an ein und demselben Thallus je nach Exposition gegenüber der Strömung sehr variabel (Parodi & Cáceres 1991, Bergey1995). Vermutlich handelt es sich bei einigen der beschriebenen Süßwasserarten der Gattung daher nur um unterschiedliche Ökotypen. B. A. Whitton (persönliche Mitteilung) vertritt die Auffassung, es handele sich bei den vielen unterschiedenen Arten letztendlich nur um die zweifelsfrei zu trennenden Arten *C. glomerata* und *C. aegagropila*. Nach den eigenen Untersuchungen an Proben aus der Ilm und anderen thüringischen Fließgewässern teilt der Verfasser diese Einschätzung. Aus diesem Grund wurde bei der Bestimmung der Arten entsprechend den Empfehlungen von B. A. Whitton verfahren.

Die Untersuchungen der vorliegenden Arbeit wurden zum überwiegenden Teil *in situ* und ausschließlich unter Verwendung natürlicher Populationen von *C. glomerata* durchgeführt. Auf Untersuchungen an Material, daß aus Kulturen stammt wurde nach Vorversuchen verzichtet. Organismus kann zwar in Batchkulturen gehalten werden - in eigenen Ansätzen gelang es auch Zoosporen zu isolieren und als Ausgangsmaterial für eine weitere Kultivierung zu verwenden - Kulturen von *C. glomerata* weisen allerdings schon nach kurzer Zeit eine Reihe (morphologischer) Abnormitäten auf (Brand 1899, in Heering 1921). Für die photophysiologischen Arbeiten erwiesen sich die Kulturen sogar als völlig ungeeignet, da der Photosyntheseapparat unter den verfügbaren Kultivierungsbedingungen nur einen geringen Anteil der Kapazität der Freilandpopulationen entwickelte.

2.2 Methodische Aspekte

Für die meisten Fragestellungen wurden Parameter von *C. glomerata* unter möglichst natürlichen Bedingungen *in situ* erfaßt. Nur so war sichergestellt, unter den auf verschiedenen zeitlichen Skalen operierenden Umweltveränderungen (insbesondere des Lichts), direkte oder, wie im Fall saisonaler Veränderungen, zeitlich integrierte Reaktionsmuster des Untersuchungsorganismus zu erhalten. Für die bearbeiteten Fragestellungen boten Messungen der pulsmodulierten Chlorophyll-Fluoreszenz ideale Voraussetzungen zur Ermittlung physiologischer Parameter. Die gelegentlich mit elektrokardiographischen Messungen (EKG) verglichene Methode ist nicht-invasiv, schnell und mit vergleichsweise geringem Aufwand

mobil einsetzbar. Sie erlaubt die Bestimmung wichtiger Photosyntheseparameter sowohl bei kontrolliertem künstlichem Anregungslicht als auch bei natürlichem Sonnenlicht.

Für die Messungen an fädigen Algen im Fließgewässer war es allerdings nötig das verwendete Fluorometer PAM-2000 (Walz, Effeltrich) zu modifizieren, da das Gerät zunächst für den Einsatz im terrestrischen Bereich und Messungen an Blättern konzipiert wurde (Abbildung 3, Bildtafel). Damit die Algenthalli während der Bestimmung der Chlorophyll-Fluoreszenz in einem definierten Abstand von der Glasfieberoptik des PAM-2000 fixiert werden konnten, wurde eine Probenhalterung mit fünf separaten Kammern konstruiert. Hierdurch konnte der Probendurchsatz zeitoptimiert und die Zahl der Vergleichsmessungen erhöht werden. An jeder dieser Kammern kann mit der Fieberoptik die Messung an der durch eine 3 mm starke Plexiglasscheibe abgedeckten Probe vorgenommen werden. Die Unterseiten der Kammern sind offen, lediglich mit einer Glasfasergaze wird die Probe gegen die Plexiglasscheibe fixiert. Diese Anordnung erlaubt die Exposition der Proben direkt am Wuchsort in Wuchshöhe unter den natürlichen Licht-, Temperatur-, Nährstoff- und mit Einschränkungen sogar unter den natürlichen Strömungsbedingungen. Über eine Schnittstelle am Steuergerät des PAM-2000 kann weiterhin ein Unterwasser-Sensor zur Messung der photosynthetisch aktiven Strahlung (Li 192-SA, Li-Cor, Lincoln, USA) angeschlossen und an der Probenaufnahme befestigt werden. Auf diese Weise konnte simultan die Chlorophyll-Fluoreszenz und die dem Organismus am Wuchsort zur Verfügung stehende photosynthetisch aktive Strahlung ermittelt werden.

3 ZUSAMMENFASSUNG DER ERGEBNISSE

3.1 Der Jahresgang von *Cladophora glomerata*: Beziehung von Umweltfaktoren und makrophytischer Besiedlungsdynamik

Die Dominanz von Makrophyten und ihre Reaktion auf verschiedene Umweltfaktoren wurde in der Ilm, einem kleinen Fließgewässer in Thüringen/Deutschland, im Verlauf eines Jahres untersucht (Kapitel 7.1, Fig. 1). Besondere Berücksichtigung fand die fädige Grünalge *Cladophora glomerata* (L.) Kützing. Die Beziehung zwischen wichtigen Umweltfaktoren und *C. glomerata* wurde mit Hilfe multivariater Verfahren untersucht.

Im Jahresgang zeigten die Makroalgen der Ilm ein deutliches Wachstumsmaximum im Frühjahr. Die dominante Art war *C. glomerata*, daneben traten als wichtige Vertreter die Makroalgen *Ulothrix zonata* Kütz., *Lemanea fluviatilis* (L.) C. Agardh und *Audouinella* sp. auf (Kapitel 7.1, Table 2). Kurz nach diesem Maximum war bereits eine rasche Abnahme der Deckungsgrade der Gewässersohle durch Makroalgen zu verzeichnen. Unter allen beobachteten Arten zeigte allein *C. glomerata* ein zweites Wachstumsmaximum im Spätsommer/Frühherbst (Kapitel 7.1, Fig. 5).

Häufige Störungen der Makroalgengemeinschaft durch Hochwasser führten wiederholt zur vollständigen Verdriftung der benthischen Organismen. Nach den Hochwassern des Sommerhalbjahres kam es nur durch *C. glomerata* zu einer Wiederbesiedlung des Substrats. In den lichtoffenen, weitgehend unbeschatteten Habitaten wurde hierbei eine schnellere Wiederbesiedlung beobachtet als in den beschatteten Habitaten. Dies wird in Beziehung zum Lebenszyklus von *C. glomerata* diskutiert (Kapitel 7.1, Figs 3-5).

Ein multiples lineares Regressionsmodell legt nahe, daß in abnehmender Wichtigkeit photosynthetisch aktive Strahlung, Strömungsgeschwindigkeit, pH, gelöster ortho-Phosphat-Phosphor und Ammonium-Stickstoff die größte Beziehung zur beobachteten Dominanz von *C. glomerata* aufweisen (Kapitel 7.1, Fig. 6).

3.2 Saisonale Veränderungen der Photosyntheseaktivität licht- und schattenakklimatisierter *Cladophora glomerata*

In der Ilm stellt *Cladophora glomerata* (L.) Kütz. die dominante filamentöse Algenart dar, die nahezu ganzjährig sowohl in offenen, weitgehend unbeschatteten als auch in beschatteten Habitaten zu finden ist. Die Frage nach Anpassungen des Photosyntheseapparates von *C. glomerata* an die variierenden Strahlungsverhältnisse wurde durch *in situ* Messungen der Chlorophyll-Fluoreszenz und mit Hilfe von Pigmentanalysen untersucht.

Über das gesamte Jahr hinweg zeigten Algen offener Habitate grundsätzlich eine geringere optimale Quantenausbeute des Photosystems II (F_v/F_m) als Vergleichsproben aus beschatteten Habitaten. Winterpopulationen wiesen höhere F_v/F_m-Werte als Sommerpopulationen auf (Kapitel 7.2, Fig. 1). Die beobachtete lichtinduzierte Abnahme der effektiven Quantenausbeute des Photosystem II war in Schattenpflanzen schon bei Strahlungsintensitäten ab 250 µmol Photonen m^{-2} s^{-1} stärker als in Lichtpflanzen (Kapitel 7.2, Fig. 2).

Das Leistungsvermögen der photosynthetischen Elektronentransportrate unterschiedlicher Populationen von *C. glomerata* wurde durch Lichtsättigungskurven (*photosynthesis-irradiance-curves*) ermittelt. Winterpopulationen offener und beschatteter Habitate zeigten keine Unterschiede der rETR. Die Lichtsättigungskurven wiesen allerdings Kennzeichen einer Schwachlichtakklimatisierung auf. Die rETR der Sommerpopulationen waren insgesamt höher, wobei die höchsten an unbeschatteten Standorten ermittelt wurden (Kapitel 7.2, Fig 3, Table 1).

Die Ergebnisse der Chlorophyll-Fluoreszenzmessungen wurden durch die Analysedaten der Photosynthesepigmente gestützt. Die Algen enthielten weniger Chlorophyll pro Trockenmasse im Winter. Im Sommer war der Chlorophyllgehalt pro Trockenmasse in *C. glomerata* offener Habitate erniedrigt. Im Winter und im Sommer erhöhte sich außerdem das Verhältnis Chl a/Chl b (Kapitel 7.2, Table 2). Neben dem Nachweis langfristiger Veränderungen der Pigmentzusammensetzung konnte auch gezeigt werden, daß *C. glomerata* die Fähigkeit zu kurzfristigen Anpassungen ihrer Photosyntheseleistung an schnelle Veränderungen ihrer Lichtumwelt besitzt. Unter Starklichteinfluß wurde die De-epoxidation von Violaxanthin zu Anthera- und Zeaxanthin nachgewiesen.

Mit einem multiplen linearen Regressionsmodell wurde versucht, die effektive Quantenausbeute von PSII mit den Umweltparametern zu verknüpfen. Das Verfahren lieferte ein Modell, welches eine Vorhersage von $\Delta F/F_m'$ durch die Meßwerte der photosynthetisch aktiven Strahlung und der Wassertemperatur erlaubt (Kapitel 7.2, Fig. 5).

3.3 Das Problem übersättigender Lichtintensitäten: Dynamische Kontrolle der Photosynthese von *Cladophora glomerata*

An Juli- und Aprilpopulationen von *C. glomerata* aus offenen und beschatteten Habitaten wurden *in situ* Tagesgänge der Photosynthese aufgezeichnet. Juliproben zeigten eine herabgesetzte optimale Quantenausbeute des Photosystems II (F_v/F_m) um die Mittagszeit (Kapitel 7.3, Fig. 1). Zwischen den Proben aus offenen und beschatteten Habitaten wurden für F_v/F_m nur geringe Unterschiede gemessen. Demgegenüber konnten deutliche Differenzen der relativen Elektronentransportrate (rETR) bei Licht- und Schattenpflanzen bestimmt werden. Im Tagesverlauf ergaben sich während der höchsten Strahlungsintensitäten geringere rETR in Schatten-

pflanzen als in Lichtpflanzen (Kapitel 7.3, Fig. 1). Weiterhin wurde bei der nicht-photochemischen Löschung von Anregungsenergie (NPQ) eine schnellere Regulierung des Auf- und Abbaus bei Licht- gegenüber Schattenpflanzen festgestellt. Lichtpflanzen wiesen gegenüber Schattenpflanzen eine größere Kapazität zur Wärmeabstrahlung von Anregungsenergie und einen um 21 % erhöhten Gehalt an Xanthophyllzykluspigmenten auf (Kapitel 7.3, Fig. 1).

Beim Vergleich der Frühjahrs- mit den Sommerpopulationen war in *C. glomerata* des Aprils eine Absenkung des Niveaus von F_v/F_m gegenüber den Juliproben zu beobachten (Kapitel 7.3, Figs 2 und 3). Dies wurde mit um 8 °C niedrigeren Wassertemperaturen und den durch herabgesetzte Stoffwechselraten entstehenden Anregungsdruck des Photosystem II in Verbindung gebracht. Der Pool der Xanthophyllzykluspigmente war um 21 % gegenüber dem der Julipflanzen erhöht und ließ auf einen Anstieg der Rate thermischen Abbaus von Anregungsenergie über de-epoxidierte Xanthophylle schließen (Kapitel 7.3, Table 1). Wie bei den Julipflanzen lag auch bei den Aprilproben der Anteil der Xanthophyllzykluspigmente in Lichtpflanzen um 20 % über dem der Schattenpflanzen (Kapitel 7.3, Table 1).

Akklimatisierung an das Lichtklima des Wuchsortes konnte bei *C. glomerata* auch durch experimentelle Induktion von NPQ nach Bestrahlung mit hohen Intensitäten aktinischen Lichtes gezeigt werden. In Algen aus den offenen Habitaten wurde am Ende einer Serie künstlicher Starklichtphasen ein um 20 % höheres NPQ und eine um 77 % höhere Komponente des reversiblen NPQ im Vergleich zu den Algen aus beschatteten Habitaten festgestellt (Kapitel 7.3, Fig. 4, Table 2).

Experimenten zum Einfluß der Temperatur auf die Photosyntheserate zeigten, daß zwischen rETR und der photosynthetischen Sauerstoffproduktion kein linearer Zusammenhang besteht. Weiterhin bestehen für beide Methoden unterschiedliche Optimumtemperaturen der maximalen Photosyntheserate. Steigende Temperaturen bewirkten eine Zunahme der rETR bis zur untersuchten Maximaltemperatur von 25 °C, während die Sauerstoffentwicklung höchste Werte bei 10 - 15 °C erreichte und Zeichen von Photoinhibition zeigte (Kapitel 7.3, Fig. 5). Hiermit verbunden war das Auseinanderdriften des DEPS der Xanthophyllzykluspigmente und des NPQ (Kapitel 7.3, Fig. 6). Dies läßt darauf schließen, daß bei höheren Temperaturen und hohen Strahlungsintensitäten zusätzliche Schutzmechanismen wie Photorespiration und Mehler-Reaktion zum nicht-photochemischen Abbau von Anregungsenergie zum Tragen kommen.

3.4 Wechselwirkung von Faktoren: Die Reaktion von *Cladophora glomerata* auf Licht und Strömung im künstlichen Fließgerinne

Cladophora glomerata (L.) Kützing ist in einer Vielzahl von Habitaten der Fließgewässer verbreitet. Licht und Strömung gehören zu den wichtigen Umweltfaktoren, die den Erfolg der Alge im Sinne von Photosynthese und Wachstum bestimmen. Um die Wechselwirkung von Licht und Strömung sowie den Einfluß der Zeit auf die Quantenausbeute der Chlorophyll-Fluoreszenz, die Zusammensetzung der Photosynthesepigmente und das Spitzenwachstum von *C. glomerata* zu untersuchen, wurden faktorielle Experimente durchgeführt.

C. glomerata aus der Ilm wurde im Freiland in künstlichen Fließgerinnen unter verschiedenen Kombinationen von Licht und Strömung herangezogen. In einem ersten Schritt wurde mittels zweifaktorieller Varianzanalyse mit Meßwiederholung (*two-way repeated measures ANOVA*) untersucht, ob die verschiedenen Faktorenstufen und Faktorenkombinationen Einfluß auf die optimale Quantenausbeute (F_v/F_m) und die Pigmentzusammensetzung haben. In einem zweiten Schritt wurde mit Hilfe multipler Regressionsanalyse beschrieben, in welcher Weise F_v/F_m von der photosynthetisch aktiven Strahlung und den Photosynthesepigmenten a) in Habitaten mit rascher Strömung und b) in Habitaten mit geringer Strömung abhängt.

Die zweifaktorielle Varianzanalyse zeigte, daß die photosynthetisch aktive Strahlung statistisch signifikanten Einfluß auf den Gesamtchlorophyllgehalt pro Trockenmasse, auf das Verhältnis Chlorophyll a zu Chlorophyll b, auf alle carotenoidabhängigen Parameter der Photosynthesepigmente und auf F_v/F_m hatte (Kapitel 7.4, Figs 2-4, Tables 2-3). Der Einfluß der Strömung hatte statistisch signifikante Bedeutung für das Verhältnis Chlorophyll a zu Chlorophyll b und für die carotenoidabhängigen Parameter der Photosynthesepigmente (Kapitel 7.4, Figs 2-4, Table 2).

Die multiple lineare Regression zeigt, daß in schnellfließenden Habitaten F_v/F_m durch einen anderen Parametersatz beschrieben werden kann als in langsamfließenden Habitaten. In den Behandlungen mit hoher Strömungsgeschwindigkeit hing die Absenkung von F_v/F_m vor allem vom De-epoxidationsgrad der Xanthophyllzykluspigmente (DEPS), der photosynthetisch aktiven Strahlung (PAR) und dem Verhältnis von Chlorophyll a zu Chlorophyll b ab (Kapitel 7.4, Table 4). In den Behandlungen mit geringer Strömung hatte ebenfalls DEPS starken Einfluß auf die Absenkung von F_v/F_m. Als zweitwichtigster Faktor wurde in diesem Habitat jedoch die Strömungsgeschwindigkeit bestimmt. Erst an dritter Stelle folgte PAR und daran anschließend der Gesamtchlorophyllgehalt pro Trockenmasse (Kapitel 7.4, Table 4). Wechselwirkungen der Effekte von Strahlungsintensität und Strömung wirkten sich auf den Gehalt an Carotenoiden pro Gesamtchlorophyll und den De-epoxidationsgrad der Pigmente des Xanthophyllzyklus aus.

Die Rate des Spitzenwachstums von *C. glomerata* stieg mit der Zunahme sowohl der Strömungsgeschwindigkeit als auch der photosynthetisch aktiven Strahlung (Kapitel 7.4, Fig. 5).
Es wird vermutet, daß bei niedrigen Strömungsgeschwindigkeiten durch größere Grenzschichtbildungen an der Zelloberfläche die Nährstoffversorgung limitiert ist, welche wiederum zu einer Begrenzung der Photosynthese führt. Ebenso dürften bei größeren Strömungsgeschwindigkeiten und turbulenter Strömung durch die Verwirbelung der Thalli die einzelnen Zellen abwechselnd kurzen Phasen der Belichtung und gegenseitiger schützender Beschattung ausgesetzt sein.

3.5 Photosynthese und Verteilungsmuster benthischer Makroalgen: *Cladophora glomerata* und *Vaucheria* sp. im Vergleich

In dieser Arbeit wurde die Entwicklung der benthischen Algenbiozönose und der Photosyntheseparameter zwischen Frühjahr und Sommer beobachtet. Untersuchungsziele waren

a) das Monitoring der benthischen Makroalgengemeinschaft im Hyporhithral der Ilm in Thüringen und die beobachtete Artenverteilung mit den für die kleinräumige Verteilung als wichtig angesehenen abiotischen Faktoren zu verknüpfen
b) die These zu überprüfen, daß die Verbreitung der in der Ilm auffälligen Makroalgen *Cladophora glomerata* (L.) Kütz. und *Vaucheria* sp. durch Eigenschaften ihrer Photosyntheseleistung begrenzt oder bestimmt wird.

Zu Beginn des Frühjahrs wurde die beobachtete Artenverteilung am besten durch die Wassertiefe und die Strömungsgeschwindigkeit erklärt (Kapitel 7.5, Figs 1-2). Das Lichtklima gewann im weiteren Verlauf der Untersuchungen an Wichtigkeit. Im Sommer und vor allem während der Wiederbesiedlungsphase nach einem Hochwasser war die Strahlungsintensität der wichtigste Umweltfaktor (Kapitel 7.5, Figs 1-2). Statistisch betrachtet konnte sie nahezu 60 % der beobachteten Varianz der Artenverteilung erklären. Demgegenüber hatte das Abflußregime keinen großen Einfluß auf die Deckung der benthischen Makroalgen. Gerade bei *C. glomerata*, der dominanten Art im untersuchten Abschnitt der Ilm, wurde ein deutlicher Rückgang sowohl der Deckungsgrade als auch der Thalluslänge bereits einige Zeit vor dem Sommerhochwasser beobachtet (Kapitel 7.5, Figs 1-2).
Messungen der photosynthetischen Elekronentransportrate des Photosystems II zeigten eine Akklimatisierung von *C. glomerata* an das Lichtklima unterschiedlicher Habitate: In *C. glomerata* aus offenen Habitaten war die maximale Elektronentransportrate ($rETR_{max}$) höher als bei Vergleichsproben aus beschatteten Habitaten (Kapitel 7.5, Table 1). Gleichzeitig zeigten die Lichtpflanzen eine Sättigung der Elektronentransportrate bei höheren Strahlungsintensitäten als

die Schattenpflanzen. Bei *Vaucheria* sp. wurde keine vergleichbare Akklimatisierung an unterschiedliche Lichtklimate beobachtet (Kapitel 7.5, Table 2).

Pigmentanalysen mittels HPLC bestätigten diese Ergebnisse: Aus offenen Habitaten stammende Proben von *C. glomerata* enthielten mehr Xanthophyllzykluspigmente pro Chlorophyll a und zeigten ein signifikant höheres Verhältnis von Chlorophyll a zu Chlorophyll b. In *Vaucheria* sp. ließen sich demgegenüber keine Unterschiede im Pigmentgehalt von Proben aus offenen und beschatteten Habitaten nachweisen (Kapitel 7.5, Tables 4-5).

Tagesgänge der Photosynthesekapazität zeigten nach Starklichtexposition in *C. glomerata* anhaltende dynamische Photoinhibition, angezeigt durch die Abnahme der F_v/F_m-Werte. In *Vaucheria* sp. kam es unter den gleichen Bedingungen hingegen nur zu einer geringfügigen Abnahme von F_v/F_m. (Kapitel 7.5, Figs 3-4) Die Untersuchungen der Photosynthesepigmente zeigten, daß *Vaucheria* sp. zu Anpassungen ihrer Photosyntheseleistung an die aktuelle Intensität der photosynthetisch aktiven Strahlung binnen Minuten in der Lage ist. Deshalb wird angenommen, daß diese Alge eine höhere Kapazität zum thermischen Abbau der lichtinduzierten Anregung besitzt als *C. glomerata*.

Zusammenfassend ist festzustellen, daß *C. glomerata* ein größeres Spektrum von Habitaten besiedeln kann, die durch unterschiedliche Lichtklimate gekennzeichnet sind. Wichtig ist hierbei vor allem die effektive Energieumwandlung selbst unter Starklichtbedingungen. Bei *Vaucheria* sp. scheint aufgrund der schnellen regulatorischen Prozesse und der geringen Photoinhibition unter Starkklichteinfluß zunächst ein Vorteil gegenüber *C. glomerata* zu bestehen. Da aber gerade unter diesen Bedingungen die Grünalge höhere Elektronentransportraten aufweist, stellt die weniger effektive Energieumwandlung der Xanthophycee letztlich einen limitierenden Faktor für ihren Erfolg dar.

4 ZUSAMMENFASSENDE DISKUSSION

Die Untersuchungen zum Erfolg von *C. glomerata* in der Ilm zeigten eine effektive Kombination von Lebenszyklus- und Photosynthesestrategien, die zu ihrem Erfolg gegenüber anderen benthischen Algen beitragen.

Fließgewässer stellen einen durch hohe Dynamik geprägten Lebensraum dar. Hochwasser können periodisch nahezu die gesamte benthische Flora auslöschen und den Prozeß der Besiedlung und der Entwicklung von Populationen erneut in Gang setzen. In der Ilm (Thüringen) stellt *Cladophora glomerata* (L.) Kütz. unter diesen Bedingungen eine auffällige Spezies der benthischen Lebensgemeinschaft dar, die nahezu ganzjährig mit vegetativen Organen präsent ist (Kapitel 7.1, Fig. 5). Hochwasser kann *C. glomerata* offenbar besser kompensieren als die übrigen beobachteten Makroalgen, sie ist die einzige Spezies, die nach diesen Störungen das Substrat in nennenswertem Umfang wiederbesiedelt. Dabei zieht sie großen Nutzen aus den Eigenschaften ihres Lebenszyklus (ganzjährige Zoosporenbildung, Akinetenbildung, Bildung dauerhafter und derber Rhizoide).

Ist *C. glomerata* erst am Standort etabliert, muß der Organismus mit den variablen Ressourcen, insbesondere den Strahlungsverhältnissen, zurechtkommen. Für die photosynthetischen Prozesse stellen die saisonalen Veränderungen des Lichtklimas und diurnale Schwankungen des Strahlungsangebots eine große Herausforderung dar. Dieses Geschehen erfährt dadurch weitere Komplexität, daß es überprägt wird von Perioden hoher Sediment- und Partikelfracht mit geringer Eindringtiefe des Sonnenlichts. Hinzu kommen die zumindest saisonal sehr variablen Wassertemperaturen (Kapitel 7.1, Fig. 3 und Fig. 5). Bei der in diesem dynamischen Lebensraum notwendigen Anpassung an die aktuelle Situation laviert der photosynthetische Apparat beständig zwischen Optimierung der Lichtsammelkapazität und der Gefahr durch übersättigende Lichtintensitäten Schäden davonzutragen. Die hierbei notwendige Kapazität zur dynamischen Anpassung ist bei *C. glomerata* zu entscheidenden Teilen durch den Xanthophyllzyklus vermittelt. Deutliche Veränderungen finden auch in der Verteilung und im Gesamtgehalt der Chlorophylle statt. Diese Veränderungen operieren träger und werden auf einer größeren zeitlichen Ebene reguliert als die Veränderungen der Carotenoide (Kapitel 7.4, Figs 2-3 und Tables 2-3). Insgesamt arbeiten diese beiden Systeme außerordentlich effizient. Die durch übersättigende Lichtintensitäten beobachteten Absenkungen der Quantenausbeute waren in allen Tagesgängen bis zum Abend reversibel und zeigten keine Anzeichen chronischer Photoinhibition. Es ist bemerkenswert, daß in der Chlorophyll c tragenden Xanthophycee *Vaucheria* sp. ebenfalls die Aktivität eines xanthophyllabhängigen Schutzmechanismus über

den Diadino-/Diatoxanthinzyklus verwirklicht ist, der aber eine von *C. glomerata* abweichende Lichtschutzstrategie offenlegt: Generell hohe Raten der nicht-photochemischen Löschung zeigen einen höchst ineffizienten Umgang mit der Ressource Licht. Da es unter Schwachlichtbedingungen auch zu keiner Steigerung der Lichtsammelkapazität durch Veränderungen des Verhältnisses Chlorophyll a zu Chlorophyll c kommt, limitiert *Vaucheria* ihre ökologische Nische auf sonnenscheinreiche Habitate und Jahreszeiten.
Die unterschiedlichen Ergebnisse an den beiden Organismen passen gut in das aktuelle Bild zur Untersuchung der Schutzmechanismen über den Xanthophyllzyklus. Es wird zunehmend deutlich, daß die Aktivität des Xanthophyllzyklus in Organismen aus verschiedenen taxonomischen Gruppen (Casper-Lindley & Björkmann 1998) und selbst in Ökotypen derselben Art unterschiedlich reguliert werden kann (Adams, Demmig-Adams,Logan, Barker & Osmond 1999).

Am Beispiel der Ilm wird zusammenfassend festgestellt, daß *C. glomerata* zwei sich ergänzende Strategien verwirklicht, die ihren Erfolg in der Ilm teilweise erklären. Die Photosynthese paßt sich den komplexen Umweltfaktoren, insbesondere Temperatur und Lichtklima, dynamisch an. Wichtige Mechanismen stellen hierbei die Regulation der Lichtsammelkapazität über die Chlorophylle und die Art der nicht-photochemischen Löschung überschüssiger Anregungsenergie dar. Der Plastizität der Photosynthese kommt im Hinblick auf den Lebenszyklus und die hohe Wiederbesiedlungskapazität nach Störungen eine große Bedeutung zu. Die Fähigkeit, die elementaren Prozesse der Photosynthese dem Angebot der Ressource Licht anpassen zu können, erweitern die ökologische Nische von *C. glomerata*. Selbst wenn durch ungünstige Umweltbedingungen die Primärproduktion verlangsamt oder die Photosynthese zeitweise inhibiert wird, können vegetative Zellen dieser Situation zeitweilig widerstehen. Die solchermaßen verwirklichte Erhaltung bereits etablierter und quasi in Wartestellung verharrender Individuen stellt eine wichtige Quelle für eine rasche Entwicklung der Population beim Eintreten günstiger Wachstumsbedingungen dar.

5 THESEN

1.1 Der Jahresgang der Besiedlungsdynamik von Makrophyten und der Einfluß verschiedener Umweltfaktoren wurde in der Ilm untersucht.

C. glomerata stellt in der Ilm die dominante Art dar. Sie zeigt bei nahezu ganzjährigem Auftreten Deckungsmaxima im Frühjahr und im Spätsommer/Frühherbst.

Im Sommer kommt es nach Störereignissen nur durch *C. glomerata* zu einer wesentlichen Wiederbesiedlung des Substrats, wobei in lichtoffenen Habitaten die Wiederbesiedlung schneller erfolgt als in beschatteten Habitaten.

1.2 Die Beziehung zwischen wichtigen Umweltfaktoren und *C. glomerata* wurde mit Hilfe multivariater Verfahren analysiert.

Ein phänomenologisches Modell zeigt, daß in abnehmender Wichtigkeit photosynthetisch aktive Strahlung, Strömungsgeschwindigkeit, pH, gelöster ortho-Phosphat-Phosphor und Ammonium-Stickstoff die größte Beziehung zur beobachteten Dominanz von *C. glomerata* aufweisen.

2.1 Anpassungen des Photosyntheseapparates von *C. glomerata* an die variierenden Strahlungsverhältnisse wurden durch *in situ* Messungen der Chlorophyll-Fluoreszenz und durch Analysen der Photosynthesepigmente untersucht.

Algen offener Habitate zeigen ganzjährig eine geringere optimale Quantenausbeute des Photosystems II (F_v/F_m) als Vergleichsproben aus beschatten Habitaten. Winterpopulationen weisen höhere F_v/F_m-Werte als Sommerpopulationen auf.

2.2 Das Leistungsvermögen der relativen photosynthetischen Elektronentransportrate (rETR) von *C. glomerata* wurde durch Lichtsättigungskurven (*photosynthesis-irradiance-curves*) ermittelt.

Winterpopulationen offener und beschatteter Habitate zeigen keine Unterschiede der rETR, die Lichtsättigungskurven weisen allerdings Kennzeichen einer Schwachlichtakklimatisierung auf. Die rETR von Sommerpopulationen sind insgesamt höher, die höchsten Raten werden an Proben aus unbeschatteten Habitaten ermittelt.

2.3 Mit einem multiplen linearen Regressionsmodell wurde die effektive Quantenausbeute des Photosystems II ($\Delta F/F_m'$) mit den Umweltparametern verknüpft.

Durch die Meßwerte der photosynthetisch aktiven Strahlung und der Wassertemperatur ist nach einem einfachen Regressionsmodell eine gute Vorhersage von $\Delta F/F_m'$ möglich.

3.1 An Juli- und Aprilpopulationen von *C. glomerata* aus offenen und beschatteten Habitaten wurden *in situ* Tagesgänge der Photosynthese beobachtet.

Niedrige Temperaturen führen in Frühjahrspopulationen gegenüber Sommerpopulationen zu einer Absenkung des Niveaus der optimalen Quantenausbeute (F_v/F_m). Der Gehalt an Pigmenten des Xanthophyllzyklus bezogen auf die Chlorophylle ist in Aprilproben gegenüber Juliproben um 20 % erhöht.

Lichtpflanzen zeigen eine schnellere Regulierung des Auf- und Abbaus der nicht-photochemischen Löschung von Anregungsenergie (NPQ) und einen um mehr als 20 % höheren Gehalt an Pigmenten des Xanthophyllzyklus als Schattenpflanzen.

3.2 Die Akklimatisierung an die Strahlungsverhältnisse des Wuchsortes wurde durch experimentelle Induktion der nicht-photochemischen Löschung von Anregungsenergie (NPQ) nach Bestrahlung mit hohen Intensitäten aktinischen Lichtes gezeigt.

Lichtpflanzen besitzen eine um 20 % höhere Kapazität zur nicht-photochemischen Löschung (NPQ) und eine um 77 % höhere Komponente des reversiblen NPQ als Schattenpflanzen.

3.3 In Laborexperimenten wurde der Einfluß der Temperatur auf die relative Elektronentransportrate (rETR) und die photosynthetische Sauerstoffproduktion untersucht.

Zwischen photosynthetischer Sauerstoffproduktion und Elektronentransportrate besteht bei Bestrahlungsintensitäten zwischen 300 bis 400 µmol Photonen m^{-2} s^{-1} kein linearer Zusammenhang mehr.

Eine Zunahme der Temperatur bewirkt eine Zunahme der Elektronentransportrate; sie erreicht die höchsten Werte bei der untersuchten Maximaltemperatur von 25 °C, die photosynthetische Sauerstoffproduktion erreicht das Maximum bereits zwischen 10 bis 15 °C.

4.1 Mittels faktorieller Experimente wurde untersucht, welchen Einfluß verschiedene Stufen der photosynthetisch aktiven Strahlung und der Strömungsgeschwindigkeit sowie Kombinationen dieser Faktoren auf die optimale Quantenausbeute (F_v/F_m) und die Pigmentzusammensetzung haben.

Das Lichtklima hat signifikanten Einfluß auf den Gesamtchlorophyllgehalt pro Trockenmasse, das Verhältnis Chlorophyll a zu Chlorophyll b, den Gehalt an Carotenoiden pro Gesamtchlorophyll, den Gehalt an den Pigmenten des Xanthophyllzyklus und am De-epoxidationsgrad der Pigmente des Xanthophyllzyklus.

Die Strömung hat signifikanten Einfluß auf das Verhältnis Chlorophyll a zu Chlorophyll b, den Gehalt an Carotenoiden pro Gesamtchlorophyll, den Gehalt an den Pigmenten des Xanthophyllzyklus und am De-epoxidationsgrad der Pigmente des Xanthophyllzyklus.

Wechselwirkungen der Effekte von Strahlungsintensität und Strömung wirken sich auf den Gehalt an Carotenoiden pro Gesamtchlorophyll und den De-epoxidationsgrad der Pigmente des Xanthophyllzyklus aus.

4.2 Mit Hilfe multipler Regressionsanalyse wurde beschrieben, in welcher Weise F_v/F_m von der photosynthetisch aktiven Strahlung und den Photosynthesepigmenten a) in Habitaten mit rascher Strömung und b) in Habitaten mit geringer Strömung abhängt.

Bei Wachstum unter hoher Strömungsgeschwindigkeit hängt die Absenkung der optimalen Quantenausbeute vor allem vom De-epoxidationsgrad der Pigmente des Xanthophyllzyklus (DEPS), der photosynthetisch aktiven Strahlung und der Zunahme des Verhältnisses Chlorophyll a zu Chlorophyll b ab.

Bei Wachstum unter geringer Strömungsgeschwindigkeit hat vor allem der DEPS Einfluß auf die Absenkung der optimalen Quantenausbeute. Der zweitwichtigste Faktor ist in diesem Habitat allerdings die geringere Strömungsgeschwindigkeit. Erst an dritter Stelle folgt die Zunahme der photosynthetisch aktiven Strahlung und anschließend die Zunahme des Gesamtgehalts an Chlorophyll bezogen auf die Trockenmasse.

5.1 Die kleinräumige Verteilung der benthischen Makroalgen im Frühjahr und im Sommer wurde mit den als wichtig angesehenen abiotischen Faktoren verknüpft.

Im Frühjahr wird die Artenverteilung der benthischen Algen in der Ilm durch die Wassertiefe und die Strömungsgeschwindigkeit am besten erklärt. Im Sommer und während der Wiederbesiedlungsphase nach einem Hochwasser ist die Strahlungsintensität der wichtigste Umweltfaktor.

5.2 Durch den Vergleich von *C. glomerata* und *Vaucheria* sp. wurde überprüft, ob die Verbreitung dieser in der Ilm auffälligen Makroalgen durch Eigenschaften ihrer Photosyntheseleistung begrenzt oder bestimmt wird.

C. glomerata kann die Elektronentransportrate im Gegensatz zu *Vaucheria* sp. an das Lichtklima des Wuchsortes anpassen. Die Analyseergebnisse der Photosynthesepigmente unterstützen diese Befunde. Während aus offenen Habitaten stammende *C. glomerata* mehr Pigmente des Xanthophyllzyklus pro Chlorophyll a besitzt als Schattenproben, gibt es in *Vaucheria* sp. keine solchen Unterschiede.

Zusammenfassend ist für *C. glomerata* festzustellen, daß die Plastizität der Photosynthesepigmente *C. glomerata* die Anpassung an die Bedingungen verschiedener Habitate erlaubt. Die Fähigkeit, die Photosynthese in Abhängigkeit von der Ressource Licht sowie von Faktoren wie

Temperatur und Strömung zu optimieren, führt zu einer wichtigen Erweiterung ihrer ökologischen Nische.

In der störungsreichen Ilm stellen die spezifischen Merkmale des Lebenszyklus von *C. glomerata* eine weitere wesentliche Komponente dar, die der Alge Vorteile bei der Wiederbesiedlung von Habitaten verschafft.

Die Eigenschaften des Lebenszyklus und die Plastizität der Photosynthese verwirklichen im variablen Lebensraum Fließgewässer eine effektive Kombination artspezifischer Strategien, die einen Großteil des Erfolgs von *C. glomerata* in der Ilm begründen.

6 LITERATUR

Adams W.W., Demmig-Adams B., Logan B.A., Barker D.H. & Osmond C.B. (1999) Rapid changes in xanthophyll cycle-dependent energy dissipation and photosystem II efficiency in two vines, *Stephania japonica* and *Smilax australis*, growing in the understory of an open Eucalyptus forest. *Plant, Cell & Environment* **22**, 125-136

Allan D.A. (1995) *Stream Ecology - Structure and function of running waters*. Chapman & Hall, London.

Barber J. & Andersson B. (1992) Too much of a good thing: light can be bad for photosynthesis. *TIBS* **17**, 61-66.

Bergey E.A. (1995) Effects of water velocity on the architecture and epiphytes of *Cladophora glomerata* (Chlorophyta). *Journal of Phycology* **31**, 264-271

Biggs B.J.F. (1996) Patterns in benthic algae of streams. In *Algal Ecology - Freshwater benthic ecosystems* (Hrsg. Stevenson R.J., Bothwell M.L. & Lowe R.L.), Seiten 31-56. Academic Press, San Dieago.

Biggs B.J.F., Smith R.A. & Duncan M.J. (1999) Velocity and sediment disturbance of periphyton in headwater streams: Biomass and metabolism. *Journal of the North American Benthological Society* **18**, 222-241.

Blum J.L. (1956) The ecology of river algae. *Botanical review* **22**, 291-341.

Casper-Lindley C. & Björkman O. (1998) Fluorescence quenching in four unicellular algae with different light-harvesting and xanthophyll-cycle pigments. *Photosynthesis Research* **56**, 277-289.

Demmig-Adams B., Gilmore A.M. & Adams III W.W. (1996) *In vivo* functions of carotenoids in higher plants. *FASEB Journal* **10**, 404-412.

Dodds (1991) Factors associated with dominance of the filamentous green alga *Cladophora glomerata*. Water Research **25**, 1325-1332.

Dodds W.K. & Gudder D.A. (1992) The ecology of *Cladophora*. *Journal of Phycology* **28**, 415-427.

Ensminger, Hagen & Braune: Response of *Cladophora glomerata* (L.) Kützing to sunlight and current in the field. *Freshwater Biology* (submitted).

Falkowski P.G. & LaRoche J. (1991) Acclimation to spectral irradiance in algae. *Journal of Phycology* **27**, 8-14.

Foerster J., Ensminger I., Hagen C. & Braune W.: Small-scale spatial distribution of benthic freshwater macroalgae in the hyporhithral of the river Ilm, Germany, related to properties of their photosynthetic performance. *Freshwater Biology* (submitted).

Hairston N.G., Smith F.E.& Slobodkin L.B. (1960) Community structure, population control, and competition. *The American Naturalist* **94**, 421-425.

Hall D.O. & Rao K.K. (1995) *Photosynthesis*. Fifth edition. Cambridge University Press, Cambridge.

Heering W. (1921) *Siphonocladiales. In Die Süßwasser-Flora Deutschlands, Österreichs und der Schweiz, Heft 7* (Hrsg. Pascher A.), Seiten 3-99. Fischer Verlag, Jena.

Hill W.R. (1996) Effects of light. In *Algal Ecology - Freshwater Benthic Ecosystems* (Hrsg. Stevenson J.R., Bothwell M.I. & Lowe R.L.), Seiten 121-144. Academic Press, San Diego.

Huner P.A., Öquist G.& Sarhan F. (1998) Energy balance and acclimation to light and cold. *Trends in Plant Science* **3**, 224-230.

Kirk J.T.O. (1994) *Light and photosynthesis in aquatic ecosystems*. Second Edition. Cambridge University Press, Cambridge.

Krause G.H. (1988) Photoinhibition of photosynthesis. An evaluation of damaging and protective mechanisms. *Physilogia Plantarum* **74**, 566-574.

Krey L. (1995) Die Ilm und ihr Einzugsgebiet. In *Modellhafte Erarbeitung ökologisch begründeter Sanierungskonzepte kleiner Fließgewässer, Fallbeispiel Ilm/Thüringen. Bd. 1.* (Hrsg. Institut für Ökologie, AG Limnologie, Friedrich-Schiller-Universität Jena), Seiten 4-20. Jena.

Neil J.H. & Jackson M.B. (1982) Monitoring *Cladophora* growth conditions and the effect of phosphorus additions at a shoreline site in northeastern Lake Erie, North America. *Journal of the Great Lakes Research* **8**, 30-34.

Öquist G., Chow W.S. & Anderson J.M. (1992) Photoinhibition of photosynthesis represents a mechanism for the long-term regulation of photosynstem II. *Planta* **186**, 450-460.

Osmond C. B. (1994) What is photoinhibition? Some insights from sun and shade plants. In *Photoinhibition of photosynthesis - From the molecular mechanisms to the field* (Hrsg. Baker N.R. & Bowyer N.R.), Seiten 1-24. BIOS Scientific Publishing, Oxford.

Parodi E.R. & Cáceres E.J. (1991) Variation in number of apical ramifications and vegetative cell length in freshwater populations of *Cladophora* (Ulvaceae, Chlorophyta)*Journal of Phycology* **27**, 628-633.

Picket S.T.A. & Collins McDonnel M.J. (1989) Changing perspectives in community dynamics: A theory of successional forces. *Trends in Ecology and Evolution* **4**, 241-245.

Power M.E. & Stewart A.J. (1987) Disturbance and recovery of an algal assemblage following flooding in an Oklahoma Stream. *The American Naturalist* **117**, 333-345.

Rosemarin A.S. (1985) Reproductive strategy in the filamentous green alga *Cladophora glomerata* (L.) Kütz. - an explanation for its widespread distribution. Verhandlungen der Internationalen Vereinigung für Limnologie **22**, 2872-2877.

Russel A.W., Critchley C., Robinson S.A., Franklin L.A., Seaton G.G.R., Chow W.S., Anderson J.M. & Osmond C.B. (1995) Photosystem II regulation and dynamics of the chloroplast D1 protein in *Arabidopsis* leaves during photosynthesis and photoinhibition. *Plant Physiology* **107**, 943-952.

Sand-Jensen K., Jeppesen E., Nielsen K., Van Der Fijl L., Hjermind L., Nielsen L.W. & Iversen T.M. (1989) Growth of macrophytes and ecosystem consequences in a lowland Danish stream. *Freshwater Biology* **22**, 15-32.

Schönborn W. (1996) Algal Aufwuchs on stones, with particular reference to the *Cladophora*-dynamics in a small stream (Ilm, Thuringia, Germany): Production, decomposition and ecosystem reorganizer. *Limnologica* **26**, 375-383.

Söderström J. (1963) Studies in Cladophora. *Botanica Gothoburgencia* 1. Göteborg.

Stevenson R.J. (1996) Patterns of benthic algae in aquatic ecosystems. In *Algal Ecology - Freshwater benthic ecosystems* (Hrsg. Stevenson R.J., Bothwell M.L. & Lowe R.L.), Seiten 31-56. Academic Press, San Diego.

Stevenson R.J. (1997) Scale-dependent determinants and consequences of benthic algal heterogeneity. Journal *of the North American Benthological Society* **16**, 248-262.

Thüringer Landesanstalt für Umwelt (1998) *Gewässergütebericht*. Schriftenreihe der Thüringer Landesanstalt für Umwelt Nr. 31, Jena.

Townsend C.R. (1989) The patch dynamics concept of stream community ecology. *Journal of the North American Benthological Society 8*, 36-50.

van den Hoeck C. (1963) *Revision of the European Species of Cladophora*. E.J. Brill, Leiden.

Weiß E. & Berry J.A. (1987) Quantum efficiency of photosystem II in relation to energy-dependent quenching of chlorophyll-fluorescence. *Biochimica et Biophysica Acta* **894**, 198-208.

Whitton B.A. (1970) Biology of *Cladophora* in freshwaters. *Water Ressearch* **4**, 81-105.

Whitton B.A. (1975) Algae. In *River Ecology* (Hrsg. Whitton B.A.), Seiten 81-105. Blackwell, Oxford.

Wong S.L., Clark B., Kirby M. & Kosciuw R.F. (1978) Water temperature fluctuations and seasonal periodicity of *Cladophora* and *Potamogeton* in shallow rivers. *Journal of the Fisheries and Research Board of Canada* **35**, 866-870.

7 WISSENSCHAFTLICHE PUBLIKATIONEN UND MANUSKRIPTE

7.1 Strategies providing success in a variable habitat: I. Relationships of environmental factors and dominance of *Cladophora glomerata*

INGO ENSMINGER, CHRISTOPH HAGEN & WOLFRAM BRAUNE

Plant, Cell & Environment (im Druck)

ABSTRACT

Dominance of macrophytes and their response to environmental factors were studied in the river Ilm, Thuringia/Germany with special reference to *Cladophora glomerata* (L.) Kütz. Macroalgae showed a growth peak in spring with *C. glomerata*, *Ulothrix zonata* Kütz., *Lemanea fluviatilis* (L.) C. Agardh and *Audouinella* sp. being the dominant species. Shortly after this peak, a rapid decline of macrophyte substrate coverage was observed. Only *C. glomerata* revealed a second growth peak in late summer/early autumn. Frequent disturbances of the macrophyte assemblage by floods resulted repeatedly in an almost complete wash out of benthic organisms. After summer floods *C. glomerata* was the species that recolonized the substrate. At high light sites, faster recovery of *C. glomerata* was observed as compared to low light sites. This is discussed in relation to the life cycle of *C. glomerata*. Amongst the physical and chemical parameters that were analysed, irradiance, current velocity, pH, ortho-phosphate-phosphorus, and ammonia-nitrogen accounted for most of the observed patterns of dominance of *C. glomerata*.

INTRODUCTION

Within the freshwaters of the temperate zones, *Cladophora glomerata* (L.) Kütz. is probably the most widespread macroalga and an important component of ecological communities (Blum 1956, Whitton 1972, Dodds & Gudder 1992). Power (1990 and 1992) emphasised the ecological importance of this filamentous green alga, as it plays an essential role in food webs and creates much of the physical structure, e.g. substrate, for colonization or refuges within northern California rivers. The substrate suitable for colonization by benthic primary producers is usually limited and thus prone to competition between different species. As a result, *C. glomerata* can be found intermixed with other macroalgae (Entwisle 1989, Power 1992) or even with mosses. Whitton & Buckmaster (1970) described the macroalga *C. glomerata* and the moss *Fontinalis antipyretica* Hedw. within the same habitats. They further mentioned that *Rhynchostegium riparioides* (Hedw.) Card. (quoted in Whitton & Buckmaster, 1970 under its former name *Eurhynchium riparioides*) often grows within tufts of *C. glomerata*.

The presence of *C. glomerata* may serve as an indicator of good water quality (Liebmann 1962), but the development of dominant stands is most often related to human impact and high nutrient loads (Blum 1956, Auer et al. 1982, Dodds & Gudder 1992). Mass development of *C. glomerata* may affect the biocoenosis in various ways and can create severe management problems (Blum 1956, Bellis & McLarty 1967, Auer & Canale 1982, Schönborn 1996). In the river Ilm/Thuringia (Germany, Fig. 1), river regulation, industrial pollution and municipal sewage effluents had a negative impact on the ecosystem until 1989. *C. glomerata* was the most dominant primary producer and frequently developed large dominant stands. Since then, the shutdown of considerable parts of industrial facilities and improvement of municipal sewage treatments at the Ilm and its catchment dramatically improved ecological conditions especially in terms of nutrient loads (Fig. 2). However, *C. glomerata* still remains the most dominant macroalga in the Ilm. Occasional mass developments of *C. glomerata* have been observed recently at different nutrient, light, and current conditions (Schönborn 1996). Despite the ecological importance of *C. glomerata*, relationships between environmental factors that regulate growth cycles as well as physiological responses of *C. glomerata* to these factors are not well understood (Whitton 1970, Dodds 1991a). It is clear that not only eutrophication may play an important role in the formation of *C. glomerata* dominated benthic assemblages. Further factors that can effect dominance of macrophytes include disturbances (Power 1992), grazers (Dodds 1991b), life cycle properties (Kiirikki M. & Lehvo 1997), and irradiance (Hanelt et al. 1997, Hanelt 1998).

The present work studied the response of *C. glomerata* to environmental factors in order to understand strategies that may provide for the alga's success in a dynamic and variable habitat. Four sampling sites, which differed in terms of light conditions and the degree of municipal effluent inlets, were used to study the seasonal cycle of physical and chemical parameters and their relationships to important benthic macrophytes in the river Ilm. On the basis of the obtained data, factors that have strong effects on the dominance of *C. glomerata* in the Ilm were determined.

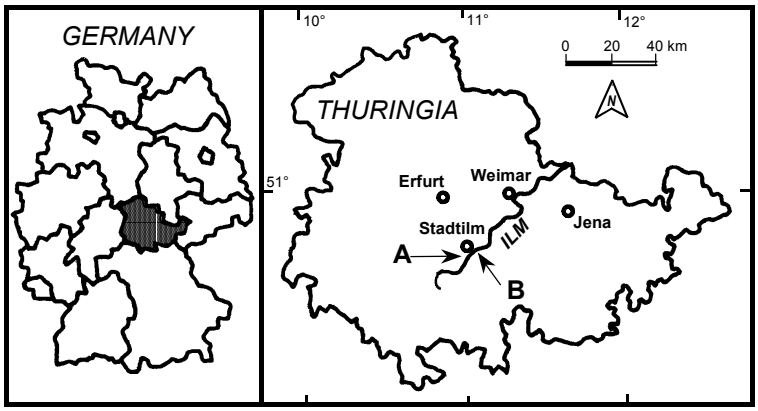

Figure 1 *Thuringia (black area), the river Ilm and the two sampling locations upstream (A) and downstream of Stadtilm (B).*

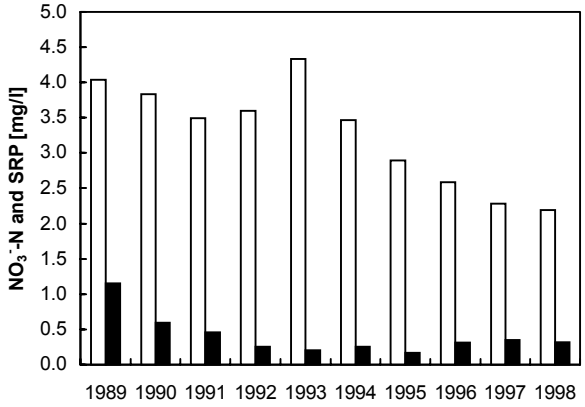

Figure 2 *Mean annual NO_3^--N and SRP concentrations of the Ilm between 1989 and 1998 upstream of Stadtilm. Data by Staatliches Umweltamt Erfurt. Open bars = NO_3^--N, closed bars = SRP.*

MATERIALS AND METHODS

Study Sites

The Ilm is a small mountain river bordered by an incomplete tree strip that creates high light and low light habitats for benthic primary producers during the summer season. The streambed is covered mostly with rocks and cobbles that are suitable as substrate for attached macrophytes. Two locations in the metarhithral of the river were selected for observation and sampling. One location was situated upstream and the second downstream of the town Stadtilm. Sampling was undertaken from May 1997 to May 1998 every 14 days. Each location was close to sites regularly gauged by the Staatliches Umweltamt Erfurt and consisted of one open site (exposed to full sunlight and therefore referred to as highlight or HL site) and one shaded site (characterised through seasonally varying degree of shade by streamside vegetation and therefore referred to as low light or LL site). Each sampling site represented a 10 m reach of the river (Fig. 1).

Macrophytes: Determination of frequency and coverage

Frequency of a certain species was estimated as the number of observations on the basis of the total number of samplings during the observation period, relative frequency of macrophytes was calculated as percentage of observations during the sampling period. Dominance of benthic macroalgae and mosses (also referred to as macrophytes) was estimated as coverage percentage of the river substrate with macrophytic thalli. A glass bottom box that covered a surface of 0.08 m^2 was used for repeated estimates of coverage of the different species. According to Entwisle (1989) and Dethier (1984) percentage coverage was determined from visual estimates. At least ten replicates of these visual estimates were then used to calculate mean substrate coverage percentage of the 10 m reach of each sampling site as a measure of dominance.

Length of thalli of *C. glomerata* was determined with a ruler with an accuracy of 0.5 cm, starting from the basal cells attached to the substrate up to the end of the filaments. Replicate measurements of five different plants at fixed points were used to calculate the mean.

Physical and chemical parameters

Field measurements of oxygen saturation, pH, conductivity, and water temperature were undertaken before noon using a WTW-multimeter (WTW, Germany). The temporal and spatial variation of flow, according to Entwisle (1989), causes formation and destruction of riffle and pool structures. For that reason, current velocity was determined at each sampling site at 5 fixed points to give an estimate of changes of flow conditions. A magnetic-inductive flowmeter Flowmate 2000 (Marsh-McBirney, Maryland, USA) was used to measure current velocity 5 cm above substrate surface and to calculate mean current velocity. Discharge rate was measured by the Staatliches Umweltamt Erfurt at Gräfinau-Angstedt, which is located 10 km upstream of Stadtilm. Daily discharge rates were used to calculate mean and maximum values for the last 14 days before sampling date. Nutrient concentrations including soluble reactive phosphorus (SRP), NO_3^--N and NH_4^+-N were measured routinely by the Staatliches Umweltamt Erfurt at the two locations at Stadtilm. Total filterable phosphate was measured only infrequently at the upstream sites and was thus not included in this work. Hence, given P values may be an underestimate at some times of the year (Jarvie, Whitton & Neal 1998).

Depth is often used to stratify rivers in order to characterise the light conditions within a habitat. In this study incident photosynthetic active radiation (PAR) was determined instead in order to give a direct estimate of the amount of available irradiance. Either a Li-190 quantum sensor or an underwater quantum sensor Li-192 (Li-Cor, Nebraska, USA) was used to measure PAR. From determinations of PAR in unshaded air and simultaneously obtained underwater PAR values at the algal surface, the attenuation c_A of PAR by streamside vegetation, light scattering and water turbidity was calculated. Data on daily global irradiance measured with a thermopile that did not select for specific spectral bands, were provided by Deutscher Wetterdienst Offenbach. The values were converted to mol photons m^{-2} d^{-1} (Larcher 1994) and used to calculate the mean of the last 14 days (I_{14}) before each sampling date. The effective irradiance (I_E) as a measure of the proportion of irradiance reaching the algal surface, was estimated as $I_E = I_{14} \times c_A$.

Statistics

The software package SPSS release 9.0 (SPSS, Illinois, USA) was used to perform statistical tests and multivariate procedures.

RESULTS

Changes of environmental factors

In order to detect effects of municipal effluents as well as effects of different light environments, one sampling location was chosen upstream and a second location downstream

of Stadtilm. Each location was divided into one open and one shaded site. Thus, our data were tested for upstream/downstream effects in nutrients, for physical and chemical parameters as well as for the influence of irradiance (Table 1). Daily sum of irradiance was highest during summer period between April and September with values between 60 - 80 mol m^{-2} d^{-1} (Fig. 3a). During the winter period between October and March, it was 10 - 20 % of the summer values. This pattern was reflected directly by the water temperature (Fig. 3a). Frequent flood events (Fig. 3b, arrows) caused almost complete wash out of benthic algae with the exception of sparse basal cell fragments of *C. glomerata* attached to unremoved boulders and stones. The first flood event was observed in late July 1997. It was followed by pronounced discharge rates of more than 2 m^3 s^{-1} until August and a further peak in October. Highest discharge rates occurred from January to mid April.

Table 1 *Annual mean, maximum and minimum values of the physical and chemical parameters of the sampling sites during the observation period 1997 - 1998.*

	Stadtilm upstream			Stadtilm downstream		
	Mean (± S.E.)	Max	Min	Mean (± S.E.)	Max	Min
Effective Irradiance [mol m^{-2} d^{-1}] [1)]	17.2 ± 2.3 8.1 ± 1.3	41.1 31.5	1.4 1.1	30.7 ± 4.2 6.5 ± 1.0	76.8 15.9	1.3 0.6
Water Temperature [°C]	9.0 ± 0.7	16.7	1.0	9.5 ± 0.07	19.3	0.6
Current velocity [m s^{-1}]	0.33 ± 0.04	1.02	0.09	0.50 ± 0.04	1.24	0.15
NH_4^+-N [mg l^{-1}]	0.14 ± 0.01	0.30	0.00	0.48 ± 0.04	1.24	0.03
NO_3^--N [mg l^{-1}]	1.83 ± 0.13	3.39	1.13	2.04 ± 0.10	2.71	1.13
SRP [mg l^{-1}]	0.24 ± 0.03	0.59	0.03	0.29 ± 0.03	0.65	0.05
N : P [2)]	16.2 ± 5.0	76.2	3.2	13.5 ± 2.6	51.0	3.2
Conductivity [µS cm^{-1}]	321.2 ± 10.1	439.0	184.5	734.6 ± 132.8	4660.0	191.0
pH	7.84 ± 0.04	8.70	7.41	7.89 ± 0.03	8.44	7.52
Oxygen Saturation [%]	106.9 ± 1.7	148.0	93.4	102.1 ± 1.1	121.0	86.0

[1)]first row from open (HL) sites, second row from shaded (LL) sites. [2)]based on the atomic ratios of PO_4^{3-}-P, NO_3^--N and NH_4^+-N.

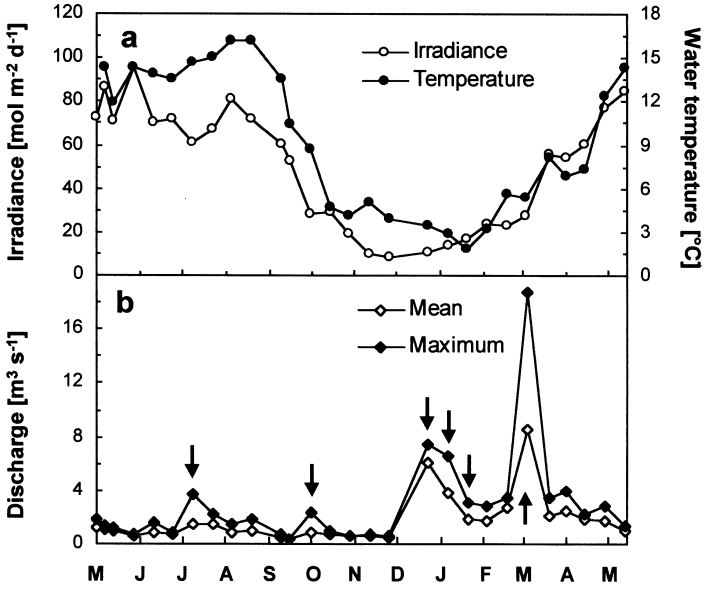

Figure 3 *Seasonal changes during the sampling period in (a) daily global irradiance and averaged water temperature; mean values of all sampling stations, and (b) discharge (arrows indicate major floods).*

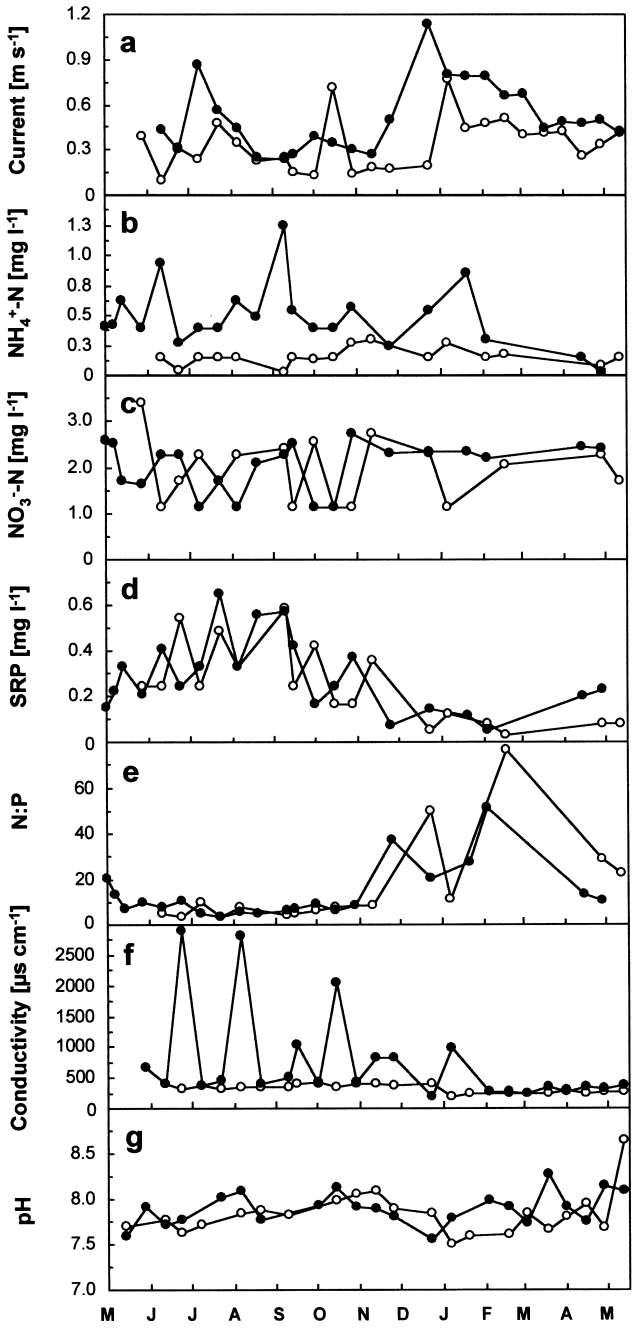

Figure 4 *Seasonal variability of physical and chemical parameters at sampling sites upstream (open circles) and downstream (closed circles) of Stadtilm. Current velocity (a), NH_4^+-N (b), NO_3^--N (c), SRP (d), N : P ratio (e), conductivity (f) and pH (g).*

Current velocity was highest during periods of high discharge (Fig. 4a) and revealed higher values at the downstream sites (Table 1, $p < 0.01$, Mann-Whitney-U-test). Levels of NH_4^+-N differed significantly between the upstream and downstream location (Table 1, student *t*-test, $p < 0.01$). No significant differences were found for NO_3^--N and SRP. The observed variation in nutrients (Figs 4b-d) reflected the hydrographic situation, e.g. high discharge rates resulted in lower concentrations due to dilution effects. No seasonal differences were found for NO_3^--N and NH_4^+-N, in contrast to SRP, which showed higher concentrations during summer (Mann-Whitney-U-test, $p < 0.01$). Mean N : P ratios, based on the atomic ratios of NH_4^+-N, NO_3^--N and SRP, ranged between 16.2 (upstream sites) and 13.5 (downstream sites) but did not express a significant difference (Fig. 4e and Table 1, Mann-Whitney-U-test, n.s.). The test for seasonal

differences showed a higher N : P ratio during summer than in winter ($p < 0.05$). Conductivity (Fig. 4f and Table 1) was higher at downstream sites ($p < 0.01$). However, this difference rather reflected the temporary outlet of effluents of a saline-works that occasionally altered conductivity to extreme values than generally higher values. Thus, single peak values occurred frequently downstream Stadtilm in June, August, September and October 1997. Neither seasonal nor upstream/downstream differences were observed for the values of pH (Fig. 4g). Oxygen, as measured early in the morning, was usually oversaturated (Table 1). Higher values were determined at the upstream compared to the downstream sites ($p < 0.05$, *t*-test), and during summer compared to winter ($p < 0.05$, Mann-Whitney-U-test). From daily curves of oxygen saturation obtained in spring and summer, it was known that O_2-levels never decreased below 95 % (data not shown). The lowest measured value of oxygen saturation occurred in November (86 %), when the Ilm was enriched in organic debris due to seasonal leaf fall.

Patterns of macrophytic association

A total of nine different macroalgae and mosses was found at the four sampling sites (Table 2). The most frequent species was *C. glomerata* that was present within 74.8 % of the total of 107 samplings, followed by *F. antipyretica* with 47.7 %. Medium frequencies were obtained for *U. zonata*, *L. fluviatilis*, *Vaucheria* sp., and *Audouinella* sp.; lowest frequencies were found for *Batrachospermum* sp., *H. foetidus,* and *R. riparioides*.

Table 2 *Frequencies of macrophytes at the four sampling sites (number of observations of a certain species on the basis of the total number of samplings during the observation period 1997 - 1998. Number of samplings = 107).*

Specie	Frequency	rel. Frequency [%]
Cladophora glomerata (L.) Kütz.	80	74.8
Fontinalis antipyretica Hedw.	51	47.7
Ulothrix zonata Kütz.	22	20.6
Lemanea fluviatilis (L.) C. Agardh.	21	19.6
Vaucheria sp.	17	15.9
Audouinella sp.	13	12.1
Batrachospermum sp.	2	1.9
Hydrurus foetidus (V.) Trev.	1	0.9
Rhynchostegium riparioides (Hedw.) Card.	1	0.9

Species diversity differed between the sites upstream and downstream of Stadtilm. The upstream sites showed a spring association composed of *C. glomerata*, *U. zonata*, *L. fluviatilis* and *Audouinella* sp. (Figs 5a, 5c) with the occasional appearance of small amounts of thalli of *Vaucheria* sp., *Batrachospermum* sp., *H. foetidus*, *F. antipyretica, and R. riparioides*. At the downstream sites only *Vaucheria* sp. was found in low numbers besides *C. glomerata* and *U. zonata*.

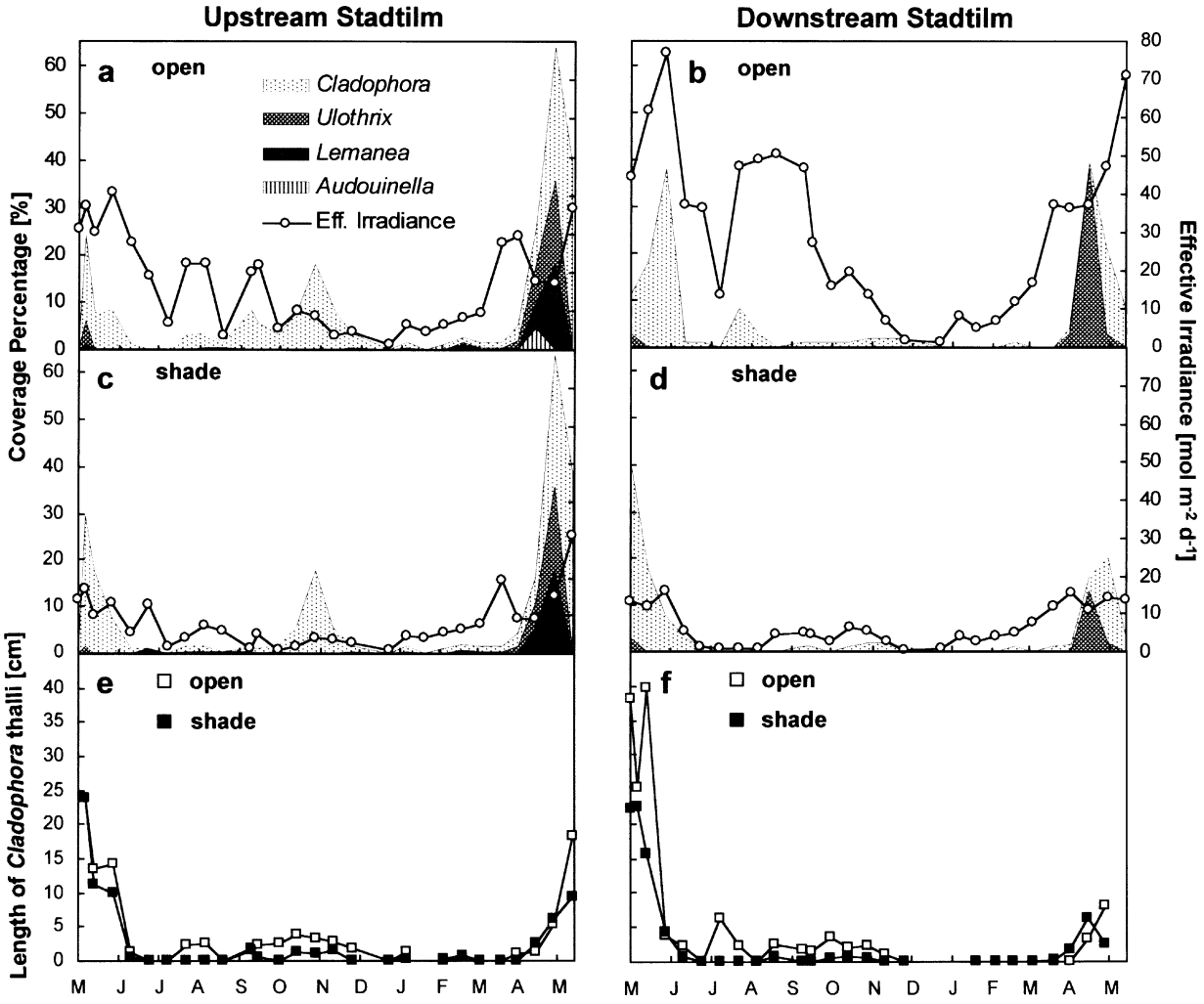

Figure 5 *Seasonal changes in cumulated coverage percentage of important (≥ 3 %) macrophytes at open (a, b) and at shaded (c, d) sampling sites, and in length of* Cladophora *thalli (e, f). Open circles = effective irradiance, open squares = length of thalli at open sites, closed squares = length of thalli at shaded sites.*

Table 3 *Summary of macrophyte dominance expressed as coverage percentage of total substrate surface at the four sampling sites.*

Site		Coverage Percentage [%]				Length of *C. glomerata* filaments [cm]	
		C. glomerata		Macrophytes			
		Mean ± S.E.	Max	Mean ± S.E.	Max	Mean ± S.E.	Max
upstream	open (HL)	6.57 ±1.76	38.00	3.13 ±1.51	38.00	3.85 ±1.19	23.80
	shade (LL)	6.28 ±1.99	38.00	2.74 ±1.44	38.00	2.59 ±1.03	23.00
downstream	open (HL)	4.30 ±1.61	38.00	2.02 ±1.41	38.00	5.34 ±2.10	39.80
	shade (LL)	3.92 ±1.66	38.00	0.89 ±0.53	13.00	2.93 ±1.25	22.60

The overall comparison of the substrate coverage data (Figs 5a-d) showed higher coverage percentages for *C. glomerata* as well as for the other macrophytes at the upstream compared to the downstream location (Table 3, Mann-Whitney-U-test, $p < 0.05$). Highest values of substrate coverage were observed in May with up to 60 % cumulated coverage due to dominant stands of *C. glomerata, U. zonata,* and *L. fluviatilis* followed by a dramatic decline of substrate coverage

shortly after this spring bloom (Figs 5a-d). Lowest coverage was observed immediately after the flood events in early July 1997, January 1998, February 1998 and March 1998 at the downstream sites (Figs 3b, 5b, 5d).

The seasonal growth of *C. glomerata* matched the overall scheme of substrate coverage mentioned above. It showed at both open and shaded sites maxima in May. These were followed by a fast decline until the end of June, and, shortly thereafter, by complete disappearance of the alga. At the open sites, *C. glomerata* developed large stands with maxima in August and, upstream, in September (Figs 5a-b). After a further pronounced maximum in substrate coverage by *C. glomerata* during November at the upstream sites, lowest values or even complete extinction of *C. glomerata* and other benthic macrophytes were found later in winter, followed by the spring development of mats consisting of *C. glomerata*, *U. zonata*, *L. fluviatilis,* and the stands of *Audouinella* sp.

The length of *C. glomerata* thalli did not show any significant differences between upstream and downstream sites, but the thalli from open sites (HL) were found to be longer than those from shaded sites (LL) (Figs 5e-f; Table 3, Mann-Whitney-U-test, $p < 0.05$).

Factors related to dominance of *C. glomerata*

Spearman's rank correlation test revealed that dominance of *C. glomerata* was significantly correlated with that of *U. zonata* and *F. antipyretica* (Table 4). Because temporal autocorrelation is often a problem in time-related data, prior to multivariate analysis temporal autocorrelation was tested for each sampling site. The mean of the D-value of the sampling sites did not show any significant autocorrelation of the data (D = 2.4, Durbin-Watson-test). For further analysis a general linear model (GLM) was used with a forward selection method within a multivariate analysis of variance to test all kinds of cross-relations between the measured parameters and their effects on the dominance of *C. glomerata*, *U. zonata*, and *F. antipyretica*. The set of physical and chemical parameters included water temperature, oxygen saturation, conductivity, pH, global irradiance, effective irradiance, current velocity, NH_4^+-N, NO_3^--N, and SRP as well as mean, maximum and minimum discharge rate. Floods (Fig. 3) were considered by the use of an index of days since the corresponding disturbance. Step by step all parameters without a statistical significant effect on dominance were eliminated from this analysis. The final set of parameters that had statistical significant influence on dominance of the three species consisted of pH, effective irradiance, current velocity, NH_4^+-N and SRP (Pillai-Spur, $p < 0.05$ for each of these five parameters).

After random selection of 50 % of the observed cases a multiple regression analysis was applied that used the five parameters determined before to describe dominance of *C. glomerata*. The resulting formula is given in Fig. 6. This phenomenological model was tested with the

remaining 50 % of cases, e.g. the predicted coverage percentage of *C. glomerata* was calculated on the basis of the corresponding physical and chemical parameters and plotted against the observed values (Fig. 6). The accuracy of the model is expressed by the R-squared value (which is in fact the squared Pearson's correlation coefficient) $R^2 = 0.69$ of the linear regression line. Using this formula, the reduced set of parameters gave reasonable estimates of trends in substrate coverage by *C. glomerata* in the Ilm.

Table 4 *Spearman's rank order correlations between substrate coverage of* C. glomerata *and other macrophytes.*

		F. antipyretica	*Vaucheria* sp.	*L. fluviatilis*	*R. riparioides*	*U. zonata*	*Batracho-spermum* sp.	*H. foetidus*	*Audoui-nella* sp.
C. glomerata	coefficient of correlation	0.256**	0.060	0.143	-0.029	0.401**	-0.041	0.060	0.121
	significance	0.008	0.538	0.142	0.766	0.000	0.672	0.541	0.214

** $p < 0.01$. Number of cases = 107

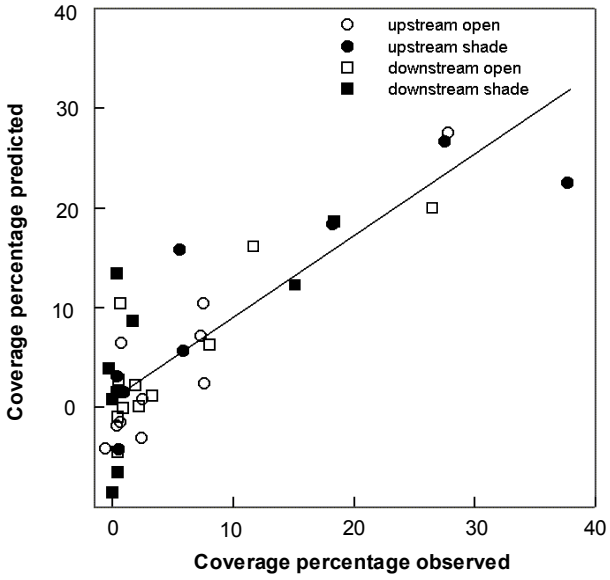

Figure 6 *Relationship between observed and predicted values of coverage percentage of* Cladophora *by the multiple regression model* $C_P = -94.017 + (13.524 \times pH) + (0.153 \times I_E) - (8.842 \times C_V) - (3.850 \times NH_4^+\text{-}N) - (20.784 \times SRP)$, *(*$C_P$ *= coverage percentage of* Cladophora*;* I_E *= effective irradiance;* C_V *= current velocity, SRP = soluble reactive phosphorus). Regression ANOVA* $p < 0.05$. *The accuracy of the fit is given as* $R^2 = 0.69$ *(a).*

DISCUSSION

Physical and chemical parameters

The physical and chemical parameters of the Ilm provide data that illustrate the dynamics of the river. Seasonal variability of the measured parameters was of greater importance than variability between upstream and downstream sites receiving different municipal sewage effluents.

Irradiance and temperature made the period between April and September the most favourable for optimum phototrophic production and growth (Fig. 3). Frequent flood disturbances caused scouring of benthic primary producers. This happened not only during winter, but also at the

beginning and during the period of optimum production in summer. Especially the early spring and summer disturbances are very important in terms of reproduction and dispersal as they do not promote the different algal life cycle strategies in the same way (see below). A further consequence of the floods is the fact that low light conditions are not exclusively restricted to the winter season due to low global irradiance levels and to sites covered by dense streamside tree vegetation in summer. Low light conditions also appeared frequently as a consequence of high discharge and related increases in water turbidity due to increased suspended sediments.

Differences in physical and chemical parameters between the two sampling locations were found for current velocity, NH_4^+-N and conductivity (Fig.4). The requirement of minimum current for the growth of some freshwater benthic algae has been demonstrated (Stevenson 1996; Dodds & Gudder 1992). Current velocity was significant in the regression model (Fig. 6), suggesting it plays a key role among the factors effecting *C. glomerata* dominance in the Ilm. As the observed mean current velocity at the sampling stations ranged well within the optimum limits of 0.5 - 0.8 m s^{-1} for *C. glomerata* (Schönborn 1996), it must be concluded, that the higher current velocity at the downstream site did not have negative effects on the dominance of *C. glomerata*.

With the exception of high discharge periods during winter absolute nutrient levels were within the ranges stated by Schönborn (1996) for optimal growth of *C. glomerata*. He quoted NO_3^--N levels of 2 - 3 mg l^{-1} and SRP levels of > 0.1 mg l^{-1} to be sufficient to induce mass production if growth is not limited by further physical and chemical parameters. Mean N : P ratios of 16 : 1 and 13 : 1 upstream and downstream, respectively, were comparable to the widely established Redfield ratio that assumes mean N : P requirements for most algae of 16 : 1 (Redfield, 1958). Taken together, this suggests that nutrients did not limit the macrophytes at the surveyed stretches for most of the observed year. High conductivity values that most probably resulted from the occasional loads of salts might be responsible as a stressor for the observed differences in species diversity as well as for the lower coverage values at the downstream sites (Figs 4 - 5). Comparison of conductivity values from measurements in the morning and in the afternoon showed, that duration of the pulses was not longer than a few hours. Hence, effects are unlikely to be assessed by this study, because it must be assumed that conductivity pulses were only observed randomly and actually occurred more frequently than was reflected within our 2 weeks sampling scheme.

Macrophyte frequency and coverage

The success of any macrophyte species at the sampling sites depended mainly on the ability to cope with (i) frequent disturbances that lead to the extinction of vegetative cells, (ii) changing light environments, and (iii) variable temperatures.

C. glomerata was found to be the most frequent and dominant macrophyte at any of the four sampling sites. Only in early spring did it seem to compete with *U. zonata*, *L. fluviatilis,* and eventually, *Audouinella* sp. for the substrate surface. In *U. zonata* zoosporogenesis of vegetative cells is stimulated at temperatures above 10 °C and usually results in the rapid decline of the population (van den Hoek, Jahns & Mann 1993). This temperature dependent zoosporogenesis of *U. zonata* may have led to the very early decline of its population, because in the end of May water temperature rose to more than 10 °C (Figs 3, 5). At the same time, *C. glomerata* showed an increased dominance (Fig. 5).

Biomass losses as a consequence of floods have been observed by Sand-Jensen et al. (1989) in a danish stream and Entwisle (1989) in two australian creeks. The high frequency of these disturbances during the observation period in the Ilm is in fact most favourable for *C. glomerata* (see Fig. 4 and Fig. 5). Compared to other algae, its life cycle provides the capacity for rapid colonization and further growth. It is characterised by two forms of reproduction, attached akinetes and zoospores. The former are developed mainly in autumn as resting cells that provide overwintering of the population. The factors that induce their formation are not clear yet. Rosemarin (1985) mentioned temperature and, to a lesser extent, reduced light and nutrient depletion. He observed in autumn at temperatures between 7 - 10 °C in a depth of 0.5 m the beginning formation of akinetes while in the fringe zone *C. glomerata* still showed vegetative cells until December. The germination of akinetes happened in May, well before the peak development of sporangia in June. Rosemarin (1985) therefore concluded that the attached akinetes result in a perenniating effect that provides an efficient spring inoculum which will give *C. glomerata* an important advantage compared to species which might grow faster or use P more efficiently.

Pronounced sporulation was found to start in *C. glomerata* in June (Rosemarin 1985), nevertheless zoosporangia seem to be produced all around the year (Hoffmann & Graham 1984). Kiirikki & Lehvo (1997) stated that propagules of *C. glomerata* are released during or just after the period of active growth. This fits well to our own observations of "bleached" cells due to zoospore release and the rapid decline of the first late spring peak of *C. glomerata* growth (Fig. 5). As zoospores contribute to the dispersal of the population they play an important role in colonization during summer, especially after disturbances. After flood disturbances, growth and recolonization of *C. glomerata* were reduced at shaded sites (Fig. 5). This may have resulted from light limited growth rates of *C. glomerata* fragments that survived on rocks at protected crevices as well as from the limitation of zoospore establishment from upstream sites. Lorenz, Monaco & Herdendorf (1991) stated that there is a minimum light requirement of 25 µmol quanta m^{-2} s^{-1} for the establishment of *C. glomerata* zoospores.

A hint at the importance of critical light values for zoosporogenesis is given by the fact of longer *C. glomerata* filaments during summer at open sites. Factors that induce zoosporogenesis are still under debate. High temperatures (15 - 20 °C) and shortened photoperiod (8 h) are mentioned by Hoffmann & Graham (1984). Mean water temperature of the Ilm during summer was usually around 13.2 °C (Fig. 4a, Table 1) and was thus within the 12 °C optimum range for growth given by Schönborn (1996). The rapid decline after the spring bloom in June 1997 was related with the highest water temperature observed (14.3 °C, Fig. 3a, Fig. 5). Temporary reductions of effective irradiance as a result of increasing streamside vegetation cover or because of periods of decreased global irradiance as observed during the early summer in 1997 may also have triggered zoosporogenesis. Small scale variations in *C. glomerata* dominance, that occurred between summer and late autumn showed a relation to the level of effective irradiance. This was most obvious at the upstream open and the upstream shaded site but also appeared at the downstream shaded site (Fig. 5a, 5c, 5d, respectively).

Determination of factors related to dominance of *C. glomerata*

Regarding the obtained linear regression formula, it is clear that the effective irradiance was found to be a factor that has significant positive effects on the dominance of *C. glomerata*. The positive statistical relationship to pH may be interpreted in two ways. On the one hand, higher coverage values usually are related to higher photosynthetic production and carbon uptake by primary producers which will in turn effect the carbon-balance and induce higher pH (Cheney & Hough 1983). This positive correlation between dissolved oxygen concentration, pH value and photosynthetic activity was confirmed by measurements of the daily course of these parameters in *C. glomerata* (unpublished data). On the other hand, *C. glomerata* is known to rely on high pH values (Whitton 1970, Schönborn 1996).

Negative regression coefficients were found for NH_4^+-N and SRP, which is surprising as *C. glomerata* development usually is associated with nutrient enrichment by phosphorus and nitrate (Sand-Jensen et al. 1989, Dodds 1991a, Schönborn 1996). According to Schönborn (1996) the observed phosphate level in the Ilm was well within the range for optimum production of *C. glomerata*. Furthermore, toxic effects of ammonia, which can result from the formation of NH_3^- at high concentrations, pH > 9, and high temperatures, can be excluded. The observed concentrations were well below critical values for *C. glomerata* mentioned by Robinson & Hawkes (1986). SRP as well as NH_4^+-N do not only effect macrophytes, but in turn these nutrients themselves are effected by uptake of benthic photoautotrophs. Because the amounts of nutrients were saturating to uptake of *C. glomerata*, they were not likely to be

limiting. Thus, the negative correlation may represent that actual demand of *C. glomerata* drives down inorganic nutrient concentrations during times of high biomass.

Within weedbeds of macrophytes current increases growth and photosynthetic rates by increased transport of nutrients (Madsen & Söndergaard 1983a, Dodds 1991b). Dodds (1991b) found at velocities higher than 8 cm s^{-1} that photosynthetic rates of *C. glomerata* decreased because of compaction and inhibition of transport of materials into and away from the algal tufts. Mean current velocity of our data was about one magnitude higher but it is very well within the optimum given by Schönborn (1996). This discrepancy might be due to the difference in measuring current velocity. Madsen et al. (1983a) and Dodds (1991b) measured this parameter within the tufts, whereas in the present study as well as that by Schönborn (1996), the parameter was measured as open water velocity above the tufts. From Madsen & Warncke (1983b), it can be concluded that current within the tufts was about the same magnitude, as they showed that current velocities in weedbeds can be reduced by more than 90 % of open water velocities. In *C. glomerata*, Dodds (1991c) found 0.4 m s^{-1} open water current velocities to be reduced to 0.3 m s^{-1} within the first 2 cm of an algal tuft.

Contrary to data published by Dodds (1991a), who described effects of floods on dominance of *C. glomerata*, floods did not show any statistical relation in our analysis. Because we did recognise visually effects of scouring by floods during the sampling period, future work should try to integrate effects of flood disturbances using additional data, e.g. the size of rocks *C. glomerata* is attached to (Dodds 1991, Power 1992).

The quality of the model for dominance of *C. glomerata* is expressed by the R-squared value in figure 6. An accuracy of $R^2 = 0.69$ of the multiple regression analysis (Fig. 6) suggests that the equation fits the data sufficiently, however there are several circumstances that limited the model's accuracy. Thus, for practical reasons a linear regression model was used instead of a non-linear model. Furthermore there are still numerous unexplained effects, for example the influence of the pulsed peaks in conductivity at the downstream sites or the influence of temperature on zoosporogenesis. Further important factors that influence *C. glomerata* and other macrophytes have not been included in this study, e.g. the effects of grazers and epiphyte grazers (Dodds 1991b). The role of spatial and seasonal changes of the irradiance environment is another area that is of great importance considering strategies providing success of benthic macrophytes . The ability of *C. glomerata* to respond to this factor in terms of photosynthesis is within the focus of a second paper presented within this volume (Ensminger, Hagen & Braune 2000).

ACKNOWLEDGEMENTS

This study was supported by the Deutsche Forschungsgemeinschaft (DFG) Graduate Study Group *Analysis of Function and Regeneration of Degraded Ecosystems*. Data on discharge and nutrients were kindly provided by Staatliches Umweltamt Erfurt. Deutscher Wetterdienst Offenbach provided data on global irradiance. Two anonymous reviewers provided helpful suggestions on the manuscript. Jens Schumacher (Jena) gave valuable critics on the statistics, and Erica Froneberg (Vienna) kindly reviewed the first English draft.

REFERENCES

Auer M.T., Canale R.P., Grundler, H.C. & Matsuoka Y. (1982) Ecological studies and mathematical modeling of *Cladophora* in Lake Huron: I. Program description and field monitoring of growth dynamics. *Journal of Great Lakes Research* **8**, 73-83.

Auer M.T., Canale R.P. (1982) Ecological studies and mathematical modeling of *Cladophora* in Lake Huron: III. The dependence of growth rates on internal phosphorus pool size. *Journal of Great Lakes Research* **8**, 93-99.

Blum J.L. (1957) An ecological study of the Saline River, Michigan. *Hydrobiologia* **9**, 361-408.

Bellis V.J. & McLarty D.A. (1967) Ecology of *Cladophora glomerata* (L.) Kütz. in southern Ontario. *Journal of Phycology* **3**, 57-63.

Cheney C. & Hough R.A. (1983) Factors controlling photosynthetic productivity in a population of *Cladophora fracta* (Chlorophyta). *Ecology* **64**, 68-77.

Dethier M.N. (1984) Disturbance and recovery in intertidal pools: Maintenance of mosaic patterns. *Ecological Monographs* **54**, 99-118.

Dodds W.K. (1991a) Factors associated with dominance of the filamentous green alga *Cladophora glomerata*. *Water Research* **25**, 1325-1332.

Dodds W.K. (1991b) Community interactions between the filamentous alga *Cladophora glomerata* (L.) Kützing, its epiphytes and epiphyte grazers. *Oecologia* **85**, 572-580.

Dodds W.K. (1991c) Micro-environmental characteristics of filamentous algal communities in flowing freshwaters. Freshwater Biology **25**, 199-209.

Dodds W.K. & Gudder D.A. (1992): The ecology of *Cladophora* in freshwaters. *Journal of Phycology* 28, 415-427

Ensminger I., Hagen C. & Braune W. (2000) Strategies providing success in a variable habitat: II. Ecophysiology of photosynthesis of *Cladophora glomerata. Plant, Cell and Environment* (in press).

Entwisle T.J. (1989) Phenology of the *Cladophora-Stigeoclonium* community in two urban creeks of Melbourne. *Australian Journal of Marine and Freshwater Research* **40**, 471-489.

Häder D.P., Herrman H., Schäfer J., Santas R. (1997) Photosynthetic fluorescence induction and oxygen production in two mediterranean *Cladophora* species measured on site. *Aquatic Botany* **56**, 253-264.

Hanelt D. (1998) Capability of dynamic photoinhibition in arctic macroalgae is related to their depth distribution. *Marine Biology* **131**, 361-369.

Hanelt D., Melchersmann B., Wiencke C. & Nultsch W. (1997) Effects of high light stress on photosynthesis of polar macroalgae in relation to depth distribution. *Marine Ecology Progress Series* **149**, 255-266.

Hoffmann J.P. & Graham L.E. (1984) Effects of selected physicochemical factors on growth and zoosporogenesis of *Cladophora glomerata* (Chlorophyta). *Journal of Phycology* **20**, 1-7.

Jarvie H.P., Whitton B.A. & Neal C. (1998) Nitrogen and phosphorus in east coast British rivers: Speciation, sources and biological significance. *The Science of the Total Environment* **210/211,** 79-109.

Kiirikki M. & Lehvo A. (1997) Life strategies of filamentous algae in the northern baltic proper. *Sarsia* **82**, 259-267.

Larcher W. (1994) *Ökophysiologie der Pflanzen*. Ulmer Verlag, Stuttgart.

Leukart P. & Hanelt D. (1995) Light requirements for photosynthesis and growth in several macroalgae from a small soft-water stream in the Spessart-Mountains, Germany. *Phycologia* **34**, 528-532.

Liebmann H. (1962) *Handbuch der Frischwasser und Abwasserbiologie, I*. Gustav Fischer Verlag, Jena.

Lorenz R.C., Monaco M.E. & Herdendorf C.E. (1991) Minimum light requirements for substrate colonization by *Cladophora glomerata*. *Journal of the Great Lakes Research* **8**, 42-53.

Madsen T.V. & Söndergaard M. (1983a) The effects of current velocity on the photosynthesis of *Callitriche stagnalis* Scop. *Aquatic Botany* **15**, 187-193.

Madsen T.V. & Warncke E. (1983b) Velocities of current around and within submerged aquatic vegetation. *Archiv für Hydrobiologie* **97**, 389-394.

Power M.E. (1990) Benthic turfs vs. floating mats of algae in river food webs. *Oikos* **58**, 67-79.

Power M.E. (1992) Hydrologic and trophic controls of seasonal algal blooms in northern California rivers. *Archiv für Hydrobiologie* **125**, 385-410.

Redfield A.C. (1958) The biological control of chemical factors in the environment. *American Scientist* **46**, 205-221.

Robinson P.K. & Hawkes H.A. (1986) Studies on the growth of *Cladophora glomerata* in laboratory continous-flow culture. *British phycological Journal* **21**, 437-444.

Rosemarin A.S. (1985) Reproductive strategy in the filamentous green alga *Cladophora glomerata* (L.) Kütz. - An explanation for its widespread distribution. *Verhandlungen der Internationalen Vereinigung für Limnologie* **22**, 2872-2877.

Sand-Jensen K., Jeppesen E., Nielsen K., van der Bijl L., Hjermind L., Wiggers-Nielsen L. & Moth-Iversen T. (1989) Growth of macrophytes and ecosystem consequences in a lowland Danish stream. *Freshwater Biology* **22**, 15-32.

Schönborn W. (1996) Algal aufwuchs on stones, with particular reference to the *Cladophora*-dynamics in small stream (Ilm, Thuringia, Germany): Production, decomposition and ecosystem reorganizer. *Limnologica* **26**, 375-383.

Stevenson R.J. (1996) The stimulation and drag of current. In (eds Stevenson R.J., Bothwell M.L. & Lowe R.L.) *Algal ecology - Freshwater benthic ecosystems*, pp. 321-340. Academic Press, San Diego.

van den Hoek C., Jahns M.H., Mann D.G. (1993) *Algen*. Thieme-Verlag, Stuttgart.

Whitton B.A. (1970) Biology of *Cladophora* in freshwaters. *Water Research* **4**, 87-114.

Whitton B.A. & Buckmaster R.C. (1970) Macrophytes of the river wear. *The Naturalist* **914**, 97-116.

7.2 Strategies providing success in a variable habitat: II. Ecophysiology of photosynthesis of *Cladophora glomerata*

INGO ENSMINGER, CHRISTOPH HAGEN & WOLFRAM BRAUNE

Plant, Cell & Environment (im Druck)

ABSTRACT

Cladophora glomerata (L.) Kütz. is the dominant filamentous algae of the river Ilm, Thuringia, Germany. For most of the year it can be found at open as well as at shaded sites. Photosynthetic acclimation of *C. glomerata* to different light intensities was detected by chlorophyll fluorescence measurements and pigment analysis. *C. glomerata* from high light sites showed decreased values of the efficiency of open photosystem II (F_v/F_m) as compared to *C. glomerata* from low light sites. Winter populations revealed higher F_v/F_m values than summer populations. Light induced decrease in the efficiency of closed photosystem II was observed at increasing irradiance intensities. The decrease was higher in *C. glomerata* from shaded sites as compared to plants from open sites. Differences in the photosynthetic electron transport rate of different populations of *C. glomerata* were shown by photosynthesis-irradiance curves. Summer populations from high light sites yielded higher maximum electron transport rates than plants from low light sites, whereas winter populations exhibited significantly decreased values compared to the summer populations. Results of the analysis of photosynthetic pigments corresponded with data from chlorophyll fluorescence measurements. In addition to these long - term acclimation effects, *C. glomerata* expressed its ability to cope with rapid changes in the light environment by the de-epoxidation of violaxanthin during exposure to high light intensities.

INTRODUCTION

Relationships between environmental factors and the dominance of *C. glomerata* in the Ilm/Thuringia (Germany) has been studied by Ensminger, Hagen & Braune (2000). It became apparent, that not only nutrients play an important role in the formation of *C. glomerata* dominated macrophyte assemblages, but also life cycle properties and the ability to grow under different light conditions. Hence, success of *C. glomerata* depends on the ability (i) of rapid and frequent dispersal of the population from vegetative cells by zoospores, (ii) to persist in a given habitat even under unfavourable conditions by akinetes, and (iii) to rapidly recolonize a habitat after disturbances. The distribution of *C. glomerata* is supposed to depend strongly on its ability

to handle changing environmental conditions including changes in the light environment. These changes include the seasonal increase and decrease of photosynthetic radiation (PAR) as well as decreases due to the development of foliage of streamside vegetation. *C. glomerata* therefore has to cope with high light stress as well as with lower levels of PAR.

Measurements of oxygen production showed in *C. glomerata* photoinhibition at irradiances above 500 µmol photons m^{-2} s^{-1} (Dodds 1991). Using fluorescence measurements, various authors reported inhibitory effects of high fluence rates on photosynthesis of marine macroalgae (Hanelt, Hupperts & Nultsch 1993; Uhrmacher, Hanelt & Nultsch 1997; Häder et al. 1997). Apart from the effects of excessive fluence rates, Huner, Öquist & Sarhan (1998) demonstrated the relation of cold temperatures to photoinhibition in unicellular green algae. The mechanism of photoinhibition is regarded as an active regulatory process to protect the photosynthetic apparatus from excessive radiation, which is accompanied by a decrease of the photosynthetic yield. A central role within this process is given to the turnover of the D1 protein, that is located in photosystem II (Sundby, McCaffery & Anderson 1993), and the turnover of the protective xanthophyll cycle (Demmig-Adams, Gilmore & Adams 1996). In marine macroalgae, Uhrmacher at el. (1995) demonstrated the light induced conversion of the xanthophyll violaxanthin into anthera- and zeaxanthin. Neidhardt et al. (1998) and Masojídek et al. (1999) showed photoadaptational processes of photosynthetic pigments in unicellular algae in relation to shifts in the light environment.

In freshwater macroalgae little work has been done on the dynamics of photoinhibition and acclimation of photosynthesis to different light environments as well as to seasonal changes. Leukart & Hanelt (1995) estimated photosynthetic rates of different freshwater macroalgae. From their results they identified typical highlight or lowlight species but they did not consider temporal or light environmental dynamics. Under laboratory conditions, various authors studied effects of light and temperature on photosynthesis of *C. glomerata* (e.g. Graham et al. 1982; Lester, Adams & Farmer 1988).

The present work analyses the response of *C. glomerata* to different light environments in its dynamic and variable habitat in order to understand strategies that may provide the alga's success in terms of photosynthesis. At four different sites (i) seasonal differences in the photosynthetic performance of *C. glomerata*, (ii) differences in photosynthetic activity between open and shaded sites, and (iii) plasticity in pigment composition that allows *C. glomerata* acclimation to different environmental conditions were studied. On the basis of the obtained data we (iv) determined main factors that have strong impact on the photosynthetic efficiency of *C. glomerata* in the Ilm.

MATERIAL AND METHODS

Study sites

A brief description of the Ilm is given in Ensminger et al. (this volume). Two locations in the metarhithral of the river were selected for observation. One location was situated upstream and the second downstream of the town Stadtilm. Sampling of algae for pigment analysis and measurements of chlorophyll fluorescence were undertaken from May 1997 to May 1998. If *C. glomerata* was available, samples were taken at each location every 14 days from one open site (exposed to full sunlight and therefore referred to as high-light or HL site) and one shaded site (characterised by seasonally varying degree of shade by streamside vegetation and therefore referred to as low-light or LL site). As samples from deep in a large tuft could experience lower light than those taken from the surface in winter, we took only upper filaments from the final 5 cm of the apical thallus end.

Chlorophyll fluorescence

A portable fluorometer (PAM-2000, Walz, Germany) was used to perform measurements of chlorophyll fluorescence of *C. glomerata* under natural sunlight *in situ* under water. A self constructed device allowed exposure of the filamentous algal thalli during determination of optimum quantum yield [$F_v/F_m = (F_m - F_0)/F_m$], which is a measure of the efficiency of open photosystem II units (PSII), and during determination of effective quantum yield [$\Delta F/F_m' = (F_v' - F_t)/F_m'$], as a measure of the efficiency of closed PSII units (Schreiber, Bilger & Neubauer 1994). First, effective quantum yield was probed by measurement of saturating flash induced maximal fluorescence (F_m') of the sample adapted to natural sunlight. Optimum quantum yield was determined after subsequent 5 min predarkening of the same sample by detection of (i) dark adapted basic fluorescence (F_0) under weak red modulated light (~0.18 µmol photons m^{-2} s^{-1}) and (ii) maximal fluorescence (F_m) during a 600 ms flash of white light (~6000 µmol photons m^{-2} s^{-1}). Light induced relative decrease of the effective quantum yield was calculated as $r(\Delta F/F_m')^* = 100 - (100 \times \Delta F/F_m')/(F_v/F_m)$.

Simultaneously with determination of effective quantum yield, incident PAR was measured close to the algal sample with a Li-192 underwater quantum sensor connected to the PAM-2000. Photosynthesis was expressed as relative electron transport rate (rETR) and was calculated as $rETR = PAR \times \Delta F/F_m' \times 0.5$ (Genty, Briantais & Baker 1989). The obtained rETR values were used to calculate photosynthesis-irradiance-curves (P-I-curves). Instead of the use of artificial light sources or filters, and in order to obtain different irradiance levels, data of repeated rETR measurements were used from different sampling dates combined with incident PAR measured simultaneously during the estimation of $\Delta F/F_m'$. The obtained steady state rETR

of the samples, thus, was calculated from measurements of 17 different sampling days during summer (April to September) and 11 sampling days during winter (October to March). These rETR data were first grouped into plants from open and from shaded sites, and afterwards each light condition was further divided into groups of summer and winter plants. Characteristic parameters of the P-I-curves (Henley 1993) are light saturated photosynthetic rate (P_{max}), initial slope of the nonsaturated photosynthetic rate (α_I), and optimum light intensity (I_k, that indicates the beginning of light saturated photosynthesis). These parameters were derived from least-square fits of the data to a model described by Eilers & Peeters (1988).

As rETR values are a ratio of fluorescence values, they are independent to biomass and can be compared across season.

Pigment analysis

Samples of *C. glomerata* (0.2 - 0.5 g freshweight) were rinsed several times in riverwater to remove loosely attached epiphytes and sediment. Then, samples were rinsed with distilled water, dry blotted for 20 s between four layers of filterpaper and immediately deepfrozen in liquid nitrogen. In the laboratory, deep frozen samples were homogenised with a mixer mill (Retsch, Germany), and pigments were extracted in 100 % acetone under dimlight conditions at 4 °C. Pigment content was determined spectrophotometrically according to Lichtenthaler (1987). The pigment pattern was further analysed by HPLC using the same extracts after addition of 15 % H_2O (Büch et al. 1994, Xyländer, Hagen & Braune 1996). Because epiphytic diatoms could not entirely be removed during the cleaning and homogenisation procedure, the amount of diatomic chlorophyll a (Chl a) was calculated from the amount of Chl c per sample. A fixed molar Chl a to Chl c ratio of 5.3 from own laboratory measurements was taken as a basis and the calculated value was subtracted from the total amount of Chl a.

Statistics

The software package SPSS release 9.0 (SPSS, Illinois, USA) was used to perform statistical tests and procedures.

RESULTS

Quantum efficiency of open PSII of *C. glomerata*

For pooled data that covered the whole sampling period measurements of optimum quantum efficiency (F_v/F_m) did not show significant differences among the four sites or between the upstream and downstream locations (one-way ANOVA, data not shown). In contrast, grouping of data from open and shaded sites into summer and winter populations revealed seasonal differences as well as differences in dependence on light conditions. Samples of HL summer

populations exhibited decreased F_v/F_m-values as compared to LL plants that were experimentally exposed for 8 min to full sunlight (Fig. 1). After 8 min of exposure to shade conditions before subsequent dark adaptation and measurement of F_v/F_m, a significant recovery from 0.649 to 0.693 (*t*-test, $p < 0.05$) was observed in HL plants. However, the values were still lower as compared to plants from shaded sites. During winter, the maximal photosynthetic capacity of all samples was increased in comparison to summer. The pattern of lower values in HL and higher values in LL plants was still observed (Fig. 1). The difference between HL and LL winter populations was significant and higher as compared with the summer populations (*t*-test, $p < 0.05$). After exposure to shade, HL and LL winter plants also exhibited increased F_v/F_m-values as shown above in summer populations.

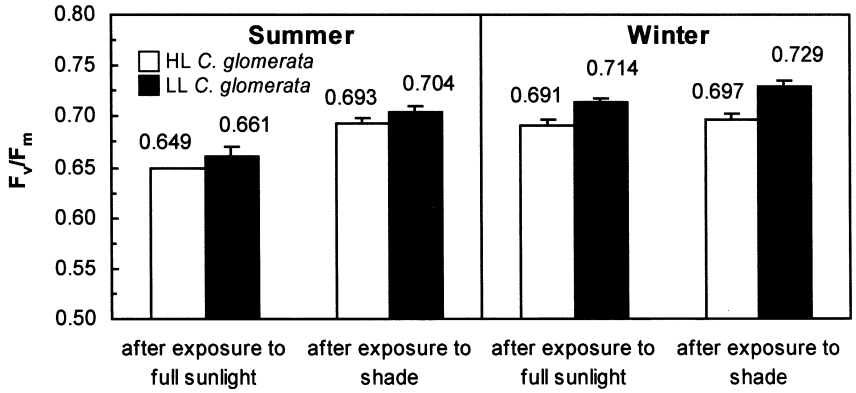

Figure 1 *Differences in efficiency of open PSII in* C. glomerata *from open sites (HL) and from shaded sites (LL) after exposure to full sunlight and shade.*

Light induced decrease of effective quantum yield

Light induced decrease of the effective quantum yield was observed in HL and LL summerplants of *C. glomerata* (Fig. 2). At low irradiance levels (< 200 µmol photons m^{-2} s^{-1}), the light induced decrease of $\Delta F/F_m'$ was almost the same in HL and in LL plants. Increased irradiance levels resulted in pronounced light induced decreases of $r(\Delta F/F_m')$* in all plants, but the decrease was higher in LL compared to HL plants.

Differences in characteristics of photosynthesis-irradiance-curves

Values of effective quantum yield were used to calculate rETR (Fig. 3). Data were analysed for seasonal and site specific differences. In summer populations of *C. glomerata* from both open and shaded sites, no differences were observed in the effectivity of rETR at non-saturating irradiances. This was indicated by values of 0.318 and 0.381 of the initial slope α_I for HL and LL plants, respectively (Fig. 3a and Table 1). At higher irradiance levels, *C. glomerata* from open sites showed higher rETR-values and a higher saturation factor I_k as compared to plants from shaded sites. This pattern was sharply contrasted by light saturation characteristics of rETR in winter populations of *C. glomerata* (Fig. 3b, Table 1). The P-I-characteristics of HL

and LL plants were almost identical. The initial slope α_I increased in winter as compared to summer populations, whereas $rETR_{max}$ decreased to almost identical values of 43.21 and 40.87 in HL and LL plants (Fig. 3b, Table 1). Similarly, the saturation factor I_K dropped down to values of 96.67 (HL) and 99.93 (LL).

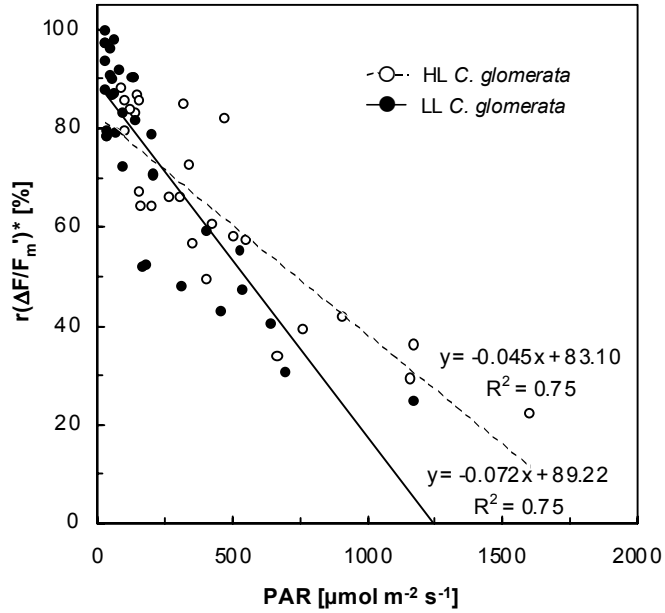

Figure 2 *Light induced decrease of the effective quantum yield of PS II in* C. glomerata *from open (open circles, dotted line) and from shaded (closed circles, full line) sites between April and September.*

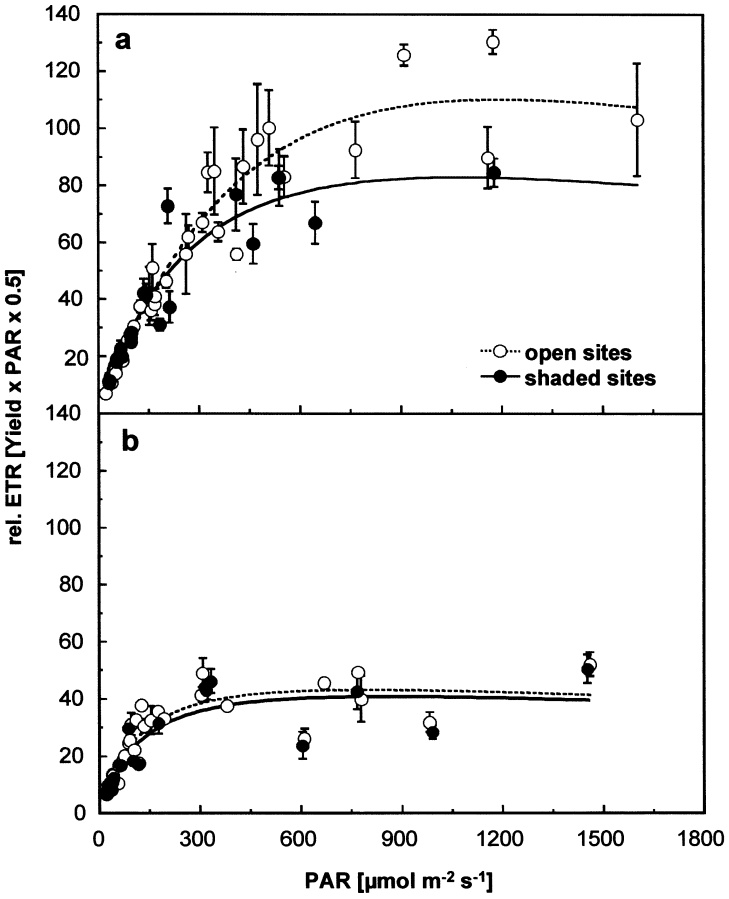

Figure 3 *Differences in photosynthesis-irradiance-characteristics of summer (a) and winter populations (b) of* C. glomerata *from open and shaded sites. Each data point represents the mean of 5 - 7 measurements ± S.E.*

Table 1 *Characteristic parameters of photosynthesis-irradiance-curves of* C. glomerata.

		α_I	$rETR_{max}$	I_K [µmol m^{-2} s^{-1}]	R_m	N
Summer	Open sites	0.318	110.17	346.45	0.970	215
	(±asymptotic S.E.)	0.032	4.04	37.11		
	Shaded sites	0.381	82.94	217.69	0.953	98
	(±asymptotic S.E.)	0.060	6.84	38.70		
Winter	Open sites	0.447	43.21	96.67	0.916	180
	(±asymptotic S.E.)	0.072	2.13	16.28		
	Shaded sites	0.409	40.87	99.93	0.861	75
	(±asymptotic S.E.)	0.144	3.64	10.70		

α_I = initial slope of the photosynthetic rate, $rETR_{max}$ = maximum relative electron transport rate, I_K = light saturation factor ($=rETR_{max}\ \alpha_I^{-1}$), R_m = multiple correlation coefficient, N = number of samples.

Changes in photosynthetic pigments

Seasonal and light dependent variations in the photosynthetic pigment pattern of *C. glomerata* were detected spectrophotometrically and by HPLC analysis (Fig. 4). Higher concentrations of Chl a and Chl b were found during summer in HL and LL plants as compared to winter populations (Fig. 4a, $p < 0.05$ for each group). Whereas in summer the Chl content in LL plants exceeded that in HL plants, the Chl content of LL winter populations declined under the value of the HL population. For the whole sampling period, as well as for summer and winter populations, the ratio of Chl a to Chl b was significantly higher in *C. glomerata* from open sites compared to plants from shaded sites (Fig. 4b, *t*-test, $p < 0.05$), significant seasonal differences were not found.

The ratio of carotenoids (car) to chlorophylls reflected higher carotenoid levels during winter in HL as well as in LL plants compared to the summer (Fig. 4c, *t*-test, $p < 0.05$). Only during summer did HL plants contain higher amounts of car than LL plants (*t*-test, $p < 0.05$) whereas in winter HL and LL plants showed equal values (Fig. 4c, *t*-test, n.s.). The ratio of the xanthophyll cycle pigments violaxanthin, antheraxanthin, and zeaxanthin (VAZ) per Chl a + Chl b was higher in HL summer populations than in HL winter populations (*t*-test, $p < 0.05$), whereas in LL plants a seasonal difference was not found and the ratio remained constant (Fig. 4d).

For the whole sampling period, the de-epoxidation state of the xanthophyll cycle pigments (0.5 A + Z)/(V + A + Z) was higher in HL than in LL plants (Fig. 4e, *t*-test, $p < 0.05$). For HL plants the seasonal comparison of the conversion state revealed higher summer values (*t*-test, $p < 0.05$) but in LL plants there was no significant difference between summer and winter samples. HL summer plants showed a higher conversion state than LL plants, but during winter no significant difference between HL and LL plants appeared.

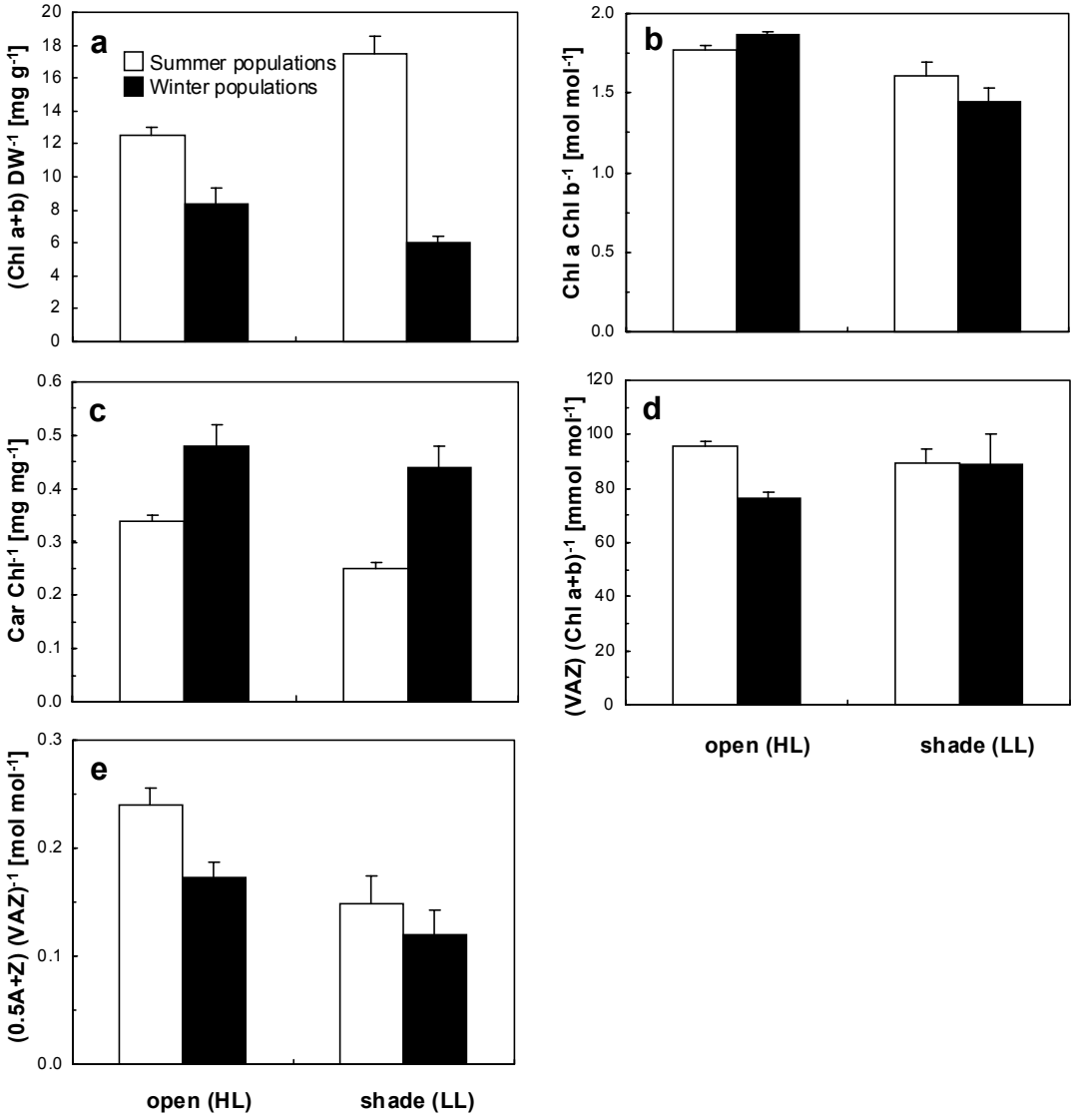

Figure 4 *Seasonal changes in pigment composition of summer (open bars) and winter populations (filled bars) of* C. glomerata *from the Ilm. Total chlorophyll (a), chlorophyll a per chlorophyll b (b), carotenoids per total chlorophyll (c), xanthophylls per total chlorophyll (d), conversion status of xanthophylls (e).*

Analysis of factors determining the photosynthetic efficiency of *C. glomerata*

Univariate analysis of variance was used to identify relationships between important environmental factors and the Chl fluorescence yield parameter $\Delta F/F_m'$, that indicates photosynthetic efficiency of *C. glomerata* under ambient light conditions. For this procedure photosynthetic active radiation (PAR, data not shown) at the algal surface during the determination of $\Delta F/F_m'$ was used in addition with the physical and chemical-parameters water temperature, oxygen saturation, conductivity, pH, global irradiance, effective irradiance, current velocity, NH_4^+-N, NO_3^--N, and SRP as well as mean, maximum and minimum discharge rate. A general linear model (GLM) with a forward selection method was applied to test all kinds of cross-relations between the measured parameters. From this complete set of physical and chemical parameters only PAR and water temperature were found to have statistically

significant effects on the photosynthetic efficiency ($p < 0.01$). A quantitative description of this relationship was achieved by using a random selection of 50 % of the observed cases of the $\Delta F/F_m'$ data of *C. glomerata* to estimate a multiple regression model (Fig. 5). The accuracy of the model was tested with the remaining 50 % of the cases. The predicted $\Delta F/F_m'$ values of *C. glomerata* were calculated on the basis of the corresponding PAR and water temperature data and were plotted against the observed values (Fig. 5). The accuracy of the model is expressed by the R-squared value $R^2 = 0.76$ of the linear regression line.

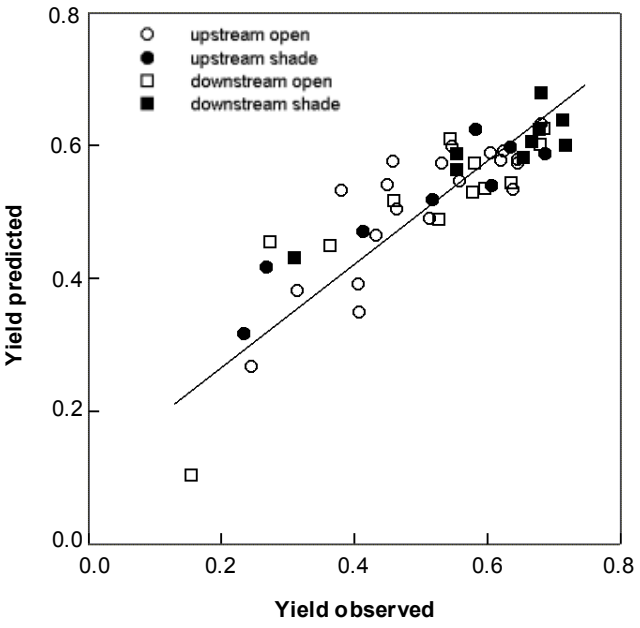

Figure 5 *Relationship between observed and predicted values of photosynthetic efficiency of* C. glomerata *by the multiple regression model* $\Delta F/F_m' = 0.522 - (I_A \times 0.00049) + (T_{H2O} \times 0.00918)$; I_A = *actual PAR during determination of effective quantum yield;* T_{H2O} = *water temperature. Regression ANOVA* $p < 0.05$. *The accuracy of the fit is given as* $R^2 = 0.76$.

DISCUSSION

Seasonal differences of the photosynthetic performance of *C. glomerata*

The optimum quantum yield given as the ratio of variable to maximal fluorescence (F_v/F_m) is a good parameter to illustrate changes in the efficiency of open PSII centres (Schreiber et al. 1994). It is widely used to assess effects of light on photosynthesis, to reflect photoinhibition and processes related to the activity of the xanthophyll cycle, and thus, allows the estimation of light stress and the regulation of photosynthetic energy conversion of PSII (e.g. Schreiber et al. 1994, Masojídek et al. 1999). According to this the results given in Fig. 1 reveal different degrees of photoinhibition in *C. glomerata*. During summer, exposure of *C. glomerata* to full sunlight resulted in decreased F_v/F_m values due to excessive light. Patterns of recovery, indicated by increased values, were observed after shorttime recovery under shade conditions. This revealed photoinhibitory up- and downregulation of PSII efficiency. This pattern was also apparent during winter when the observed irradiance levels amounted only 10 - 20 % of the values observed during April to September. Huner et al. (1998) and Ottander et al. (1993)

discussed photosynthesis and its inhibition not only in terms of light stress but also in relation to cold temperatures. Energy imbalances between the light energy absorbed through photochemistry versus energy utilised through metabolism are considered to cause over-excitation of PSII and thus, photoinhibition. Low temperature acclimation, that was assumed for winter populations of *C. glomerata*, therein resulted in the observed depression of photosynthetic efficiency after exposure to full winter sunlight.

Differences of photosynthetic parameters at open and at shaded sites

C. glomerata from open and from shaded sites revealed different abilities to regulate quantum efficiency of PSII to provide protection of the photosystem from damages of excessive photon fluence rates (Fig. 2). HL plants possessed a higher capacity to cope with high irradiance levels, as indicated by the higher percent values of the light induced decrease of $\Delta F/F_m'$ relative to F_v/F_m. At low irradiance levels, HL plants already revealed slightly decreased values compared to LL plants. This results coincided with the increased levels of carotenoids and xanthophyll cycle pigments observed in HL plants, as is discussed below. Thus, the less efficient use of the light resource by HL plants exhibited the properties of a photosystem that was used to deal with superfluous high fluence rates. The lesser light induced decrease of $\Delta F/F_m'$ at low light intensities, that was revealed by LL plants, accounted for their acclimation to low fluence rates and the need to make efficient use of the limited light resource. At fluence rates higher than 200 $\mu mol\ m^{-2}\ s^{-1}$, a higher decrease further reflected that these plants were not optimised to deal with high fluence rates (Fig. 2). Again, these results were related to carotenoid and xanthophyll cycle levels in the samples, as LL plants did not need to make intensive use of the protective and regulatory properties of this pigments (see below). These differences in light induced decrease of photosynthetic efficiency between plants from open and from shaded sites indeed demonstrate the ability of *C. glomerata* to develop different capacities of energy conversion in PSII in dependence on its light environment.

Acclimation to seasonal changes of environmental conditions were further shown in P-I-curves (Fig. 3). During summer P-I-characteristics differed between HL and LL plants (Fig. 3a). I_k and $rETR_{max}$ were higher in *C. glomerata* from open sites compared to plants from shaded sites and thus illustrated the acclimation of the HL plants to high fluence rates (Table 1). Winter plants strongly reflected seasonal changes of environmental conditions. Lower temperature and irradiance levels and lower irradiance maxima in addition to less pronounced differences of the light regime between open and shaded sites resulted in changes of the photosynthetic efficiency of *C. glomerata* (Fig. 3b, Table 1). During winter the values of $rETR_{max}$ and I_k strongly decreased and there were no more differences between HL and LL plants, together indicating the down-regulation of photosynthesis. Higher α_I-values as compared to summer expressed the

acclimation to low irradiance levels and the effective energy conversion under these light conditions.

The model that was used to calculate the P-I-curves did not detect any photoinhibition, as it would be indicated by a negative slope at supersaturating irradiance levels. This is presumably due to sampling and immediate measurement under natural light conditions. Thus, samples reflected steady state performance of the photosynthetic apparatus. This handling avoided cumulative effects by measurements at increasing irradiance steps, as it is usually done in P-I-experiments (Henley 1993). It does not implicate, that there was no inhibition of photosynthetic rates at all. As Henley (1993) pointed out, that patterns of photoinhibition are time dependent and P-I-changes encompass several mechanisms, most of them are better classified as photoregulation/protection or dynamic photoinhibition rather than damage to PSII (Krause & Weis 1991). Hence, only after prolonged exposure to supersaturating irradiances, maximum photosynthetic rate as well as saturation constants might show a decrease. In this respect, the implication of our results is that PSII electron transport capacity exceeds carboxylation capacity at saturating irradiances until a critical percentage of PSII centres have been deactivated. Thereafter, photosynthetic rate is limited by PSII electron transport at all irradiances (Henley 1993). This is consistent with the observed decline of $rETR_{max}$ in summer LL compared to summer HL samples, and in winter compared to summer samples.

Plasticity in pigment composition allows acclimation to different light regimes

The changes in photosynthetic efficiency were concomitant with the changes in pigment composition. Acclimation to low light environments was shown by increased amounts of Chl per dryweight in summer *C. glomerata* from shaded sites (Fig. 4a). The lower values observed in winter plants from shaded and from open sites may be due to two different processes: (a) Under low light conditions and cold temperatures *C. glomerata* is able to form resting cells. That is accompanied by degradation of chlorophylls and the formation of thicker cell walls. This process, of course, influences the ratio of Chl/dry weight. Thus, during winter occasionally darkgreen and brownish appearing filaments were found. (b) The photosynthetic apparatus acclimates to different environmental conditions by varying the amount of Chl. In this way *C. glomerata* seems to downregulate its light capture efficiency in an unfavourable environment. Higher Chl a to Chl b ratios in LL plants during summer as given in Fig. 4b were mainly due to higher amounts of Chl b. Localised in the antenna complex, Chl b increases the absorption efficiency of the photosystem and indicates the acclimation of the alga's photosystem to shade conditions. Such increases in the amount of Chl b were shown in unicellular green algae, that were converted from high light to low light growth conditions e.g. by Berner et al. (1989). In contrast, in winter, when biochemistry, not photochemistry, is limited by low ambient

temperatures, it might be useful to reduce the absorption efficiency in HL as well as in LL plants (Huner et al. 1998). This will minimise photoinhibition and potential damage from irradiance levels that result from excessive excitation energy under cold temperatures. The overall increase in carotenoids per Chl shown in Fig. 4c emphasises the above mentioned, as it indicates two important changes in pigmentation, the reduction of the total amount of Chl and an increase of carotenoids that serve as a protective agent to increase heat dissipation of absorbed energy (Demmig-Adams et al. 1996). Further, acclimation to light stress is indicated in Fig. 4d by the increased xanthophyll pool size in HL compared to LL summer plants. Acclimation to cold temperatures is expressed by the high levels of xanthophylls in winter plants from open and from shaded sites. It becomes obvious that in HL plants in summer and in HL and LL plants in winter, increased VAZ levels serve to protect the antenna from over excitation. The use that *C. glomerata* makes of this protective energy dissipating system is well illustrated in Fig. 4e by higher de-epoxidation levels in summer in HL plants compared to LL plants.

Taken together, the observed change of pigment composition in response to growth at low temperatures or high irradiances results in a reduction in light harvesting capacity together with an increased capacity to dissipate excess light as heat through carotenoids and the xanthophylls antheraxanthin and zeaxanthin.

Factors determining photosynthetic efficiency of *C. glomerata*

The linear regression formula suggests that photosynthetic efficiency of *C. glomerata* was mostly effected by PAR and water temperature (Fig. 5). As it was shown in Fig. 2, $\Delta F/F_m'$ decreases at increasing light intensities, and thus, explains the negative regression coefficient found for PAR. In addition, positive effects of an increase in water temperature, as suggested by the positive regression coefficient, indicate the importance of a balanced energy flux. At cold temperatures photochemical reactions are slowed down and photosynthetic efficiency has to be reduced to avoid photodamage. This reduction of photosynthetic efficiency depends on the pigmentation of *C. glomerata* and was shown to be evident in Fig. 4.

The quality of the model is given by the R-squared value $R^2 = 0.76$ (Fig. 5). It shows that reasonable estimates can be obtained from the regression equation. In fact, there are still factors that have not been included in the analysis, but which potentially account for unexplained variations of photosynthetic efficiency. Amongst these are for instance different levels of vitality of algal samples due to different cell ages, or stress caused by pulses of saline effluents (Ensminger et al. 2000, this volume).

In conclusion, the results demonstrate the complexity of influences environmental factors on photosynthetic activity of *C. glomerata*. Considering the properties of its life cycle (cf.

Ensminger et al. 2000), the plasticity of the photosynthetic apparatus allows the acclimation to different habitat conditions, and might provide an important advantage compared to species without these abilities. It is the aim of future studies to focus on differences in ecophysiology of photosynthesis of *C. glomerata* and other macroalgae.

ACKNOWLEDGEMENTS

This study was supported by grants of the Deutsche Forschungsgemeinschaft (DFG) Graduate Study Group *Analysis of Function and Regeneration of Degraded Ecosystems* (Förderkennzeichen GRK 266/1-96). Data on discharge and nutrients were kindly provided by Staatliches Umweltamt Erfurt. Deutscher Wetterdienst Offenbach provided data on global irradiance. Two anonymous reviewers provided helpful suggestions on the manuscript. Erica Froneberg (Vienna) kindly gave valuable comments on the English draft.

REFERENCES

Berner T., Dubinsky Z., Wyman K. & Falkowski P.G. (1989) Photoadaption and the "package" effect in *Dunaliella tertiolecta* (Chlorophyceae). *Journal of Phycology* **25**, 70-78.

Büch K., Stransky H., Bigus H.J. & Hager A. (1994) Enhancement by artificial electron acceptors of thylakoid lumen acidification and zeaxanthin formation. *Journal of Plant Physiology* **144**, 641-648.

Demmig-Adams B., Gilmore A.M., Adams W.A. (1996) In vivo functions of carotenoids in higher plants. *The FASEB Journal* **10**, 403-412.

Dodds W.K. (1991) Community interactions between the filamentous alga *Cladophora glomerata* (L.) Kuetzing, its epiphytes, and epiphyte grazers. *Oecologia* **85**, 572-580.

Eilers P.H.C. & Peeters J.C.H. (1988) A model for the relationship between light intensity and the rate of photosynthesis in phytoplankton. *Ecological Modeling* **42**, 199-215.

Ensminger I., Hagen C. & Braune W. (2000) Strategies providing success in a variable habitat: I. Relationships of environmental factors and dominance of *Cladophora glomerata. Plant, Cell and Environment* (in press).

Genty B.E., Briantais J.M. & Baker N.R. (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta* **990**, 87-92.

Graham J.M., Auer M.T., Canale R.P. & Hoffmann J.P. (1982) Ecological studies and mathematical modeling of *Cladophora* in Lake Huron: 4. Photosynthesis and respiration as functions of light and temperature. *Journal of Great Lakes Research* **8**, 100-111.

Häder D.P., Herrman H., Schäfer J. & Santas R. (1997) Photosynthetic fluorescence induction and oxygen production in two mediterranean *Cladophora* species measured on site. *Aquatic Botany* **56**, 253-264.

Hanelt D., Hupperts K. & Nultsch W. (1993) Daily course of photosynthesis and photoinhibition in marine macroalgae investigated in the laboratory and field. *Marine Ecology Progress Series* **97**, 31-37.

Henley W.J. (1993) Measurement and interpretation of photosynthetic light-response curves in algae in the context of photoinhibition and diel changes. *Journal of Phycology* **29**, 729-739.

Huner P.A., Öquist G. & Sarhan, F. (1998) Energy balance and acclimation to light and cold. *Trends in Plant Science* **3**, 224-230.

Krause G.H. & Weis E. (1991) Chlorophyll fluorescence and photosynthesis: the basics. *Annual Review of Plant Physiology and Plant Molecular Biology* **42**, 13-49.

Lester W.W., Adams M.S. & Farmer A.M. (1988) Effects of light and temperature on photosynthesis of the nuisance alga *Cladophora glomerata* (L.) Kutz from Green Bay, Lake Michigan. *New Phytologist* **109**, 53-58.

Leukart P. & Hanelt D. (1995) Light requirements for photosynthesis and growth in several macroalgae from a small soft-water stream in the Spessart-Mountains, Germany. *Phycologia* **34**, 528-532.

Lichtenthaler H.K. (1987) Chlorophylls and Carotenoids - Pigments of photosynthetic biomembranes. *Methods in Enzymology* **148**, 350-382.

Liebmann H. (1962) *Handbuch der Frischwasser und Abwasserbiologie*, *I*. Gustav Fischer Verlag, Jena.

Masojídek J., Torzillo G., Koblízek J., Kopecký J., Bernardini P., Sacchi A. & Komenda J. (1999) Photoadaptation of two members of the chlorophyta (*Scendesmus* and *Chlorella*) in laboratory and outdoor cultures: changes in chlorophyll fluorescence quenching and the xanthophyll cycle. *Planta* **209**, 126-135.

Neidhardt J., Benemann J.R., Zhang L. & Melis A. (1998) Relationship between chronic photoinhibition, light-harvesting chlorophyll antenna size and photosynthetic productivity in *Dunaliella salina* (green algae). *Photosynthesis Research* **56**, 175-184.

Ottander C., Hundall T., Andersson B., Huner P.A. & Öquist G. (1993) Photosystem II reaction centres stay intact during low temperature photoinhibition. *Photosynthesis Research* **35**, 191-200.

Schreiber U., Bilger W. & Neubauer C. (1994) Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of in vivo photosynthesis. In *Ecophysiology of photosynthesis* (eds Schulze E.D. & Caldwell M.M.), pp. 49-70. Springer-Verlag, Berlin..

Sundby C., McCaffery S. & Anderson M. (1993) Turnover of the photosystem II D1 protein in higher plants under photoinhibitory and nonphotoinhibitory irradiance. *Journal of Biological Chemistry* **268**, 25476-25482.

Uhrmacher S., Hanelt D. & Nultsch W. (1997) Zeaxanthin content and the degree of photoinhibition are linearly correlated in the brown alga *Dictyota dichotoma*. *Marine Biology* **123**, 159-165.

Xyländer M., Hagen C. & Braune W. (1996) Mercury increases light susceptibility in the green alga *Haematococcus lacustris*. *Botanica Acta* **109**, 222-228.

7.3 Strategies providing success in a variable habitat: III. Dynamic control of photosynthesis in *Cladophora glomerata*

INGO ENSMINGER, MARCO XYLÄNDER, CHRISTOPH HAGEN &
WOLFRAM BRAUNE

Plant, Cell & Environment (in Begutachtung)

ABSTRACT

Diurnal patterns of photosynthesis were studied in July and April populations of *Cladophora glomerata* (L.) Kütz. from open and from shaded sites. Summer samples showed decreased efficiency of open photosystem II at noon and only slight differences between samples from open and shaded sites. Electron transport rate was limited in shade plants at highest fluence rates, non-photochemical quenching (NPQ) revealed faster up- and downregulation in samples from open sites. Daily course of de-epoxidation was not linearly correlated with the course of NPQ. The comparison of samples from open and from shaded sites revealed higher capacity of thermal energy dissipation, and increased amounts of xanthophyll-cycle pigments (21 %) in samples from open sites. In April, downregulation of the efficiency of open photosystem II was observed and related to lower water temperature, and hence, increased excitation pressure. Increases of 21 % in the pool size of xanthophyll-cycle pigments in April compared to summer suggested higher levels of thermal energy dissipation via de-epoxidised xanthophylls. Similar to summer samples, the amount of xanthophyll-cycle pigments was 20 % higher in samples from open sites. Acclimation of *C. glomerata* to growth light conditions was further shown by experimental induction of NPQ, indicating increases of NPQ of 23 % and of the reversible component of NPQ of 77 % in open site samples. Temperature effects on photosynthetic rate exhibited non-linear relationships and different optimum temperatures of electron transport rate and oxygen evolution.

INTRODUCTION

Cladophora glomerata (L.) Kütz. is one of the most widespread freshwater macroalgal species (Dodds & Gudder 1992, Whitton 1970). It can be found almost all over the year and in various habitats, such at sites exposed to the full sunlight as well as at shaded sites (Ensminger, Hagen & Braune 2000a). Thus, in our sampling region, the river Ilm, in May daily sum of global irradiance amounted to more than 2800 J m^{-2}, whereas in December values as low as 32 J m^{-2} were measured (for a brief description see Ensminger et al. 2000a). This pattern can be

modified by development and abscission of foliage of streamside vegetation or water turbidity due to increased discharge and sediment transport. In open habitats, photosynthetic active radiation effective on the alga's surface reached a fraction of almost 80 % of PAR measured outside the stream, whereas in shaded habitats at the same time it rarely exceeded 8 % of PAR (Ensminger et al. 2000a). Water temperature ranged between 1 °C in January and almost 20 °C in July (Ensminger et al. 2000a). In comparison to PAR, diurnal variations in temperature were rather small, not exceeding 3 to 4 degrees between morning and evening.

These spatial and temporal variations of environmental conditions demand constant adjustment of primary and secondary photosynthetic processes of *C. glomerata*, thus providing success in a variable habitat. Logan et al. (1998) showed that higher plants growing in light exposed locations often experience an imbalance between energy absorption and subsequent conversion through electron transport on the one hand, and photosynthetic light utilisation by carbon fixation on the other hand, because they cannot utilise all of the light absorbed for photosynthesis. The light absorbed in excess potentially causes photooxidative damage of photosystem II (PSII). At low temperatures, this dangerous imbalance gets even worse, because electron transport and reactions of the Calvin cycle are more strongly limited than temperature independent processes of light absorption (Huner et al. 1993). Hence, plants have to adjust rapidly their photosynthetic apparatus to these changing conditions in order to protect PSII photochemistry from excessive excitation and potential damage (Huner et al. 1993). Protective mechanisms include the activity of the xanthophyll cycle, which is characterised in *C. glomerata* by increased de-epoxidation of violaxanthin into anthera- and zeaxanthin under excessive light conditions (for a review see e.g. Demmig-Adams, Gilmore & Adams III 1996). Formation of de-epoxidised xanthophylls results in increased thermal dissipation of absorbed energy (non-photochemical quenching, NPQ). Shifts in the amount of antenna proteins and chlorophylls serve to obtain absorption control. In the green alga *Dunaliella* sp. Thompson, Guo & Harrison (1992) found light harvesting to be decreased by reduced chlorophyll concentrations in order to compensate for reduction in CO_2 fixation at low temperatures. Increases in the amount of chlorophyll b were shown in unicellular green algae, that were converted from high light to low light growth conditions (e.g. Berner et al. 1989).

Taken together, plasticity of photosynthesis is a major attribute that enables photoautotrophs to balance energy conversion and energy consumption by acclimation of the photosynthetic apparatus in order to persist under variable environmental conditions. In contrast to marine species, little work has been done regarding the regulation of photosynthesis in freshwater macroalgae under different light and temperature conditions. Diurnal patterns of photosynthesis were studied for example by Hanelt, Huppertz & Nultsch (1993) or Hanelt, Li & Nultsch

(1994), and acclimation of photosynthesis to different depth and thus, different light environments, was investigated by Silva et al. (1998) in the marine genus *Gelidium*. Similarly, most of the autecological response of algae to temperature was obtained from research on marine phytoplankton, as was outlined by DeNicola (1996).

In this paper, plasticity of photosynthesis in *C. glomerata* from open and from shaded sites was assessed by measurements of chlorophyll fluorescence, pigment composition, and oxygen production in order to determine diurnal patterns and seasonal differences. In particular, we aimed on regulatory processes including non-photochemical energy dissipation and the composition of photosynthetic pigments in *C. glomerata* from different habitats as well as on effects of temperature that might prevent overexcitation and extended reduction of photosynthetic efficiency by photodamage.

MATERIALS AND METHODS

Site description, plant material and diurnal field measurements

In situ measurements of chlorophyll fluorescence were performed at a location in the hyporhithral of the river Ilm, Thuringia/Germany. Samples of *C. glomerata* were taken from an open (exposed to full sunlight and therefore referred to as high-light or HL site) and a shaded site (characterised by seasonally varying degree of shade by streamside vegetation and therefore referred to as low-light or LL site). Diurnal patterns of photosynthesis and the activity of the xanthophyll cycle were investigated twice (July 1998 and April 1999) by collecting *C. glomerata* attached to stones at the open as well as at the shaded site. In order to describe sustained differences of fluorescence parameters due to growth in different light environments, HL and LL samples were used simultaneously. Therefore, the day prior to the measurements, a number of thalli from the open HL site was collected and exposed in the shaded LL habitat, and vice versa. All algae were allowed to adapt at the same water depth to the new environment during the night, and measurements were started early in the morning. As samples from deep in a large tuft could experience lower light than those taken from the surface, we took only upper filaments from the final 5 cm of the apical thallus end. Measurements of chlorophyll fluorescence were undertaken about every hour, at least every two hours samples were preserved for later analysis of photosynthetic pigments (see below). Daily course of underwater photosynthetic active radiation (PAR) was measured simultaneously at HL and LL sites using two photodiodes (BPW-21, Centronics, UK) calibrated prior to a Li-192 underwater quantum sensor (LiCor, Lincoln, USA). Water temperature in the field was monitored with a WTW-multimeter (WTW, Germany).

Chlorophyll fluorescence in the field

Field measurements of chlorophyll fluorescence of *C. glomerata* were performed using a portable fluorometer (PAM-2000, Walz, Germany) under natural sunlight *in situ* under water. (Ensminger et al. 2000b). Algal thalli were exposed in a self constructed device during determination of optimum quantum yield [$F_v/F_m = (F_m - F_0)/F_m$], a measure of the efficiency of open PSII units, and during determination of effective quantum yield [$\Delta F/F_m' = (F_m' - F_t)/F_m'$], as a measure of the efficiency of closed PSII units (Schreiber, Bilger & Neubauer 1994). First, effective quantum yield was probed by measurement of saturating flash induced maximal fluorescence (F_m') of the sample adapted to natural sunlight. Subsequently samples were darkened for 5 min and optimum quantum yield of the same sample was determined by detection of (i) dark adapted basic fluorescence (F_0) under weak red modulated light (~0.18 µmol photons m^{-2} s^{-1}) and (ii) dark adapted maximal fluorescence (F_m) during a 600 ms flash of white light (~6000 µmol photons m^{-2} s^{-1}). Non-photochemical quenching, as a measure of radiationless dissipation of absorbed light energy, was calculated for July samples according to Bilger & Björkman (1990) as $NPQ = (F_m - F_m')/F_m'$.

Incident PAR was measured close to the algal sample simultaneously with determination of effective quantum yield (Li-192 underwater quantum sensor connected to the PAM-2000). The rate of photosynthesis by means of chlorophyll fluorescence was expressed as relative electron transport rate (rETR) and was calculated as $rETR = PAR \times \Delta F/F_m' \times 0.5$ (Genty, Briantais & Baker 1989). This assumes: $\Delta F/F_m'$ represents the effective photochemical quantum yield, PAR corresponds to the flux density of incident photosynthetic active radiation and transport of two quanta, as two photosystems are involved (factor 0.5). Furthermore, as rETR values are a ratio of fluorescence values, they are independent to biomass and can be compared across season.

Effects of high fluence rates on quenching characteristics of HL and LL samples

Samples of *C. glomerata* from HL and LL sites were transferred to an aquaculture facility (Forellengrund, Mellingen) close to the sampling site and protected against direct sunlight under a hatcheries roof outside. Samples were kept in an open plastic tank overnight to allow complete recovery. During recovery and experiments continuous flow of fresh Ilm water was provided through the tank. All experiments were undertaken within the tank, in order to control light conditions. Chl fluorescence was measured with the PAM-2000 as described for the diurnal measurements, except that a white light source (Tungsten halogen, OSRAM, Germany) was used as actinic light. Induction of quenching characteristics was achieved by the following routine: Each sample was illuminated for 5 s with weak far red light (15 µmol m^{-2} s^{-1}, $\lambda > 700$ nm) and subsequently predarkened for 5 min prior to the estimation of F_0 and F_m to calculate

F_v/F_m. This procedure was repeated three times. Afterwards, samples were exposed to high fluence rates (1550 µmol photons m^{-2} s^{-1}, 12 min) followed by dim light conditions (15 - 20 µmol photons m^{-2} s^{-1}, 12 min). This sequence was repeated twice and followed finally by 30 min of high fluence rate and 21 min of dim light. Every three minutes F_0', F_t and F_m' were measured by PAM-2000 routine settings to calculate $\Delta F/F_m'$ and NPQ. The reversible component of NPQ was determined according to Demmig-Adams (1998) by subtracting the sustained component of NPQ (= F_m/F_mrec - 1, where $F_m$rec is the partially recovered F_m after 21 min of final recovery under dim light) from the maximum NPQ during high light (= $F_m/F_m' - 1$). Non-photochemical quenching of F_0 was calculated as $(F_0 - F_0')/F_0'$ and used to describe quenching capacity of the antenna of PSII (Demmig-Adams 1998).

Comparison of Chl fluorescence and O_2-evolution rates in the laboratory

Photosynthesis-irradiance-curves (P-I-curves) were derived from laboratory measurements of Chl fluorescence and O_2-evolution rates to study the effect of temperature on photosynthesis of a December population of *C. glomerata* (mean water temperature 5 °C). Pulse modulated *in vivo* Chl fluorescence of algal samples (Schreiber 1986) was recorded after at least 3 h storage of the samples at the selected temperature and 20 min predarkening in a thermostatically controlled O_2-measuring suspension cuvette (DW 2/2, Bachofer, Reutlingen, Germany) using a PAM 101-103 fluorometer (Walz, Effeltrich, Germany). At each temperature (1, 5, 10, 15, 20 and 25 °C), P-I-curves were obtained from measurements of 3 different samples. F_0 was determined by a weak, modulated light of approximately 0.1 µmol photons m^{-2} s^{-1}, and F_m by a saturating 600 ms flash of 5500 µmol photons m^{-2} s^{-1} (white light). After a lag phase of 60 s, Chl fluorescence transients (F_t) were induced by continuous actinic irradiation of 6 min at each of the following fluence rates of 6.5, 11, 20, 33, 80, 190, 450, 790, 1100, 1660 and 1950 µmol m^{-2} s^{-1}. Actinic light was provided by a halogen white light source (KS 1500, Schott, Germany) equipped with a cut-off filter ($\lambda_{1/2}$ = 600 nm). Simultaneously with triggering of actinic illumination, the modulation frequency of the measuring light was automatically increased from 1.6 kHz to 100 kHz. To determine F_m', saturating pulses were applied every 3 min and were used to calculate effective quantum yield $\Delta F/F_m'$. NPQ was expressed according to Bilger & Björkman (1990) as mentioned before. During the predarkening period respiration and during actinic light exposure O_2-evolution were measured by a Clark-type oxygen electrode connected to the cuvette. Respiration and O_2-evolution were used to calculate gross photosynthetic O_2-production per Chl. From both, Chl fluorescence and O_2-evolution measurements, P-I-curves including characteristic parameters were calculated, such as light saturated photosynthetic rate (P_{max}), initial slope of the non-saturated photosynthetic rate (α_I), and optimum light intensity

(I_k, that indicates the beginning of light saturated photosynthesis; Henley 1993). These parameters were derived from least-square fits of the data to a model described by Eilers & Peeters (1988).

Pigment analysis

After determination of optimal quantum yield, the same samples of *C. glomerata* (0.05 - 0.1 g freshweight) were rinsed several times in riverwater to remove loosely attached epiphytes, subsequently rinsed with distilled water, dry blotted for 20 s between four layers of filter-paper and immediately deepfrozen in liquid nitrogen (Ensminger et al. 2000b). Deep frozen samples were homogenised with a mixer mill (Retsch, Germany) in the laboratory, and pigments were extracted in 100 % acetone under dimlight conditions at 4 °C. The pigment pattern was analysed by HPLC after addition of 15 % H_2O (Büch et al. 1994, Xyländer, Hagen & Braune 1996). Because epiphytic diatoms could not entirely be removed during the cleaning and homogenisation procedure, the amount of diatomic chlorophyll a (Chl a) was calculated from the amount of Chl c per sample. A fixed molar Chl a to Chl c ratio of 5.3 from own laboratory measurements was taken as a basis and the calculated value was subtracted from the total amount of Chl a. Molar amounts of pigments were used to calculate the ratio of Chl a per Chl b, the amount of the xanthophylls violaxanthin, antheraxanthin and zeaxanthin per Chl (VAZ Chl^{-1}), and of the de-epoxidation status (DEPS) of the xanthophylls [$(0.5A+Z)\ (VAZ)^{-1}$].

RESULTS

Diurnal changes of photosynthetic activity

July populations of *C. glomerata*

July populations of *C. glomerata* from open and shaded sites showed distinct diurnal patterns of photosynthetic activity. That was accompanied by slight diurnal changes with mean water temperature of 18.2 °C (± 0.3 °C S.E.). In algae exposed to HL conditions, F_v/F_m decreased slightly with increases in PAR (Fig. 1A). Lowest values were obtained around 13:30, when PAR was highest. Shifts in PAR, as observed during the afternoon, were accompanied by moderate inverse shifts of F_v/F_m. In the evening, F_v/F_m levels were similar to the level observed in the morning. There was only a slight difference between samples from HL compared to those from LL sites.

A closer relation to increasing PAR was revealed by rETR (Fig 1B). At 12:30 for example, values of LL plants revealed a maximum. Similar rates in LL and HL were demonstrated at moderate insulation (400 µmol photons $m^{-2}\ s^{-1}$), whereas HL plants revealed higher values after PAR had reached at least 1000 µmol photons $m^{-2}\ s^{-1}$. During the afternoon, at decreasing fluence rates, rETR levels again reached similar values as measured during the morning.

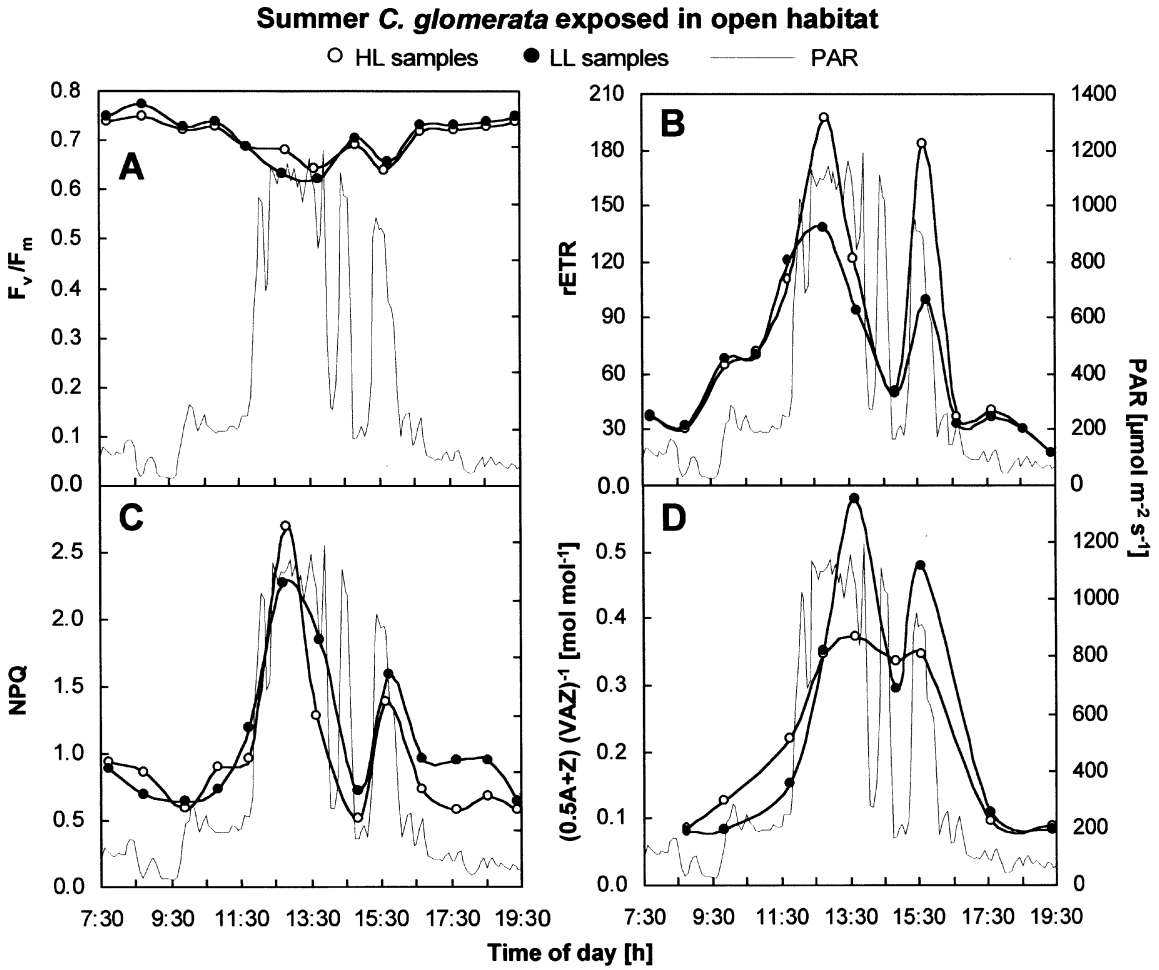

Figure 1 *Diurnal course of photosynthetic active radiation (PAR), photosynthetic parameters and xanthophyll pattern in July populations of* C. glomerata *from open (HL) and shaded (LL) sites. All samples were exposed to full sunlight (HL). Optimum PSII quenching efficiency at open units, F_v/F_m (A); relative electron transport rate, rETR (B); non-photochemical quenching, NPQ (C); de-epoxidation state of the xanthophyll cycle, (0.5A+Z) $(VAZ)^{-1}$ (D). Each datapoint represents the mean of n = 2 - 3 measurements.*

NPQ increased at increasing fluence rates, with HL plants showing highest values when PAR was highest (Fig. 1C). Fluctuations from high fluence rates to PAR less than 1000 µmol photons m^{-2} s^{-1} resulted in a faster subsequent decrease of NPQ in HL plants as was apparent in LL plants. Both, HL and LL *C. glomerata* finally showed values of NPQ that were well below the values observed during the morning.

The de-epoxidation status of the xanthophyll cycle pigments (DEPS) of *C. glomerata* (Fig. 1D), that is expressed by (0.5A+Z) $(VAZ)^{-1}$, showed straight increases in HL plants upon exposure to increasing fluence rates. At noon DEPS reached a plateau that was followed by a straight decline to values already observed during the morning. In contrast, LL *C. glomerata* revealed slower increases of DEPS, reached a higher maximum DEPS at high fluence rates as compared to HL plants, and did not develop a plateau. Short fluctuations in fluence rate during the day resulted in fast reactions of DEPS in LL plants.

Due to sunflecks, July *C. glomerata* exposed to shade conditions temporary experienced shorttime (min) maximum fluence rates of 600 µmol photons m^{-2} s^{-1} (data not shown). The diurnal course of these low levels of PAR did not result in distinct patterns of the photosynthetic parameters measured and of DEPS. In general, the level of rETR in HL plants was slightly reduced compared to the level in LL plants. In addition, DEPS showed only weak activity of the xanthophyll cycle and no distinct difference between HL and LL samples.

April populations of *C. glomerata*

Due to lacking foliage of streamside tree vegetation in April, the daily course of PAR showed distinct diurnal changes at the HL as well as at the LL site (Fig. 2 and Fig. 3). Maximum levels were 1000 µmol photons m^{-2} s^{-1} at the HL and about 700 µmol photons m^{-2} s^{-1} at the LL site, whereas water temperature was at 10.5 °C (± 0.3 °C S.E.).

In contrast to July populations, the diurnal course of F_v/F_m clearly responded inversely to the fluence rate, with HL samples most times showing higher values than LL samples (Fig. 2A). At fluence rates higher than 400 µmol photons m^{-2} s^{-1}, April samples showed already a decrease of F_v/F_m below 0.5, whereas from July *C. glomerata* values higher than 0.7 were derived upon exposure to the same fluence rate (Fig. 1A). Relative electron transport rate increased with increases in PAR in April *C. glomerata*, with slightly higher rates in HL compared to LL samples (Fig. 2B). However, at similar fluence rates, these values were lower in April samples as compared to July samples. At 11:30 and at a fluence rate of 400 µmol photons m^{-2} s^{-1}, rETR in April samples was 82 and 67 in HL and LL, respectively (Fig. 2B). At the same fluence rate in July, at 10:00 and at 400 µmol photons m^{-2} s^{-1}, rETR reached 110 and 120 in HL and LL samples, respectively (Fig. 1B).

F_v/F_m values of April *C. glomerata* measured at LL conditions exhibited a diurnal course similar to the values observed at the HL site (Fig. 3A). Because maximum PAR at noon was not as high as observed at the HL site (Fig. 3A), F_v/F_m was reduced to only 0.50 in LL and 0.58 in HL plants at about 500 µmol photons m^{-2} s^{-1}. The relative electron transport rate described a similar course as was derived from measurements at the open site. Obtained values, as for example at 12:30 at 500 µmol photons m^{-2} s^{-1} were 90 for HL and 76 for LL samples and thus, comparable to those measured at the open HL site (Fig. 2B and Fig. 3B).

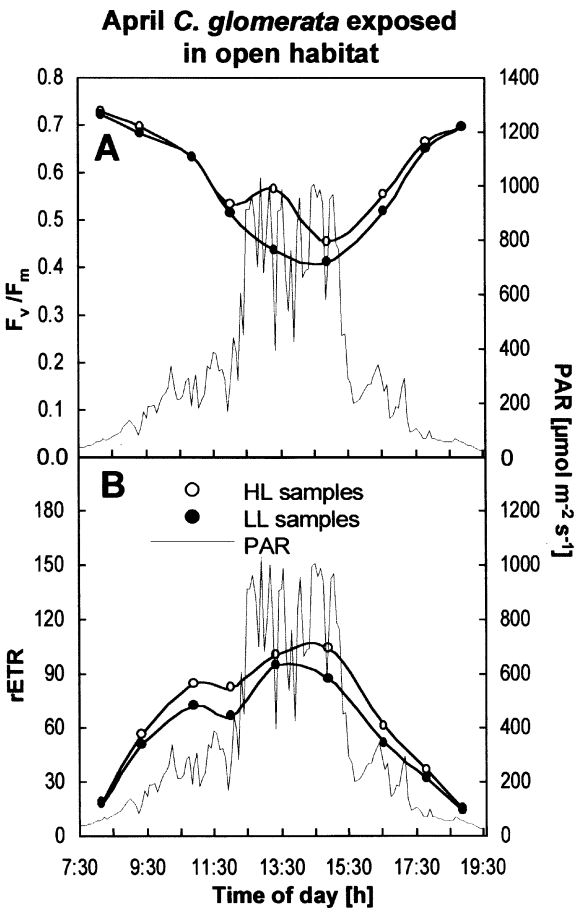

Figure 2 *Diurnal course of photosynthetic active radiation (PAR), and of photosynthetic parameters of April populations of* C. glomerata *from open (HL) and shaded (LL) sites exposed to open conditions (HL). Optimum PSII efficiency at open units, F_v/F_m (A); relative electron transport rate, rETR (B). Each datapoint represents the mean of n = 2 - 3 measurements.*

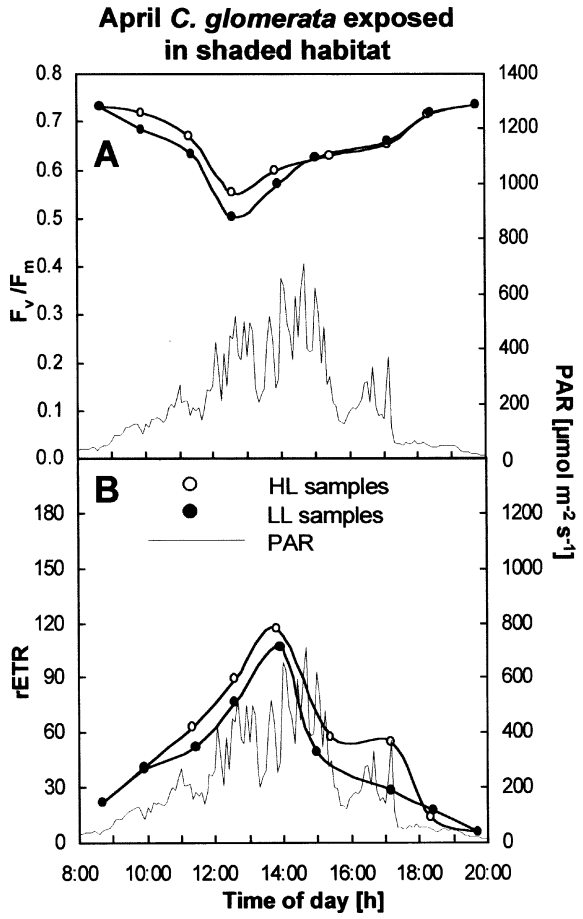

Figure 3 *Diurnal course of photosynthetic active radiation (PAR), and of photosynthetic parameters of April populations of* C. glomerata *from open (HL) and shaded (LL) sites exposed to shade conditions (LL). For abbreviations see Fig. 2.*

Photosynthetic pigments of July and April *C. glomerata*

Spectrophotometrical determination and HPLC analysis were used to detect light dependent variations in photosynthetic pigments.

The overall comparison of July and April *C. glomerata* revealed no statistically significant difference in the total amount of Chl a and Chl b (*t*-test, n.s.). However, in July the ratio of Chl a per Chl b was increased by 9 % compared to April samples, whereas the ratio of VAZ per Chl was decreased by 21 % in July compared to April samples (*t*-test, each $p < 0.05$).

In July, *C. glomerata* from HL and from LL sites did not show any difference in the total amount of Chl a and Chl b (Table 1, *t*-test, n.s.). In HL samples the ratio of Chl a per Chl b was increased by 14 % (Table 1, *t*-test, $p < 0.1$), and the ratio of VAZ per Chl was increased by 21 % compared to LL samples (Table 1, *t*-test, $p < 0.05$).

In April, in *C. glomerata* from LL sites the total amount of Chl a and Chl b was 36 % higher compared to HL samples, whereas in HL algae the ratio of Chl a per Chl b as well as the ratio

of VAZ per Chl were increased by 9 % and by 20 %, respectively (Table 1, *t*-test, each $p < 0.05$).

Table 1 *Mean values of total chlorophyll per dryweight ((Chl a + Chl b) DW^{-1}), the ratios chlorophyll a per chlorophyll b (Chl a Chl b^{-1}) and xanthophylls per total chlorophyll (VAZ Chl $(a+b)^{-1}$) of* C. glomerata *from open (HL) and from shaded (LL) sites. Given values are means of n = 15 - 20 ± S.E.*

	HL		LL	
	July	April	July	April
(Chl a + Chl b) DW^{-1} [mg g^{-1}]	10.38 ± 0.62	10.99 ± 1.21	10.54 ± 0.53	14.99 ± 1.21
Chl a Chl b^{-1} [mol mol^{-1}]	1.63 ± 0.02	1.85 ± 0.03	1.49 ± 0.08	1.70 ± 0.04
VAZ Chl $(a+b)^{-1}$ [mmol mol^{-1}]	93.57 ± 2.47	112.31 ± 4.50	77.24 ± 5.21	93.49 ± 7.27

Effect of artificial high light exposure on energy conversion of HL and LL grown *C. glomerata*

Using alternating sequences of high fluence rates and subsequent recovery of PSII under dim light conditions, quenching characteristics of HL and LL grown *C. glomerata* under controlled conditions were assessed (Fig. 4). F_v/F_m levels of 0.79 (in HL) and 0.78 (in LL) prior to the experiment indicated no difference in optimum quantum efficiency, and demonstrated complete recovery of PSII in HL samples (Fig. 4A). Exposure to 1550 µmol photons m^{-2} s^{-1} resulted in decreases of $\Delta F/F_m'$ in HL as well as in LL samples. During the recovery periods under dim light (15 - 20 µmol photons m^{-2} s^{-1}) the decrease of $\Delta F/F_m'$ was mostly revertible. The amount of revertible $\Delta F/F_m'$ was slightly higher in LL compared to HL *C. glomerata* and decreased with increasing exposure duration to high light conditions. During each dim light period the obtained values of $\Delta F/F_m'$ constantly decreased compared to the values of the respective former dim light period.

Differences in the protection efficiency of PSII between HL and LL samples of *C. glomerata* were also reflected by non-photochemical quenching behaviour (Fig. 4B). Exposure of *C. glomerata* to high fluence rates resulted in a fast increase of NPQ. Under subsequent dim light conditions NPQ became reduced, but if again exposed to high fluence rates, NPQ was re-established very fast up to the level developed during the former exposure to 1550 µmol photons m^{-2} s^{-1}. NPQ thus, demonstrated a saturation kinetic. The kinetics of NPQ in samples of HL and LL *C. glomerata*, showed distinct differences especially during the rate of up- and downregulation of NPQ. HL plants expressed fast and high level performance of building up NPQ, whereas LL plants acted slower. Data for quenching characteristics after the final 21 min of illumination with 1550 µmol photons m^{-2} s^{-1} are given in table 2. These data indicate higher levels of energy dissipation in PSII in HL samples. At the end of the last exposure to high fluence rates, 23 % higher NPQ was obtained from HL (2.43) compared to LL plants (1.97, Fig.

4B and Table 2). Similarly, 77 % higher level of energy dissipation as shown by the reversible portion of NPQ in HL (1.17) versus LL plants (0.659). Furthermore, the level of quenching of initial fluorescence $(F_0 - F_0')/F_0'$ indicated greater values in HL *C. glomerata* after exposure to high fluence rates, and hence, higher quenching of the PSII antenna fluorescence in HL compared to LL samples (Table 2).

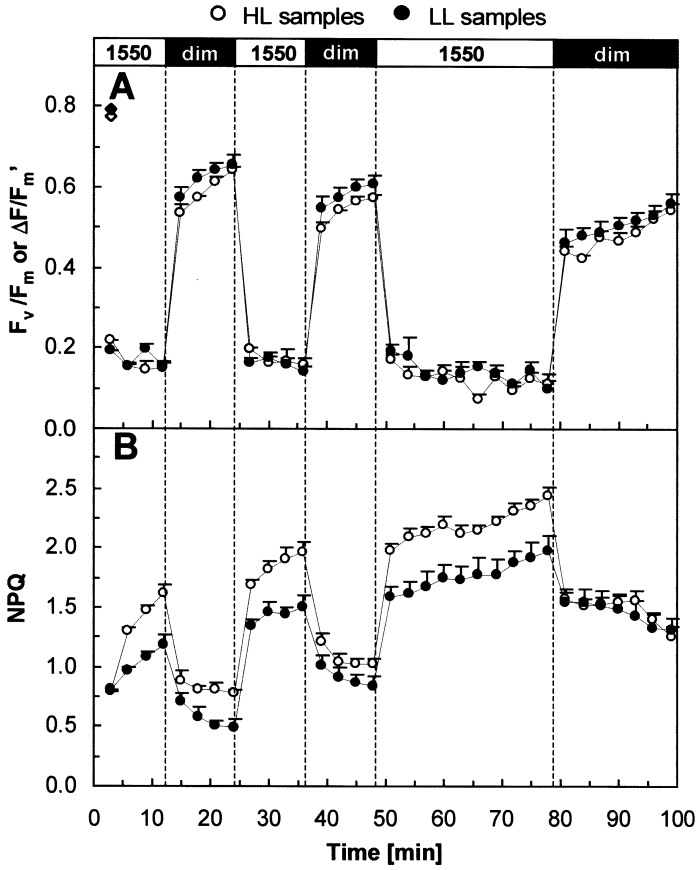

Figure 4 *Changes in the time course of chlorophyll fluorescence characteristics upon repeated exposure and recovery cycles to high fluence rates (1550 µmol photons m^{-1} s^{-1}) and dim light (15 - 20 µmol photons m^{-2} s^{-1}) in C. glomerata. Optimum PSII efficiency at open units, F_v/F_m (diamonds) and PSII efficiency at closed units (circles, A); non-photochemical quenching, NPQ (B). Given values are means of n = 5 ± S.E.*

Table 2 *Chlorophyll fluorescence characteristics of HL and LL grown* C. glomerata *after final 21 min exposure to 1550 µmol photons m^{-2} s^{-1}. Each value represents the mean ± S.E. of n = 5.*

	HL	LL	Significance (*t*-test)
NPQ	2.43 ± 0.06	1.97 ± 0.13	$p < 0.05$
revers. NPQ	1.17 ± 0.03	0.66 ± 0.05	$p < 0.05$
$(F_o-F_o')/F_o'$	0.12 ± 0.02	-0.02 ± 0.02	$p < 0.05$

Effects of temperature on photosynthesis-irradiance-characteristics

From measurements of chlorophyll fluorescence rETR was calculated and used to derive photosynthesis-irradiance-curves which express energy conversion characteristics of PSII (Fig. 5A). From these curves an increase in energy conversion with increasing temperature was apparent. Table 3 shows highest $rETR_{max}$ at 25 °C. Despite the differences in α_{IF} and $rETR_{max}$, energy conversion at PSII did not show any pattern of photoinhibition, as would be indicated by a negative slope of the curve after reaching maximum rates of rETR.

Photosynthesis-irradiance-curves derived from parallel photosynthetic oxygen-evolution measurements revealed a maximum of α_I and P_{max} at 10 °C, and a slightly lower P_{max} at 15 °C. (Fig. 5B and Table 4). In general, at fluence rates higher than 400µmol photons m^{-2} s^{-1}, O_2-evolution decreased, indicated by a negative slope of the curve after reaching the maximum rate of photosynthesis at all temperatures except of 1 °C (Fig. 5B).

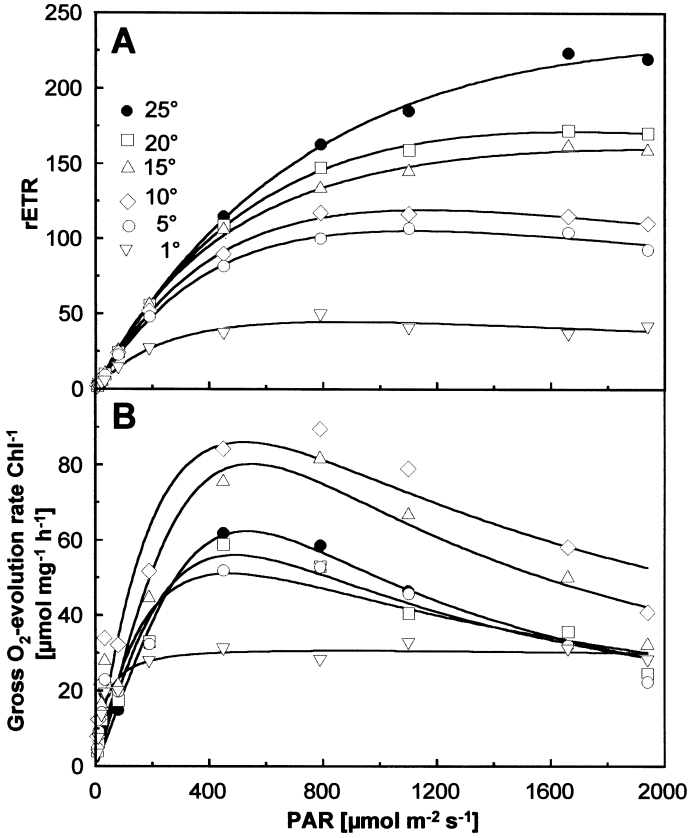

Figure 5 *Temperature-dependence of photosynthesis-irradiance-curves of* C. glomerata *derived from measurements of chlorophyll fluorescence (A) and from estimations of gross O_2-evolution-rates per Chl (B).*

Increasing electron flow at increasing temperatures was not strictly paralleled by a reduced DEPS of xanthophyll cycle pigments (Fig. 6). During the experiments, high levels of de-epoxidation of the xanthophylls were developed at 5 °C and at 10 °C, whereas at higher temperatures the level decreased, thus revealing reduced non-photochemical energy dissipation. However, NPQ increased with increases in temperature and developed highest values at 15 °C. Only at temperatures higher than 15 °C NPQ began to decrease, and thus, did not show any clear relationship to DEPS (Fig. 6).

Table 3 *Temperature dependence of parameters of photosynthesis-irradiance-curves of* C. glomerata *from chlorophyll fluorescence measurements. α_{IF} = initial slope of the nonsaturated relative electron transport rate, $rETR_{max}$ = maximum relative electron transport rate, asym. S.E. = asymptotic standard error.*

Temperature [°C]	α_{IF} ± asym. S.E.	$rETR_{max}$ ± asym. S.E.	R_m
1	0.22 ± 0.04	44.85 ± 1.80	0.99
5	0.31 ± 0.02	105.76 ± 1.21	0.99
10	0.33 ± 0.01	119.50 ± 0.10	0.99
15	0.38 ± 0.02	160.19 ± 2.02	0.99
20	0.35 ± 0.01	171.60 ± 0.97	0.99
25	0.34 ± 0.02	228.83 ± 7.03	0.99

Table 4 *Temperature dependence of parameters of photosynthesis-irradiance-curves of* C. glomerata *from estimations of gross O_2-production-rates per Chl. α_I = initial slope of the nonsaturated photosynthetic rate, P_{max} = maximum photosynthetic rate, asym. S.E. = asymptotic standard error.*

Temperature [°C]	α_I	P_{max} [µmol O_2 mg Chl^{-1} h^{-1}]	R_m
1	1.03 ± 0.18	30.61 ± 1.01	0.98
5	0.37 ± 0.13	51.16 ±5 .34	0.92
10	0.52 ± 0.17	86.12 ± 7.82	0.94
15	0.30 ± 0.09	80.39 ± 4.92	0.95
20	0.27 ± 0.08	56.05 ± 4.92	0.95
25	0.20 ± 0.05	62.43 ± 4.84	0.97

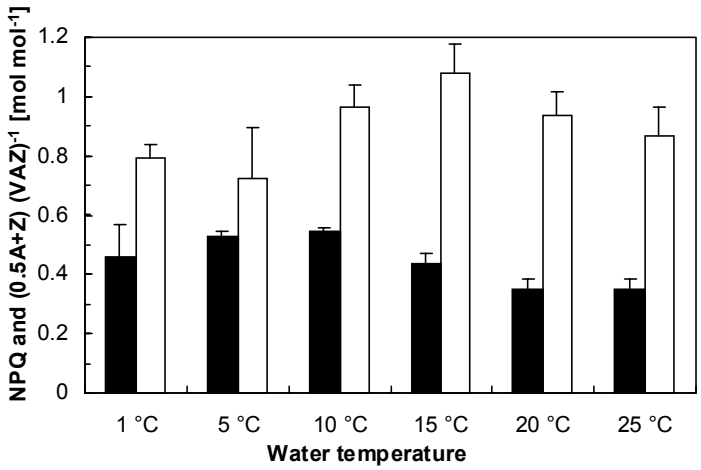

Figure 6 *De-epoxidation status of xanthophyll cycle pigments, $(0.5A+Z)\ (VAZ)^{-1}$ (closed bars) and non-photochemical quenching, NPQ (open bars) upon final exposure of* C. glomerata *to high fluence rates of 1950 µmol photons m^{-2} s^{-1} in P-I-curve measurements. Given values are means of n = 3 ± S.D.*

DISCUSSION

Diurnal patterns of photosynthetic activity

In July, diurnal changes of photosynthetic activity of *C. glomerata* revealed only little effects on the optimum efficiency of PSII quenching of dark adapted samples, which is expressed by the overall stress parameter of Chl fluorescence F_v/F_m (Schreiber et al. 1994). In fact, slight decreases of the F_v/F_m level at noon revealed increased excitation pressure on photosystem II (Fig. 1A). As complete recovery of the values was observed until late afternoon in HL as well

as in LL samples, no sustained photodamage is concluded. This was supported by the measured values of rETR. Obvious differences in HL and LL samples in the capacity of PSII energy conversion at high fluence rates expressed the limited rate of electron transfer of LL samples and the higher capacity in HL plants (Fig. 1B).

Upon exposure to high fluence rates, the non-photochemical dissipation of excessive energy captured by the antenna apparatus is reflected by the parameter NPQ (Demmig-Adams et al. 1996). In our experiments, this non-photochemical quenching was shown by rapid increases of NPQ in HL as well as in LL samples. It was most obvious at noon (Fig. 1C). In contrast to LL *C. glomerata*, HL samples revealed faster up- and downregulation of NPQ. This resulted in HL plants in an earlier reversal of NPQ during the afternoon to levels obtained during the morning. The experimental exposure of samples to artificial high fluence rates underlined this faster reaction of HL *C. glomerata* (Fig. 4B, see below).

Different capacities of the photoprotective xanthophyll cycle (Demmig-Adams et al. 1996) were observed in HL and LL samples of *C. glomerata* (Fig. 1D). However, the course of DEPS did not directly correlate with the course of NPQ. This is surprising, as in higher plants in general direct relationships between the formation of anthera- and zeaxanthin and the parameter NPQ were reported (e.g. Demmig-Adams 1998). Nevertheless, the results obtained from the temperature experiments similarly showed a diverging kinetic of the temperature dependent course of DEPS and NPQ (Fig. 6). Thus, quenching mechanisms other than via thermal dissipation and linear electron transport must additionally play an important role in *C. glomerata* (see below).

In April, with daily mean water temperature being almost 8 °C below the temperature level during July, C. *glomerata* revealed downregulation of the efficiency of open PSII during the day. F_v/F_m substantially decreased already after exposure to PAR of 400 µmol photons m^{-2} s^{-1} (Figs 2A and 3A). In comparison to the levels of F_v/F_m derived from July plants, this clearly expressed enhanced excitation pressure on PSII during April. According to Huner et al. (1993, 1998), this was probably related to the prevailing lower water temperatures. Due to slow-down of enzymatic processes of carbon fixation and reduction within the Calvin-cycle, an imbalance evolves between energy conversion and consumption. This imbalance causes excitation pressure already at moderate levels of PAR. Thus, at lower temperatures the importance of protective quenching mechanisms increases, and is reflected e.g. by altered pools of xanthophyll-cycle pigments in April (Table 1). Increased thermal dissipation of energy absorbed via anthera- and zeaxanthin results in reduced rETR in April compared to July rates (Figs 1B, 2B and 3B). From this point of view it becomes obvious, that higher levels of the

ratio of Chl a per Chl b in April *C. glomerata* illustrate an active process of downregulation of light capture efficiency due to the increased risk of overexcitation of PSII (Table 1).

Differences in energy conversion of HL and LL grown *C. glomerata* upon exposure to artificial high light

Differences between HL and LL plants relied primary on differences in NPQ and the fast recovery of NPQ in HL plants. Decreases in the efficiency of PSII units ($\Delta F/F_m'$) upon exposure to high fluence rate were accompanied by increased levels of NPQ (Fig. 4). In HL samples the light induced formation and reversal of NPQ was more rapid compared to LL samples of *C. glomerata* (Fig. 4B, Table 2). Despite the lower rates in LL plants compared to HL plants, the kinetics of induction and relaxation of NPQ were very similar, exhibiting patterns of light activation (Ruban & Horton 1999). This was apparent from samples upon second or third exposure to high fluence rates and their instant onset of levels of NPQ with values as high as those developed at the end of the former exposure period. According to data from *Dunaliella tertiolecta* (Casper-Lindley & Björkman 1998) this may reflect induction of de-epoxidation of xanthophylls at the beginning of exposure, without immediate epoxidation of zeaxanthin at subsequent dim light. Higher levels of NPQ and reversible NPQ of HL plants were accompanied by higher levels of F_0'-quenching. As $(F_0 - F_0')/F_0'$ indicates quenching efficiency of the PSII antenna (Demmig-Adams 1998), increased values in HL *C. glomerata* revealed their higher capacity in antenna-quenching. The differences in the formation of NPQ, together with the decline of NPQ during dim light exposure and the different levels of F_0'-quenching depicted the observed diurnal changes of HL samples. Hence, *C. glomerata* from HL sites revealed smarter quenching-patterns of absorbed excessive light, whereas LL algae responded very sensitive to fluctuations in PAR (Figs 1-3).

Effects of temperature on photosynthesis-irradiance-characteristics

Plants have to acclimate their photosynthetic physiology to temperature by balancing photon capture (chlorophyll a concentration for light reactions) with carbon fixation (concentration of Calvin cycle enzymes) (DeNicola 1996, Huner et al. 1998). In most algae, acclimation of photosynthetic metabolism to lower temperature, which affects reaction rates of enzymes, is achieved by altering the enzyme and chlorophyll concentrations (Thompson et al. 1992). In this respect, the experiments with *C. glomerata* grown at 5 °C (Fig. 5A) revealed that temperature dependent increases in energy conversion of PSII and rETR were probably due to higher activity of the enzymatic apparatus involved in CO_2-reduction (Huner et al. 1993) and the temperature dependence of the repair cycle of PSII, which is inhibited by low temperatures (Öquist, Greer & Ögren 1987). However, the first step of photoinhibition depends on the

reduction status of Q_A and is temperature independent (Ottander et al 1993). Therefore, at similar fluence rates, photoinhibition will occur sooner at low temperatures as at higher temperatures.

Oxygen evolution revealed an increase up to temperatures of 10 °C to 15 °C and a subsequent decrease at higher temperatures (Fig. 5B). It is suggested, that at temperatures below 10 °C this reflected limitations of the enzymatic rates as well as low rates of mitochondrial respiration, whereas at 10 °C to 15 °C photosynthetic rate had increased due to increased enzymatic activity. Similarly, Rhee & Gotham (1981) found that net photosynthesis varied little in a range from 10 °C to 20 °C because of compensation in cell enzyme content. However, at higher temperatures the enzymatic activity was outmatched by increased mitochondrial respiration, increases in pseudocyclic electrontransport (Mehler-reaction), and enhanced photorespiration. In addition, the latter reactions are probably responsible for non-linear relationships of O_2-evolution and rETR, because electron flow rate increased steadily with increasing temperatures.

The experiments did not show direct relationships between rETR and oxygen-evolution rate, but demonstrated different mechanisms to apply at varying temperatures and at varying fluence rates. This was also apparent from figure 6, where DEPS and NPQ diverged at increasing temperature. These results are in opposite to studies showing linear relationships of O_2-evolution and rETR (or $\Delta F/F_m'$) (e.g. Beer, Larsson, Poryan & Axelsson 2000), which are obviously given only at moderate temperatures, low O_2-concentrations (Genty et al. 1989), and up to moderate/saturating fluence rates.

In conclusion, the results showed different diurnal courses of photosynthesis in July and April *C. glomerata*, and these were accompanied by characteristic differences in photosynthetic parameters.

Algal cells may acclimate light-harvesting efficiency by increasing total chlorophyll concentrations (increase of size number and photosynthetic units) at higher temperatures to compensate for increased CO_2 fixation (Thompson et al. 1992), and similarly, as was found for *Dunaliella*, may decrease light harvesting (turning off photosynthetic units) at low temperatures to compensate for reductions in CO_2 fixation (Levasseur, Morissette, Popovic & Harrison 1990). Indeed, temperature effects were one source inducing plasticity of the photosynthetic apparatus, apparent by the downregulation of F_v/F_m and of rETR, increased thermal energy dissipation, increased xanthophyll content and higher Chl a/Chl b in April samples. These mentioned features were used to minimise the risk of overexcitation in *C. glomerata*.

Plasticity of photosynthesis, in response to the light environment, with e.g. focus on minimising overexcitation in HL habitats, was proven by comparison of quenching characteristics of HL

and LL grown *C. glomerata*. This revealed higher capacity of non-photochemical quenching in HL samples.

All these mechanisms provide *C. glomerata* a substantial extension of its ecological niche. Instead of a disassembly of photosynthetic pigments, the photosynthetic apparatus acclimates to unfavourable conditions. Hence, even if primary production is slowed down or photosynthesis is inhibited for some part, vegetative cells are able to withstand a wide range of environmental conditions. This provides a great advantage, because already established thalli of *C. glomerata* represent a source for rapid growth and development of a population if environmental conditions turn more favourable.

ACKNOWLEDGEMENTS

This study was supported by grants of the Deutsche Forschungsgemeinschaft (DFG) Graduate Study Group *Analysis of Function and Regeneration of Degraded Ecosystems* (Förderkennzeichen GRK 266/1-96). Deutscher Wetterdienst Offenbach provided data on global irradiance.

REFERENCES

Beer S., Larsson C., Poryan O. & Axelsson L. (2000) Photosynthetic rates of *Ulv*a (Chlorophyta) measured by pulse amplitude modulated (PAM) fluorometry. *European Journal of Phycology* **35**, 69-74.

Berner T., Dubinsky Z., Wyman K. & Falkowski P.G. (1989) Photoadaptation and the "package" effect in *Dunaliella tertiolecta* (Chlorophyceae). *Journal of Phycology* **25**, 70-78.

Bilger W. & Björkman O. (1990) Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. *Photosynthesis Research* **25**, 173-185.

Büch K., Stransky H., Bigus H.J. & Hager A. (1994) Enhancement by artificial electron acceptors of thylakoid lumen acidification and zeaxanthin formation. *Journal of Plant Physiology* **144**, 641-648.

Casper-Lindley C. & Björkman O. (1998) Fluorescence quenching in four unicellular algae with different light-harvesting and xanthophyll-cycle pigments. *Photosynthesis Research* **56**, 277-289.

Demmig-Adams B., Gilmore A.M. & Adams III, W.W. (1996) *In vivo* functions of carotenoids in higher plants. *FASEB Journal* **10**, 404-412.

Demmig-Adams B. (1998) Survey of thermal energy dissipation and pigment composition in sun and shade leaves. *Plant and Cell Physiology* **39**, 474-482.

DeNicola D.M. (1996) Periphyton responses to temperature at different ecological levels. In *Algal ecology - Freshwater benthic ecosystems* (eds. Stevenson R.J., Bothwell M.L. & Lowe R.L.), pp. 149-181. Academic Press, San Diego.

Dodds W.K. & Gudder D.A. (1992) The ecology of Cladophora in freshwaters. *Journal of Phycology* **28**, 415-427.

Eilers P.H.C. & Peeters J.C.H. (1988) A model for the relationship between light intensity and the rate of photosynthesis in phytoplankton. *Ecological Modelling* **42**, 199-215.

Ensminger I., Hagen C., Braune W. (2000a) Strategies providing success in a variable habitat: I. Relationships of environmental factors and dominance of *Cladophora glomerata*. *Plant, Cell & Environment* (in press).

Ensminger I., Hagen C., Braune W. (2000b) Strategies providing success in a variable habitat: II. Ecophysiology of photosynthesis of *Cladophora glomerata*. *Plant, Cell & Environment* (in press).

Genty B.E., Briantais J.M. & Baker N.R. (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta* **990**, 87-92.

Henley W.J. (1993) Measurement and interpretation of photosynthetic light-response curves in algae in the context of photoinhibition and diel changes. *Journal of Phycology* **29**, 729-739.

Hanelt D., Huppertz K. & Nultsch W. (1993) Daily course of photosynthesis and photoinhibition in marine macroalgae investigated in the laboratory and field. *Marine Ecology Progress Series* **97**, 31-37.

Hanelt D., Li J. & Nultsch W. (1994) Tidal dependence of photoinhibition of photosynthesis in marine macrophytes of the south china sea. *Botanica Acta* **107**, 66-72.

Huner P.A., Öquist G., Hurry V.M., Krol M., Falk S. & Grifith M. (1993) Photosynthesis, photoinhibition and low temperature acclimation in cold tolerant plants. *Photosynthesis Research* **37**, 19-39.

Huner P.A., Öquist G. & Sarhan F. (1998) Energy balance and acclimation to light and cold. *Trends in Plant Science* **3**, 224-230.

Levasseur M., Morissette J., Popovic R. & Harrison P.J. (1990) Effects of long term exposure to low temperature on the photosynthetic apparatus of *Dunaliella tertiolecta* (Chlorophyceae). *Journal of Phycology* **26**, 497-484.

Logan B., Stephen C.G., Adams III W.W. & Demmig-Adams B. (1998) Seasonal differences in xanthophyll cycle characteristics and antioxidants in *Mahonia repens* growing in different light environments. *Oecologia* **116**, 9-17.

Ottander C., Hundal T., Andersson B., Huner N.P.A. & Öquist G. (1993) Photosystem II reaction centres stay intact during low temperature photoinhibition. *Photosynthesis Research* **35**, 191-200.

Öquist G., Greer D.H.& Ögren E. (1987) Light stress at low temperature. In: *Photoinhibition, Topics in Photosynthesis Vol. 9* (eds Kyle D.J., Osmond C.B. & Arntzen C.J.), pp. 67-87. Elsevier Science Publishers, Amsterdam.

Rhee G.Y. & Gotham I.J. (1981) The effect of environmental factors on phytoplankton growth: Temperature and the interactions of temperature with nutrient limitation. *Limnology and Oceanography 26*, 635-648.

Ruban A.V. & Horton P. (1999) The xanthophyll cycle modulates the kinetics of nonphotochemical energy dissipation in isolated light-harvesting complexes, intact chloroplasts, and leaves of spinach. *Plant Physiology* **119**, 531-542.

Schreiber U., Bilger W. & Neubauer C. (1994) Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of *in vivo* photosynthesis. In: *Ecophysiology of photosynthesis* (eds. Schulze E.D. & Caldwell M.M.), pp. 49-70. Springer, Berlin.

Schreiber (1986) Detection of rapid induction kinetics with a new type of high-frequency modulated chlorophyll fluorometer. *Photosynthesis Research* **9**, 261-272.

Silva J., Santos R., Serodio J. & Melo R.A. (1998) Light response curves for *Gelidium sesquipedale* from different depths, determined by two methods: O_2-evolution and chlorophyll fluorescence. *Journal of Applied Phycology* **10**, 295-301.

Thompson P.A., Guo M. & Harrison P.J. (1992) Effects of variation in temperature. I. On the biochemical composition of eight species of marine phytoplankton. *Journal of Phycology* **28**, 481-488.

Whitton B.A. (1970) Biology of *Cladophora* in freshwaters. *Water Research* **4**, 87-114.

Xyländer M., Hagen C. & Braune W. (1996) Mercury increases light susceptibility in the green alga *Haematococcus lacustris*. *Botanica Acta* **109**, 222-228.

7.4 Response of *Cladophora glomerata* (L.) Kützing to sunlight and current in the field

INGO ENSMINGER, CHRISTOPH HAGEN & WOLFRAM BRAUNE

Freshwater Biology (in Begutachtung)

SUMMARY

1. *Cladophora glomerata* (L.) Kützing is abundant in various habitats in flowing freshwaters. Light and current are amongst the important factors, that determine the alga's success in terms of photosynthesis and growth. Factorial experiments were used to determine temporal effects and interactions of light and current on photosynthetic quantum yield, composition of photosynthetic pigments and tip growth of *C. glomerata*.
2. Experimental channels were used outsides to grow *C. glomerata* under different combinations of light and current. A repeated measures two-way ANOVA was used to identify treatment effects on optimum quantum yield and pigmentation. A multiple regression analysis was applied to assess different relationships between quantum yield, photosynthetic active radiation and photosynthetic pigments in high current and low current treatments.
3. Light treatments had significant effects on the ratio chlorophylls to dry mass, the ratio chlorophyll a to chlorophyll b, all carotenoid related parameters, and the optimum quantum yield. The current treatments had significant effects on the ratio chlorophyll a to chlorophyll b and the carotenoid related parameters.
4. In high current treatments, optimum quantum yield was strongly related to the de-epoxidation of the xanthophyll cycle pigments, photosynthetic active radiation and the ratio of chlorophyll a per chlorophyll b, whereas in low current treatments the de-epoxidation of the xanthophyll cycle pigments, current velocity, photosynthetic active radiation and the ratio of chlorophylls to dry mass explained changes of the optimum quantum yield.
5. Growth rates of tips of *C. glomerata* were enhanced by increases in current as well as by increases in irradiance.

INTRODUCTION

Within the freshwaters of the temperate zones *Cladophora glomerata* (L.) Kützing is the most widespread alga (Whitton 1970). It is abundant in a variety of habitats, such under high current or low current conditions as well as in shaded or unshaded stretches. Colonization of the

different habitats requires acclimation to specific environmental and physical conditions. Amongst these, current is an important determinant of benthic algal ecology in freshwater habitats (Stevenson 1996) as well as light, that directly effects photosynthetic parameters (Pfeifer & McDiffett 1975, Dodds 1991a, Ensminger, Hagen & Braune 1998).

Effects of current include enhancement of periphyton and macrophyte growth at moderate current velocities, and retard of colonization and organic matter accrual at high velocities (Biggs 1996). Current affects nutrient supply in macrophyte beds (e.g. Werner & Weise 1982, Madsen & Sand-Jensen 1991) or benthic algae (Dodds 1989, Stevenson 1996), and in *C. glomerata*, morphology was subject of current induced changes (Power 1990, Parodi & Cáceres 1991). The influence of current velocity on photosynthetic rates of *C. glomerata* was investigated by Dodds (1991a). From measurements of photosynthesis at different current velocities he considered increased current velocity to be responsible for increased photosynthesis. Dodds (1991a) explained these results by decreases of the boundary layer thickness at increasing current velocities, and hence, higher import of limiting nutrients. Under low current conditions, light attenuation due to selfshading was assumed to be more important than current in controlling photosynthesis (Dodds 1991b).

In spite of the well established hypothesis of direct effects of current on nutrient supply, considerable few work has focussed on current-light relationships in freshwaters (Madsen, Enevoldsen & Jörgensen 1993, Steinman & McIntire 1986). None of these studies adressed changes in photosynthetic pigments and energy conversion, which necessarily have to match different requirements within various freshwater habitats (for example differences in photosynthetic quantum yield of samples from high light or low light habitats). In marine species of *Cladophora* from high light and low light sites, Häder et al. (1997) found different abilities to cope with high fluence rates. These can be related to photoinhibition, a dynamic process due to overexcitation of the photosystem, which shows different patterns in marine macroalgae from different habitats (Hanelt, Hupperts & Nultsch 1992, Häder et al. 1997). In flowing freshwaters, it is suggested, that these processes will be modified by complex interactions of the combination of light and current. For instance, high light and high, turbulent current possibly prevent overexcitation and photoinhibition of individual chloroplasts, because of continuous mixing of the filaments. In contrast, high light and low current with an almost laminar flow lead to a minimum movement of the thalli, and thus, demand downregulation of light harvesting processes to prevent photodamage from overexcitation. This downregulation can be achieved e.g. in unicellular green algae by decreases in the amount of chlorophylls (Berner et al. 1989) or by increases in the activity of energy dissipating mechanisms like the xanthophyll cycle (Demmig-Adams, Gilmore & Adams 1996).

This study aims on the influence of simultaneous current and light manipulations on photosynthetic activity, pigment composition and growth of *C. glomerata*. Factorial experiments were conducted that included different types of habitats in terms of current (high current vs. low current) as well as in terms of light (low, intermediate and high light).

It is supposed, that different light conditions result in differences in photosynthetic capacity, pigmentation and growth of *C. glomerata* and that these patterns will be modified by changes in current. In different habitats, the control of the photosynthetic quantum yield is given to different sets of environmental factors and pigmentation, revealing plasticity of photosynthesis and photosynthetic pigments of *C. glomerata*.

MATERIAL AND METHODS

Experimental channels

Six experimental channels made of PVC were used outsides at an aquaculture facility near Mellingen, a small town located within the hyporhithric zone of the Ilm (Thuringia/Germany, for details see Ensminger, Hagen & Braune, in press). Each channel was 0.15 m wide and 2.20 m long. Water was supplied by continuous flow of fresh and untreated water that came directly from the Ilm via the facility's channel system.

Plant material - *C. glomerata* from the Ilm

In a first experiment, the effect of light and current on photosynthesis and pigment composition was investigated. Complete *C. glomerata* thalli that were attached to flat stones of about 5 - 7 cm in diameter and up to 10 cm in length were collected from a nearby site at the Ilm and exposed in the channels during the experiment.

In a second experiment the effect of light and current on tip growth of *C. glomerata* was observed. In that case we used the last five centimetres of previously collected thalli of *C. glomerata* which were tightened together and fixed within the channels with nylon strings.

Experimental design

Prior to each experiment, the channels were equipped with algae from the Ilm, which were then initialised for a period of 8 days. During this period, black nylon mesh (grid width approximately 2 mm) was placed over the six channels in order to reduce photosynthetic active irradiance (PAR) to a portion of 40 %. Measurement of PAR was undertaken with a Li-Cor 192 SA (Li-Cor, Nebrasca, USA). Current was set to velocities of about 0.4 m s^{-1} by adjusting the inclination of the channels. Current velocity was measured with a Flowmate 2000 (Marsh-McBirney, Maryland, USA). After the initialisation period, the inclination of the channels was changed. Thus, high current (HC) sections were created with turbulent flow and current

velocities between 0.47 and 0.54 m s^{-1} as well as low current sections (LC) with current velocities between 0.29 to 0.34 m s^{-1} and an almost laminar flow (Table 1). The water column above the algal thalli depended on the thickness of the stones on which the algae were attached to and varied between 2 - 4 cm. Different light conditions were achieved by modification of the nylon mesh layers. One pair of channels was supplied with additional layers of the mesh, which resulted in low light conditions (LL) with a portion of 10 % of PAR at the water surface. A second pair of channels remained at 40 % of PAR (intermediate light treatment, IL). High light (HL) conditions with 100 % of PAR were obtained in the third pair of channels by removing any nylon mesh. The final settings resulted in six different treatments: Low light/high current (LL/HC), low light/low current (LL/LC), intermediate light/high current (IL/HC), intermediate light/low current (IL/LC), high light/high current (HL/HC) and high light/low current (HL/LC). Samples for pigment analysis were taken and measurements of chlorophyll fluorescence were performed on days 1, 14 and 28. Samples for tip growth measurements were taken once (see below).

Table 1 *Experimental design of the different current and light treatments. LL = low light, IL = intermediate light, HL = high light, HC = high current, LC = low current*

		Light condition		
		LL	IL	HL
Current velocity [m s^{-1}]	HC	0.50 ±0.05	0.47 ±0.04	0.54 ±0.05
	LC	0.29 ±0.03	0.29 ±0.03	0.34 ±0.02
Effective irradiance [% PAR]		10	40	100

Chlorophyll fluorescence

With a portable fluorometer (PAM-2000, Walz, Germany), measurements of chlorophyll fluorescence of *C. glomerata* were performed. A self constructed device allowed the exposure of the filamentous algal thalli *in situ* during determination of optimum quantum yield [$F_v/F_m = (F_m - F_0)/F_m$], which is a measure of the efficiency of open photosystem II units (PSII; Schreiber, Bilger & Neubauer 1994). F_v/F_m was determined after 5 min predarkening of the sample by detection of (i) dark adapted basic fluorescence (F_0) under weak red modulated light (~0.18 µmol photons m^{-2} s^{-1}) and (ii) maximal fluorescence (F_m) during a 600 ms flash of white light (~6000 µmol photons m^{-2} s^{-1}).

Pigment analysis

Samples of *C. glomerata* (0.05 - 0.1 g freshweight) were taken directly from the channels and rinsed several times in riverwater to remove loosely attached epiphytes. Finally, samples were rinsed with distilled water, dry blotted for 20 s between four layers of filterpaper, weighed on an analytical balance and immediately deepfrozen in liquid nitrogen. Subsamples were stored

for a few hours in a cooler until determination of dry mass (after 24 h drying at 105 °C) at the laboratory. Homogenisation of deep frozen samples was accomplished with a mixer mill (Retsch, Germany). Pigments were extracted in 100 % acetone under dimlight conditions at 4 °C. Pigment content was determined spectrophotometrically according to Lichtenthaler (1987). Further analysis of the pigment pattern was done by HPLC using the same extracts after addition of 15 % H_2O (Büch et al. 1994; Xyländer, Hagen & Braune 1996). Because epiphytic diatoms could not entirely be removed during the cleaning and homogenisation procedure, the amount of diatomic chlorophyll a (Chl a) was calculated from the amount of Chl c per sample. A fixed molar Chl a to Chl c ratio of 5.3 was assumed and the calculated value was subtracted from the total amount of Chl a.

Growth of *C. glomerata* tips

Tip growth of *C. glomerata* was assessed by the vital fluorescent stain calcofluor white M2R (Sigma, Germany) which stains plant cell walls (Waaland & Waaland 1975, Kasten 1981). After initialisation of *C. glomerata* for the growth experiment, thalli were transferred to a solution of 0.01 % calcofluor white in filtered (0.2 µm, Sartolab P20 plus, Sartorius, Germany) Ilm water and stained for 30 min. Afterwards, thalli were retransferred to the experimental channels. Growth conditions were adjusted to the different light and current treatments as described above. After 5 days, thalli were examined with fluorescent microscopy (excitation maximum at λ= 437 nm, emission maximum at λ = 490 nm). Growth was measured by the length of unstained tips that represented new growth since staining. From each treatment 3 samples were taken and from each sample at least 15 tips were measured giving a total of 45 tips per treatment.

Statistics

For the photosynthesis and pigment experiment a two-way repeated measures analysis of variance (ANOVA) was used to analyse the data for any time dependent effects. Main effects were light conditions (open, shade or intermediate) and current (low current or high current type). If there were significant effects of the factor light conditions, Bonferronis post-hoc test was used to further identify the specific differences between the three different light treatments. Additionally, differences between the treatments after 28 days were analysed with a one-way ANOVA. If significant effects of light were detected, again Bonferronis post-hoc test was used to identify differences between the light treatments. Because the two-way ANOVA only identifies differences due to time and factorial treatments, a multiple regression analysis was used to describe the kind of relationship between photosynthetic capacity, indicated by the parameter F_v/F_m, and environmental as well as physiological parameters. Growth data were

analysed with a simple one-way ANOVA. The software package SPSS release 9.0 was used to perform statistical tests and multivariate procedures (SPSS, Illinois, USA).

RESULTS

Effects of time, light and current on *C. glomerata*

During the experimental period the weather situation changed repeatedly. Thus, the initial phase was characterised by high insulation. At noon, PAR was around 1900 µmol m^{-2} s^{-1}, and the daily sum of global irradiance amounted to an average of more than 2400 J cm^{-2} (Fig. 1). Right after, at the beginning of the experiments, weekly mean of global irradiance decreased to about 1600 J cm^{-2}. Between day 15 and 21, again there was an increase to almost 2300 J cm^{-2}. Concomitant with these changes in global irradiance, effects of ageing of the filaments have to be taken into account but were not further assessed (but see Pfeiffer & McDiffett 1975). Effects of this comprehensive time factor were significant on the estimated parameters of photosynthetic pigments as well as on F_v/F_m (Table 2).

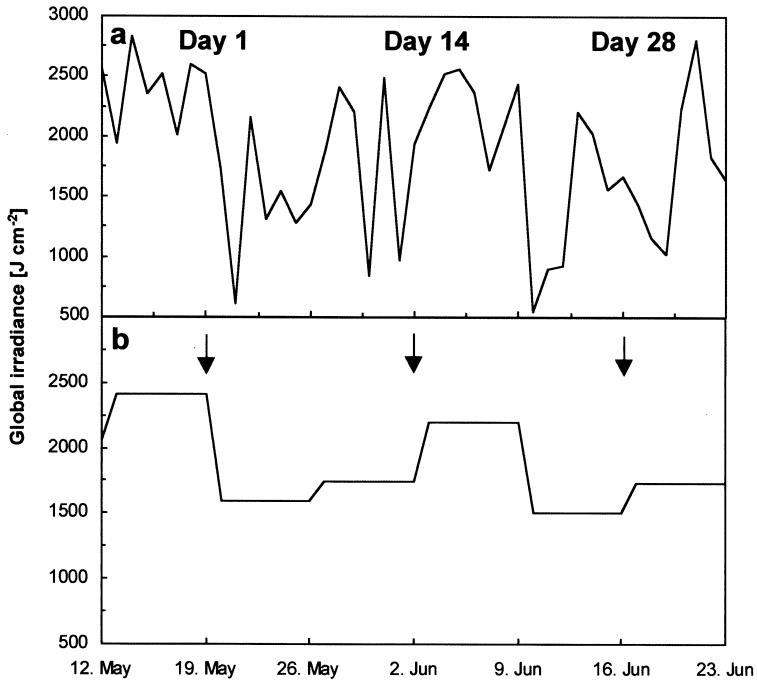

Figure 1 *Global irradiance during the experimental period. Daily sum of global irradiance (a); weekly mean of global irradiance (b) characterising the general weather situation. Black arrows indicate sampling and measurement on Day 1 (begin of factorial treatments), day 14 and day 28 of the experiments of light and current. Following day 28, tip growth experiments were conducted. Data of global irradiance were measured with a thermopile without selection of specific spectral bands and were kindly provided by Deutscher Wetterdienst Offenbach.*

Effects of light on the ratio Chl (a+b) per dry mass (Chl (a+b)/DM) were significant for all sampling dates (Fig. 2a, Table 2). On day 1 these effects where due to significant differences between LL and IL channels ($p < 0.05$, Table 3), whereas on day 14 LL channels revealed significant higher amounts of Chl (a+b)/DM compared to HL channels ($p < 0.05$, Table 3). On day 28 *C. glomerata* from LL as well as from IL channels contained more Chl compared to samples from HL channels ($p < 0.01$, and $p < 0.05$, respectively, Table 3). Despite the fact, that

there was no significant effect of current on Chl (a+b)/DM, significant interaction of light and current was assessed on day 14 ($p < 0.05$, Table 2).

Table 2 *Results of the repeated measures two-way ANOVA for photosynthetic quantum efficiency of open PSII, and photosynthetic pigments. For parameters where there was a significant treatment x time interaction, ANOVA results are given on each sampling date.* F-*values (first row) and* p-*values (second row). Significance of* p-*Values is indicated (** $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$*). Chl (a+b) DM^{-1} = total chlorophyll per dry mass, Chl a Chl b^{-1} = ratio of chlorophyll a per chlorophyll b, Car Chl (a+b)$^{-1}$ = ratio of carotenoids per total chlorophyll, VAZ (Chl a+b)$^{-1}$ = xanthophylls per total chlorophyll, (0.5A+Z) (VAZ)$^{-1}$ = de-epoxidation status (DEPS) of xanthophyll cycle pigments, F_v/F_m = optimum quantum yield of PSII.*

	Time effect		Treatment effect		
Parameter		Date	Light	Current	Light x current
Chl (a+b) DM^{-1} [mg g^{-1}]	8.264	Day 1	4.362	0.158	0.101
	0.002 **		0.022 *	0.694	0.904
		Day 14	3.715	0.163	4.003
			0.036 *	0.689	0.029 *
		Day 28	8.837	3.191	1.008
			0.001 **	0.084	0.377
Chl a Chl b^{-1} [mol mol^{-1}]	26.034	All[a]	3.745	6.211	0.736
	0.000 ***		0.036 *	0.019 *	0.488
Car Chl (a+b)$^{-1}$ [mg mg^{-1}]	29.480	Day 1	4.058	0.297	0.932
	0.000 ***		0.052	0.590	0.406
		Day 14	1.839	0.008	4.306
			0.177	0.929	0.023 *
		Day 28	36.525	8.498	1.009
			0.000 ***	0.007 **	0.377
VAZ (Chl a+b)$^{-1}$ [mol mol^{-1}]	25.734	Day 1	1.705	2.926	0.921
	0.000 ***		0.200	0.098	0.410
		Day 14	1.803	1.563	2.548
			0.182	0.221	0.095
		Day 28	62.766	13.405	1.118
			0.000 ***	0.001 **	0.340
(0.5A+Z) (VAZ)$^{-1}$ [mol mol^{-1}]	32.373	Day 1	1.777	0.160	0.211
	0.000 ***		0.188	0.692	0.811
		Day 14	30.236	0.622	0.258
			0.000 ***	0.437	0.775
		Day 28	31.678	5.836	3.375
			0.01 *	0.022 *	0.048 *
Fv/Fm	96.371	Day 1	1.090	0.722	1.786
	0.000 ***		0.349	0.402	0.185
		Day 14	35.355	2.696	2.022
			0.000 ***	0.111	0.150
		Day 28	25.150	1.447	3.026
			0.000 ***	0.238	0.064

The ratio Chl a per Chl b (Chl a/Chl b, Fig. 2b) was significantly effected by light and current over time. Because there was no significant interaction of time with one of the two treatment factors, the results of the repeated measures ANOVA are given for all sampling dates instead of the individual sampling dates (Table 2). Effects of the light treatments accounted for the significance of the difference between LL and HL treated *C. glomerata* ($p < 0.05$,Table 3).

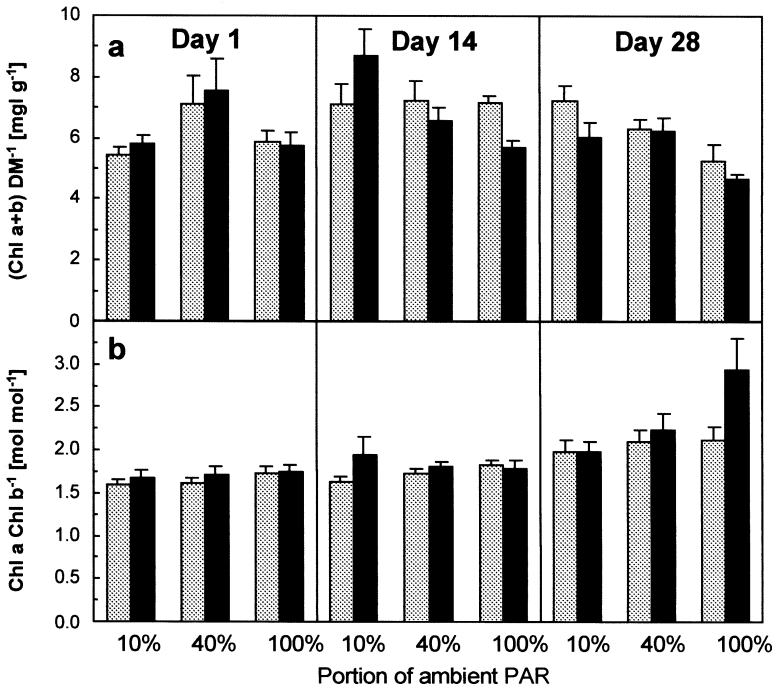

Figure 2 *Differences in the total chlorophyll to dry mass ratio (Chl DM^{-1}, a) and the chlorophyll a to chlorophyll b ratio (Chl a Chl b^{-1}, b) from different experimental habitats. High current = scattered bars, low current = filled bars; LL = 10 %, IL = 40 % and HL = 100 % of ambient irradiance. Each bar represents the mean of n = 12 ± standard error.*

Table 3 *Results of the Bonferroni post-hoc tests for the different light treatments for photosynthetic pigments, and photosynthetic quantum efficiency of open PSII. Significance of* p-*Values is indicated (** $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$*). For details see table 2.*

		Light treatment		
Parameter	Date	LL vs IL	LL vs HL	IL vs HL
(Chl a+b) DM^{-1} [mg g^{-1}]	Day 1	0.034 *	1.000	0.083
	Day 14	1.000	0.036 *	0.246
	Day 28	1.000	0.001 **	0.011 *
Chl a Chl b^{-1} [mol mol^{-1}]	All	1.000	0.039 *	0.158
Car (Chl a+b)$^{-1}$ [mg mg^{-1}]	Day 1	0.081	0.105	1.000
	Day 14	1.000	0.243	0.463
	Day 28	0.000 ***	0.000 ***	0.001 **
VAZ (Chl a+b)-1 [mol mol-1]	Day 1	0.233	0.878	1.000
	Day 14	1.000	0.309	0.357
	Day 28	0.004 **	0.000 ***	0.000 ***
(0.5A+Z) $(VAZ)^{-1}$ [mol mol^{-1}]	Day 1	0.211	0.971	1.000
	Day 14	0.018 **	0.000 ***	0.000 ***
	Day 28	0.000 ***	0.000 ***	0.169
Fv/Fm	Day 1	0.984	1.000	0.479
	Day 14	0.01 *	0.000 ***	0.000 ***
	Day 28	0.000 ***	0.000 ***	1.000

Carotenoids per Chl (Car/Chl (a+b), Fig. 2c) were significantly effected by the interaction of current and light on day 14 ($p < 0.05$, Table 2). On day 28 effects of both, current and light, were observed (Table 2), the carotenoid content was lower in LL compared to IL and HL channels (both $p < 0.001$, Table 3) and lower in IL compared to HL channels ($p < 0.05$,

Table 3). In *C. glomerata* from LC channels the content of carotenoids was higher ($p < 0.05$) compared to HC channels (Fig. 3a).

The ratio xanthophylls per total Chl (VAZ/Chl (a+b)) was effected by light and current (Fig. 3b, Table 2). However, differences between the treatments were not significant on day 1 and 14. On day 28, *C. glomerata* from LL channels showed lower values than IL and HL treatments ($p < 0.01$ and $p < 0.001$, respectively, Table 3), in IL treatments the values were lower compared to *C. glomerata* from HL channels ($p < 0.001$, Table 3). Effects of current resulted in higher VAZ/Chl (a+b) in LC compared to HC channels ($p < 0.01$, Table 2).

Light effects were significant on the de-epoxidation state (DEPS) of the xanthophyll cycle pigments on day 14 (Fig 3c, Table 2). These effects accounted for differences between LL and IL ($p < 0.01$, Table 3), LL and HL as well as between IL and HL channels (both $p < 0.001$, Table 3). On day 28 light effects were still significant and resulted in a lower de-epoxidation state of LL compared to IL and LL compared to HL channels (both $p < 0.001$, Table 3). Effects of current resulted in lower values in LL/HC and HL/HC channels compared to the corresponding LC channels ($p < 0.05$, Table 3). Additionally, the interaction of light and current had significant effects on the DEPS of the xanthophyll cycle on day 28 ($p < 0.05$, Table 2).

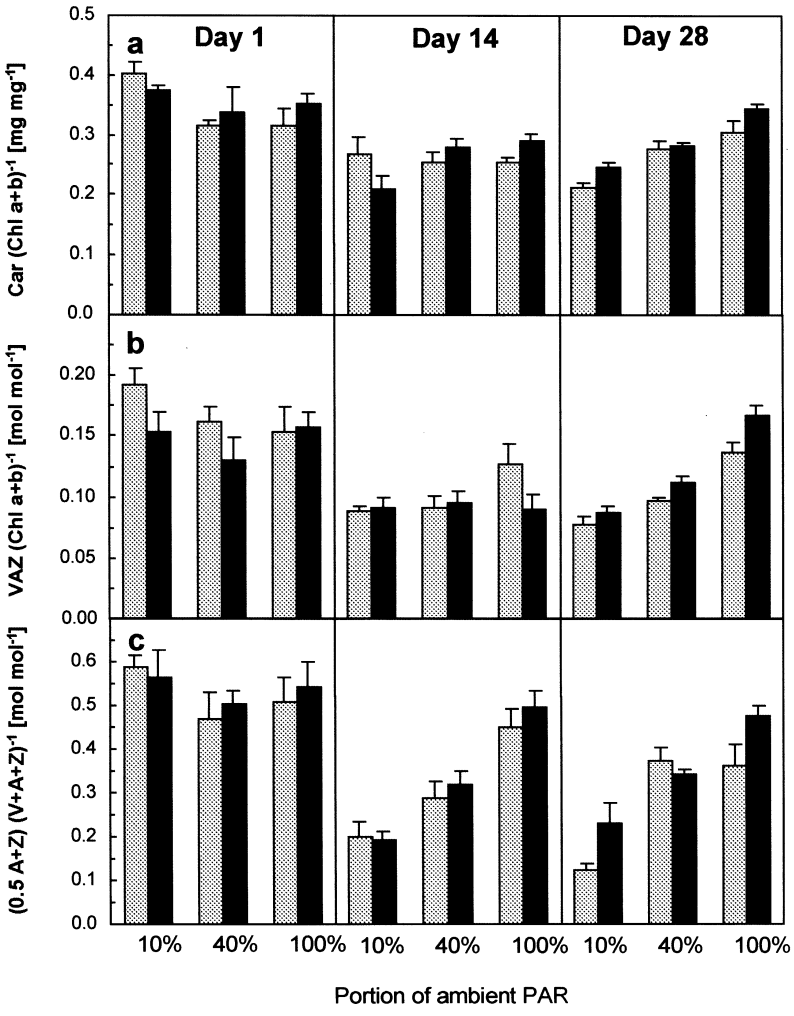

Figure 3 *Differences in the carotenoids to total chlorophyll ratio (Car Chl $(a+b)^{-1}$), in the xanthophylls to total chlorophyll ratio (VAZ Chl $(a+b)^{-1}$), and in the de-epoxidation status (DEPS) of the xanthophyll cycle pigments ($(0.5A + Z)\ (VAZ)^{-1}$) in C. glomerata from different experimental habitats. Car Chl $(a+b)^{-1}$ (a), VAZ Chl $(a+b)^{-1}$ (b), $(0.5A + Z)\ (VAZ)^{-1}$ (c). For abbreviations see Fig. 2.*

F_v/F_m was significantly effected by the light treatments (Fig. 4, Table 2). After 14 days, the values were significantly higher in LL compared to IL and HL, as well as in IL compared to HL channels ($p < 0.05$, $p < 0.001$ and $p < 0.001$, respectively, Table 3). Current did not have significant effects on the F_v/F_m, despite the lower mean values of the low current treatments of most of the channels on day 14 and 28.

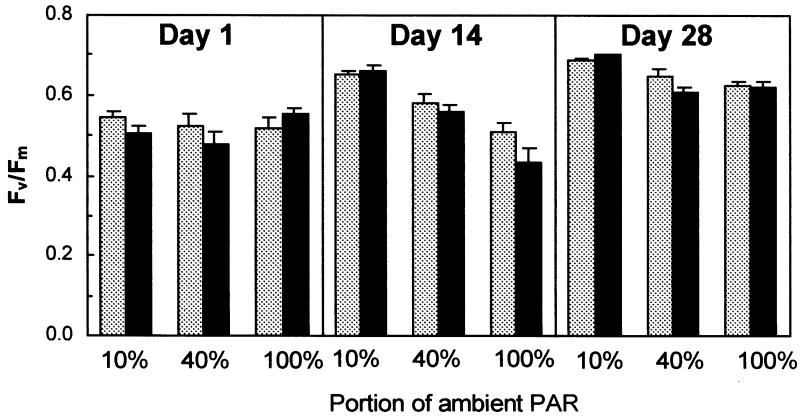

Figure 4 *Changes in the efficiency of open PSII (F_v/F_m) in C. glomerata from different experimental habitats. For abbreviations see Fig. 2.*

Relationships of the habitat type and the parameters determining F_v/F_m

Values of F_v/F_m from day 14 and day 28 were separated by high or low current treatment. Each of the two groups was subjected to a multiple regression analysis that used a forward selection method (Table 4). The parameters PAR (measured immediately before the determination of F_v/F_m), current velocity, Chl (a+b)/DM, Chl a/Chl b, Car/Chl (a+b), VAZ/Chl (a+b) and DEPS of xanthophyll cycle pigments were included into the procedure.

Table 1 *Multiple regression analysis of optimum quantum yield (F_v/F_m) of* C. glomerata *from different experimental habitats. Independent variables are numbered in order of their significance to quantum yield.* B = *regression coefficient,* β = *standardised regression coefficient.*

	B	*β*	*T*	Significance of *T* (*p*)
F_v/F_m in high current				
Constant	0.610		13.972	0.000
1. (0.5A+Z) $(VAZ)^{-1}$	-0.293	-0.592	-5.749	0.000
2. PAR	-0.00006409	-0.445	-4.462	0.000
3. Chl a Chl b^{-1}	0.06876	0.283	2.868	0.007
$R^2 = 0.744$, ANOVA $P < 0.001$				
F_v/F_m in low current				
Constant	0.759		17.683	0.000
1. (0.5A+Z) $(VAZ)^{-1}$	-0.430	-0.592	-7.828	0.000
2. current velocity	0.279	0.332	4.259	0.000
3. PAR	-0.00005239	-0.321	-4.308	0.000
4. total Chl	-0.01334	-0.226	-2.852	0.008
$R^2 = 0.853$, ANOVA $P < 0.001$				

The analysis revealed, that F_v/F_m of *C. glomerata* in both treatments depended mostly on the DEPS of the xanthophyll cycle pigments, as indicated by the β regression coefficients of -0.592 in high current as well as in low current channels (Table 4). In high current treatments, PAR was the second important factor followed by Chl a/Chl b (β = -0.445, β = 0.283, respectively, Table 4). In contrast, in low current treatments current velocity was the second important factor, followed by PAR and Chl (a+b)/DM (β = 0.332, β = -0.321, β = -0.226, respectively, Table 4).

Tip growth of *C.* glomerata

Tip growth of *C. glomerata* was highest in HL channels and lowest in LL channels (Fig. 5). Both, effects of current and light conditions were found to have significant effects on growth (two-way ANOVA, $p < 0.01$). Tip growth increased in the order LL < IL < HL channels, the differences between LL and IL, LL and HL, IL and HL were significant (Bonferroni, $p < 0.05$, $p < 0.01$, $p < 0.01$, respectively). Within each light treatment, high current treated plants revealed significant higher tip growth than low current treated plants (*t*-test, $p < 0.05$ for each treatment). No interaction of the two factors was detected.

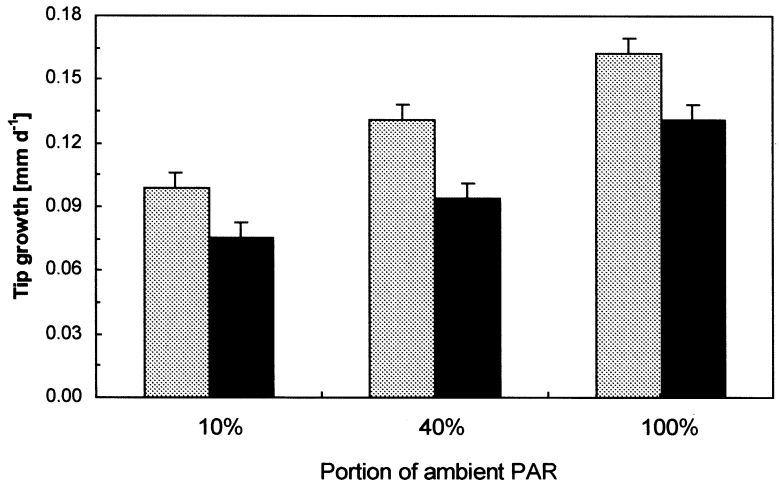

Figure 5 *Differences in tip growth per day of* C. glomerata *from different experimental habitats. For abbreviations see Fig. 2.*

DISCUSSION

Effects of time on *C. glomerata*

The factor time was considered to integrate two important features: Ageing of the individual thalli of *C. glomerata*, and variability of global irradiance, as a consequence of changes of the weather situation during the experiment. Other factors, like pH and temperature, show greater diurnal variation than changes over the short period of the 4 weeks during June and July. Under almost natural conditions this integrative factor time was responsible for time dependent changes of physiological parameters in *C. glomerata* as was demonstrated by significant effects on any of the observed parameters (Tables 2 and 3). Most obvious was its influence in the timecourse of DEPS of the xanthophyll cycle pigments (Fig. 3c). Compared to day 1 the level

of the values of days 14 and 28 constantly decreased due to the decrease of global irradiance (Fig. 1), whereas differences among the treatments of days 14 and 28 increased.

Effects of light and current on C. *glomerata*

Superimposed by this temporal effects, *C. glomerata* developed spatial characteristics in dependence to light and current. The estimated parameters of *C. glomerata* showed different susceptibility to the factorial treatments. Chl (a+b)/DM was influenced by the factor light (and the interaction of light and current on day 14). A feature of HL grown alga is their lower pigment content compared to LL grown alga (Neidhardt et al. 1998). This acclimation to different light environments resulted e.g. on day 28 in highest Chl (a+b)/DM values in LL/HC, somewhat smaller values in LL/LC and IL channels and lowest values in HL channels (Fig. 2a). The changes in Chl a/Chl b developed an inverse pattern. On day 28 highest values appeared in HL and lowest values in LL treatments and were related mostly to increases in the amount of Chl b. Decreases of Chl a/Chl b are a typical indicator of increased light capture efficiency at lower light intensities of LL-acclimated photoautotrophs, as Berner et al. (1989) showed in unicellular algae. In turn, acclimation to high light environments results in the reduction of the antenna system that contains most of the Chl b (Neidhardt et al. 1998).

In contrast to the light harvesting role of chlorophylls, most carotenoids serve as photoprotective agents (Demmig-Adams et al. 1996) that prevent the formation of reactive oxygen species in photosystem II by energy transfer from excited Chl a under environmental stress. In higher plants (Demmig-Adams et al. 1996) as well as in algae (Casper-Lindley & Björkman 1998) Car/Chl (a+b), and the fraction of carotenoids represented by the xanthophyll cycle pigments is increased in HL compared to LL photosynthetic tissues. The de-epoxidized xanthophylls (zeaxanthin and antheraxanthin) facilitate the harmless dissipation of excess excitation energy directly within the light-harvesting antenna (Demmig-Adams et al. 1996).

In our experiments all parameters related to carotenoids were effected by both, light and current. Car/Chl (a+b) increased with increasing light and was higher in LC compared to HC channels (Fig. 3a). It thus showed, that increased irradiance as well as lower current velocities increased the demand of photoprotective properties. Similarly, this pattern became obvious in VAZ/Chl (a+b) (Fig. 3b) and was finally underlined by the higher conversion state of the xanthophylls at increasing irradiance (Fig. 3c).

The optimum quantum yield F_v/F_m is the parameter which exhibits the properties of the photosynthetic apparatus and its pigment composition (Neidhardt et al. 1998, Uhrmacher, Hanelt & Nultsch 1997). Changes in F_v/F_m are supposed to occur faster than changes in most photosynthetic pigments, because it depends on fast, light dependent processes like acidification of the thylakoid lumen and the DEPS of the rapid xanthophyll cycle. For that reason,

changes between the different light habitats became already visible on day 14 (Fig. 4), but the level of F_v/F_m was maintained the same in LC and HC treatments. This was in contrast to the observed differences in the carotenoid patterns. Regulatory processes including cyclic electron transport around PSI, and shifts in the composition of the photosynthetic pigments associated with carotenoids therefore are supposed to account for the maintenance of the energy conversion at PSII, indicated by F_v/F_m, under the different current treatments.

Relationships of the habitat type and the parameters determining F_v/F_m

Whereas the ANOVA results showed the significance of the factors light and current on *C. glomerata*, the multiple regression analysis was used to describe the relationships between light, current, pigment composition, and F_v/F_m of *C. glomerata*.

There was a strong negative relationship between F_v/F_m and the DEPS of the xanthophyll cycle pigments in both, HC as well as in LC treatments, that illustrated the demand of *C. glomerata* on xanthophyll cycle related regulatory processes to protect its photosynthetic apparatus from overexcitation by PAR. (Table 4). In HC treatments, the value of F_v/F_m further decreased with increases of the parameter PAR. The positive relation to Chl a/Chl b (the higher the portion of Chl b, the higher the observed quantum yield) indicated that *C. glomerata* possesses a high potential to acclimate the absorption cross section of the photosynthetic antenna to the light environment.

In LC treatments, F_v/F_m was found to increase with increases in current velocity, whereas lower current velocities resulted in decreased quantum efficiency (Fig. 4). Nutrient depletion in the diffusion boundary layer within the thalli at low current velocities (Stevenson 1996; Raven 1992), which will include nitrate or CO_2 (Dodds 1991b) has to be considered as an explanation. Depleted CO_2 limits the Calvin cycle, and hence, causes energy imbalances between the photosynthetic electron transport chain and secondary processes of photosynthesis. Under this condition, reduced energy conversion through the photosystem is strongly demanded to prevent the photosystem from overexcitation and photodamage. Such processes were demonstrated by increased activity of the xanthophyll cycle. In HL treatments turbulent current might have played an additional role in preventing dynamic photoinhibition and decreases of F_v/F_m simply by movement of the thalli. If the filaments get mixed up, the individual chloroplast is constantly transferred from high light to attenuated light conditions. This avoids excitation pressure which develop easier in chloroplasts of thalli exposed to low and laminar current, where the upper filaments are permanently exposed to high fluence rates.

Ecological implications

Higher growth rates in HL compared to IL and LL exposure as well as in HC compared to LC treatments demonstrated the stimulation of growth by increases in irradiance and current

velocity. The importance of current, especially under light saturation conditions, was demonstrated by highest growth rates in the HL/HC treatment. In contrast, F_v/F_m revealed more complex responses to high current velocities and the different irradiances. Explanations have to take into account, that F_v/F_m expresses the capacity and regulation of the energy conversion process. This includes effects of photoprotective mechanisms related to the carotenoids and implicates increases of quantum efficiency in LL treatments as well as decreases in HL treatments due to enhanced protection against photodamage by increased non photochemical quenching via carotenoids.

In conclusion, *C. glomerata* clearly responds to changes in irradiance and current velocity. The plasticity of its pigmentation allows the adjustment of the photosystem to the requirements of the different habitat types, and thus, is an essential feature that provides success in dynamic freshwater habitats. Optimum quantum yield was found to be a good indicator of irradiance effects, whereas effects of current velocity on optimum quantum yield only became clearly visible if additional information on the carotenoid pattern was considered.

From our results it is concluded that the most favourable habitat for *C. glomerata* in the river Ilm is characterised by high current and high light conditions. During spring and autumn considerable parts of the river stretches match this requirements, because flow regime is characterised by moderate discharge rates without flood or drought disturbances, and there is no pronounced shading by dense tree canopies. During midsummer, flow generally is lowered and light is often attenuated by dense canopies of streamside tree vegetation. These conditions favour decreased growth rates and my contribute to the frequently observed summer decline of *C. glomerata* (e.g. Ensminger et al. 1998).

ACKNOWLEDGEMENTS

This study was supported by grants of the Deutsche Forschungsgemeinschaft (DFG) Graduate Study Group *Analysis of Function and Regeneration of Degraded Ecosystems* (Förderkennzeichen GRK 266/1-96). Data of discharge and nutrients were kindly provided by Staatliches Umweltamt Erfurt. Deutscher Wetterdienst Offenbach provided data of global irradiance.

REFERENCES

Berner T., Dubinsky Z., Wyman K. & Falkowski P.G. (1989) Photoadaptation and the "package" effect in *Dunaliella tertiolecta* (Chlorophyceae). *Journal of Phycology*, **25**, 70-78.

Biggs B.J.F. (1996) Hydraulic habitat of plants in streams. *Regulated Rivers: Research & Management*, **12**, 131-144.

Büch K., Stransky H., Bigus H.J. & Hager A. (1994) Enhancement by artificial electron acceptors of thylakoid lumen acidification and zeaxanthin formation. *Journal of Plant Physiology*, **144**, 641-648.

Casper-Lindley C. & Björkman O. (1998) Fluorescence quenching in four unicellular algae with different light-harvesting and xanthophyll-cycle pigments. *Photosynthesis Research*, **56**, 277-289.

Demmig-Adams B., Gilmore A.M., Adams W.A. (1996) In vivo functions of carotenoids in higher plants. *The FASEB Journal*, **10**, 403-412.

Dodds W.K. (1991a) Community interactions between the filamentous alga *Cladophora glomerata* (L.) Kützing, its epiphytes and epiphyte grazers. *Oikos*, **85**, 572-580.

Dodds W.K. (1991b) Micro-environmental characteristics of filamentous algal communities in flowing freshwaters. *Freshwater Biology*, **25**, 199-209.

Dodds W.K. (1989) Photosynthesis of two morphologies of *Nostoc parmelioides* (Cynobacteria) as related to current velocities and diffusion patterns. *Journal of Phycology*, **25**, 258-262.

Ensminger I., Hagen C. & Braune W. (1998) Photosynthetic activity and changes in the pigment pattern of *Cladophora glomerata* in dependence on light intensity in its natural habitat (River Ilm, Thuringia/Germany. *Photosynthesis: Mechanisms and Effects Vol. III*. (Ed. G. Garab), pp. 2333-2336. Kluwer Academic, Dorderecht.

Ensminger I., Hagen C. & Braune W. Strategies providing success in a variable habitat: I. Relationships of environmental factors and dominance of *Cladophora glomerata*. *Plant, Cell & Environment*, (in press).

Genty B.E., Briantais J.M. & Baker N.R. (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta*, **990**, 87-92.

Häder D.P., Herrman H., Schäfer J. & Santas R. (1997) Photosynthetic fluorescence induction and oxygen production in two mediterranean *Cladophora* species measured on site. *Aquatic Botany*, **56**, 253-264.

Hanelt D., Hupperts K. & Nultsch W. (1992) Daily course of photosynthesis and photoinhibition in marine macroalgae investigated in the laboratory and field. *Marine Ecology Progress Series*, **97**, 31-37.

Kasten F.H. (1981) Methods for fluorescence microscopy. *Staining procedures* (Ed. G. Clark), pp. 39-103. Williams & Wilkins, Baltimore & London.

Lichtentaler H.K. (1987) Chlorophylls and Carotenoids - Pigments of photosynthetic biomembranes. *Methods in Enzymology*, **148**, 350-382.

Madsen T.M & Sand-Jensen K. (1991) Photosynthetic carbon assimilation in aquatic macrophytes. *Aquatic Botany*, **41**, 5-40.

Madsen T.V., Enevoldsen H.O.& Jörgensen T.B. (1993) Effects of water velocity on photosynthesis and dark respiration in submerged stream macrophytes. *Plant, Cell & Environment*, **16**, 317-322.

Neidhardt J., Benemann J.R., Zhang L.P. & Melis A. (1998) Photosystem II repair and chloroplast recovery from irradiance stress: relationship between chronic photoinhibition, light-harvesting chlorophyll antenna size and photosynthetic productivity in *Dunaliella salina* (green algae). *Photosynthesis Research*, **56**, 175-184.

Parodi E.R. & Cáceres E.J. (1991) Variation in number of apical ramifications and vegetative cell length in freshwater populations of *Cladophora* (Ulvaceae, Chlorophyta). *Journal of Phycology*, **27**, 628-633.

Pfeifer F. & McDiffett W.F., (1975) Some factors affecting primary productivity of stream riffle communities. *Archiv für Hydrobiologie*, **73**, 306-317.

Power M.E. (1990**)** Effects of fish in river food webs. *Science*, **250**, 811-814.

Raven J.A. (1992) How benthic macroalgae cope with flowing freshwater: Resource acquisition and retention. *Journal of Phycology*, **28**, 13-146.

Schreiber U., Bilger W. & Neubauer C. (1994) Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of in vivo photosynthesis. *Ecophysiology of photosynthesis* (Eds E.D. Schulze & M.M. Caldwell) pp. 49-70. Springer, Berlin.

Steinman A.D. & McIntire C.D. (1986) Effects of current velocity and light energy on the structure of periphyton assemblages in laboratory streams. *Journal of Phycology*, **22**, 352-361.

Stevenson R.J. (1996) The stimulation and drag of current. *Algal ecology - Freshwater benthic ecosystems* (Eds R.J. Stevenson, M.L. Bothwell & R.L. Lowe) pp. 321-340. Academic Press, San Diego.

Uhrmacher S., Hanelt D. & Nultsch W. (1997) Zeaxanthin content and the degree of photoinhibition are linearly correlated in the brown alga *Dictyota dichotoma*. *Marine Biology*, **123**, 159-165.

Waaland S.D. & Waaland J.R. (1975) Analysis of cell elongation in red algae by fluorescent labelling. *Planta* (Berlin), **126**, 127-138.

Werner I. & Weise G. (1982) Biomass production of submersed macrophytes in a selected stretch of the River Zschopau (South GDR) with special regard to orthophosphate incorporation. *Internationale Revue der gesamten Hydrobiologie*, **67**, 45-62.

Whitton B.A. (1970) Biology of *Cladophora* in freshwaters. *Water Research* **4**, 87-114.

Xyländer M., Hagen C. & Braune W. (1996) Mercury increases light susceptibility in the green alga Haematococcus lacustris. *Botanica Acta*, **109**, 222-228.

7.5 Small-scale spatial distribution of benthic freshwater macroalgae in the hyporhithral of the river Ilm, Germany, related to properties of their photosynthetic performance

JULIA FOERSTER, INGO ENSMINGER, CHRISTOPH HAGEN & WOLFRAM BRAUNE

Freshwater Biology (in Begutachtung)

SUMMARY

1. The aim of this paper was (i) to monitor the benthic macroalgal assemblages in the hyporhithral of a mountain stream (river Ilm) in Thuringia, Germany, and to relate the observed species distribution to abiotic factors which were presumed to be valid on a small spatial scale and (ii) to test whether prominent benthic macroalgae, *Cladophora glomerata* (L.) Kütz and *Vaucheria* sp., were limited in their distribution by properties of their photosynthetic performance.
2. In early spring, the observed species distribution was best explained by water depth and current velocity. Over time, light intensity increased in importance. In summer and especially during the recolonization phase after a flood disturbance, light intensity was the most important environmental factor which accounted for almost 60 % of the explainable variation in the species distribution. Discharge rates did not exert a great impact on benthic macroalgal cover. Especially for *C. glomerata*, the dominant macroalgae in the examined stretch, a profound reduction in coverage as well as in length of thalli was observed already some time before the summer-flood occurred.
3. Measurements of photosynthetic electron transport rates at PS II indicated that *C. glomerata* dynamically acclimates to different light intensities: *C. glomerata* taken from high light habitats reached higher maximal electron transport rates and the resulting photosynthesis-irradiance-curve saturated at higher light intensities as compared to samples of *C. glomerata* taken from low light habitats. For *Vaucheria* sp., no such acclimation to different light intensities was observed.
4. Pigment analysis using HPLC corroborated these findings: samples of *C. glomerata* taken from high light habitats showed a significantly higher ratio of chlorophyll a to chlorophyll b and contained more xanthophylls per chlorophyll a. For *Vaucheria* sp., no differences in the pigment content between samples taken from different habitats were found.

5. Diurnal measurements of photosynthetic capacities revealed that under high light stress, *C. glomerata* shows pronounced dynamic photoinhibition as indicated by a decrease in F_v/F_m-values. For *Vaucheria* sp. held under the same conditions, only a minor decrease in F_v/F_m-values was observed. *Vaucheria* sp. was able to regulate its photosynthesis within minutes and presumably has a higher capacity for thermal dissipation of absorbed light energy than *C. glomerata*.
6. In conclusion, *C. glomerata* can grow over a wider range of light intensities and can be found even in shaded habitats. *Vaucheria* sp. shows no acclimation to different light intensities but presumably has an advantage over *C. glomerata* under high irradiance conditions.

INTRODUCTION

In fast-flowing freshwater streams, benthic algae are important primary producers and play a crucial role for the whole ecosystem. As outlined by Stevenson (1997), the distribution of benthic algae is caused by proximate, intermediate and ultimate factors which are interwoven in a hierarchical system. Stevenson proposed climate, geology and land-use as important ultimate factors that may explain the distribution of algae on a larger spatial scale, whereas proximate factors (i.e. those which directly effect life, metabolism and function of the organisms) are important in structuring the community on a small spatial scale.

In our study, focusing on the small-scale spatial distribution of benthic macroalgae, the following abiotic factors were considered: (i) substratum size: since bigger stones and blocks tend not to be turned over by current as easily as smaller stones and pebbles (Power & Stewart 1987), they offer more stable conditions for settlement. Dodds & Gudder (1992) described a positive correlation between the size of the substratum and the abundance of *Cladophora* sp., and for *Rhizoclonium* sp. Power & Stewart (1987) reported similar observations. (ii) Current velocity: the current may promote algal growth since it provides a constant nutrient supply. On the other hand, current causes mechanical stress, and increased current velocities can harm and eventually tear off algae (Biggs 1996; Stevenson 1997). Therefore, species distribution in relation to current velocity is likely to resemble some sort of optimum curve. For *Cladophora* sp., Schönborn (1996) stated optimal growth rates at velocities between 0.5 and 0.8 m s^{-1}. (iii) Light conditions: for photoautotrophic organisms, absorption of sufficient light energy is crucial for maintaining the metabolism. On the other hand, excessive light may result in over-excitation and photoinhibition of photosynthesis which finally can cause chronic damage of photosynthetic units as well as the whole plant cells (Powles 1984; Krause 1988; Osmond 1994). Recent research has provided much insight into the mechanisms by which plants try to

cope with excessive incident light and how they protect their photosynthetic units against harm caused by high light intensities (Dau 1994; Osmond 1994; Niyogi 1999). One important protecting mechanism consists of dissipating the surplus energy as heat. This process is positively correlated to the amount of zeaxanthin (Demmig-Adams & Adams 1992; Horton, Ruban & Walters 1994; Gilmore 1997) or, in some algal taxa, diatoxanthin (Olaizola & Yamamoto 1994) present in the plant cells. These xanthophylls are formed by de-epoxidation of precursors (violaxanthin or diadinoxanthin, respectively) during periods of high-light stress in the so-called xanthophyll-cycles (Hager & Holocher 1994 and references therein). Long, Humphries & Falkowski (1994) pointed out that there are costs attributed to the development and maintenance of these protecting mechanisms. They argue that the knowledge about the various mechanisms of photoinhibition that has been gained in laboratory experiments can now be taken to the field and might prove useful in understanding the distribution of plants. Hanelt (1998) found that depth distribution of arctic macroalgae is related to their capability of dynamic photoinhibition.

Benthic freshwater algae live in an ever-changing light environment: the incident light intensity varies considerably over the course of the year, and the patchiness of the riparian vegetation adds to the resulting pattern of different habitats. If benthic algae are divided into sun- and shade-species (Leukart & Hanelt 1995) and thus specialised to certain light conditions, the changes in the available light intensity should be followed by changes in the species distribution. On the other hand, Ensminger, Hagen & Braune (1998) observed acclimation to different light intensities in *Cladophora glomerata* (L.) Kütz. over the course of a year and in relation to the light intensities at the natural habitat. For other benthic algae, no information concerning their phenological plasticity and their capacity to acclimate to the naturally changing light environment is available. Therefore, in addition to mapping the species distribution observed in the stream, ecophysiological experiments in order to test for the algae's capacities to acclimate to different light intensities were conducted on samples of *C. glomerata*, the dominant macroalgae in the Ilm, and *Vaucheria* sp., a macroalgae belonging to a different algal division with a different pigment composition (see below) but morphologically resembling *C. glomerata* concerning the characteristics of a filamentous macroalgae.

MATERIALS AND METHODS

Study site

The Ilm is a small mountain river with headwaters in the *Thüringer Wald* in Thuringia, Germany, flowing through new red sandstone as well as through lime stone. Keeping the ultimate factors (climate and geology) sensu Stevenson (1997) equal, all research was carried

out in the hyporhithral of the Ilm. Two different types of habitats were chosen: shaded habitats (low light, LL) where the stream was bordered by trees and bushes, and open habitats (high light, HL) where due to lack of riparian vegetation, full sunlight reached the stream.

For some of the ecophysiological measurements, algal patches were taken from sunny sites in the stream and kept for 10 - 14 days in flow through channels. Water was supplied from the Ilm, current velocity was maintained at approx. 0.5 m s^{-1}. Black nylon mesh was used to cover the channels in order to create shaded habitats where algae received only 40 % (two layers of nylon mesh) or 20 % (four layers of nylon mesh) of the incident photosynthetic active radiation (PAR).

Distribution of macroalgae and diatoms

Along a 1 km stretch in the hyporhithral of the river Ilm upstream of the village Buchfart, 20 transects were established across the stream. These included open as well as shaded sites and sites of different depths (up to 0.8 m). Benthic vegetation was monitored at one meter intervals along each transect using a glass-bottom tube (Aqua-Scope, diameter of round plexiglas screen 0.1 m). Macroalgae were determined at least to the level of genus, and the percentage of cover per view was estimated. The presence of epilithic as well as epiphytic diatoms was noted when they appeared in macroscopically visible amounts. For *C. glomerata*, the maximum length of thalli was estimated to the nearest 0.1m. At every meter, current velocity was measured 0.03 m above ground using a Flow-Mate 2000 (Marsh-McBirney Inc., U.S.A.). The type of substratum was monitored on a 6-step scale (Schönborn 1992), and the light conditions were estimated on a 5-step scale (from open, potentially fully sunny sites down to completely shaded sites). Daily discharge rates as measured about 5 km downstream of the study site were provided by Staatliches Umweltamt Erfurt. Daily sums of global irradiance were measured with a thermopile at the weather station Weimar by Deutscher Wetterdienst Offenbach (DWD). From these data, weekly means were calculated. The weekly mean of the global irradiance was combined with the estimated light condition per site to give a semi-quantitative parameter that incorporated seasonal changes of the light intensity from spring to summer as well as the decrease of available light intensity at sites where the developing foliage of the riparian vegetation caused shade.

Transects were marked permanently by means of ironbars, and mapping was repeated on a weekly basis during spring and summer of 1999 in order to assess the extent of seasonal variations and the impact of disturbances (flooding events). Water temperature (°C), conductivity (µS cm^{-1}), pH, O_2-content (mg l^{-1}) and O_2-saturation (%) were assessed weekly by means of a universal meter (WTW, Germany), and the contents of NO_3^-, NO_2^-, NH_4^+ and o-PO_4 were determined using Merck equipment (Merck, Germany).

Mapped vegetational patterns were correlated to measured abiotic variables by means of Canonical Correspondence Analysis (CCA, ter Braak 1986) using the software program Canoco 4.0 for Windows including CanoDraw and CanoPost. Significance of the obtained CCA model was assessed by Monte Carlo tests (199 permutations). Running an additional CCA with a manual selection of the environmental variables allowed for calculating the relative importance of each variable.

Photosynthetic activity of *C. glomerata* and *Vaucheria* sp.

Photosynthesis was determined *in situ* via chlorophyll fluorescence using a portable PAM-2000 (Walz, Germany). A self-constructed clip was used to hold the algal sample in the flowing water during measurements. For each experiment, samples of *C. glomerata* and *Vaucheria* sp. from HL and LL habitats were used. Photosynthetic activity was measured on two different time-scales: On a time-scale of minutes, photosynthetic efficiency was determined by measuring photosynthesis-irradiance-curves (P-I-curves). Samples of *C. glomerata* and *Vaucheria* sp. were taken from HL- and LL - sites from the stream or flow through channels in the morning hours before they could have experienced any damage from the incident sunlight. The following routine was applied to each sample: after 5 min of pre-darkening and application of 20 s of far red light (λ = 700 nm), the optimal quantum yield (F_v/F_m) (nomenclature following van Kooten & Snel 1990) was determined by application of a saturation flash. After that, actinic white light was applied to the sample. Photosynthetic active radiation (PAR) was increased stepwise to about 800 µmol m^{-2} s^{-1}. At every light intensity, after 40 s of illumination, a saturation flash was given and the effective quantum yield ($\Delta F/F_m'$) was determined. From these data, the relative electron transport rate (rETR) was calculated with rETR = PAR x $\Delta F/F_m'$ x 0.5. The obtained data-points were fitted according to a model developed by Eilers & Peeters (1988) using the software program KyPlot 2.0. From these curves, the relevant parameters α (the initial slope of the curve), $rETR_{max}$ (maximum rate of electron transport), I_k (characteristic light intensity, given by $rETR_{max}$ x α^{-1}) and ω (a parameter denoting photoinhibition) were obtained.

Diurnal patterns of photosynthetic activity of *C. glomerata* and *Vaucheria* sp. were monitored on two clear, sunny days (01 June 1999 and 29 July 1999). At both days, PAR at midday reached more than 2000 µmol m^{-2} s^{-1}. Algal samples taken from HL and LL habitats were exposed at a HL site in the streambed. Every hour, photosynthetic performance was determined: the effective quantum yield ($\Delta F/F_m'$) of each sample under the natural sun light was measured *in situ*. Subsequently, the optimal quantum yield (F_v/F_m) of the same sample was determined after darkening the sample for 5 min and applying far red light for 20 s.

Photodiodes (BPW-21, Centronics, GB) which were calibrated against a Li-192 SA-sensor (LiCor, USA) were used to record incident PAR over the course of the day.

Pigment analysis

For pigment analysis, samples of *C. glomerata* and *Vaucheria* sp. were taken on the same dates as the P-I-curves. Samples were dry-blotted for 20 s between four layers of filter paper and then immediately frozen in liquid nitrogen. In the laboratory, pigment analysis was performed by HPLC as described by Xyländer, Hagen & Braune (1996), modified according to Ensminger, Hagen & Braune (2000).

The examined algae differ in their pigment compositions: *C. glomerata* belongs to the division Chlorophyta and contains the chlorophylls a and b (Chl a and Chl b) and a xanthophyll-cycle consisting of violaxanthin (V), antheraxanthin (A) and zeaxanthin (Z). *Vaucheria* sp. represents the division Heterokontophyta and contains the chlorophylls a and c (Chl c) but no Chl b. Its xanthophyll-cycle consists of diadinoxanthin (Ddx) and diatoxanthin (Dtx). For statistical analysis, the following parameters were calculated: for samples of *C. glomerata*, the ratio of Chl a per Chl b (Chl a/Chl b) and the amount of xanthophyll-cycle pigments per Chl a (VAZ/Chl a). Analogous, for samples of *Vaucheria* sp., the ratio of Chl a per Chl c (Chl a/Chl c) and the sum of Ddx and Dtx per Chl a (Ddx+Dtx/Chl a) were calculated.

During the diurnal measurements of photosynthesis (see above), additional samples of *C. glomerata* were taken every hour. Pigment analysis was conducted as described here, and the de-epoxidation state (DEPS) of each sample was determined with DEPS = (0.5A+Z)/(V+A+Z).

Statistics

For each algal species, parameters derived from P-I-curves and pigment analysis were compared in a one-way ANOVA testing for significant differences between HL and LL habitats. Spearman's rank correlations were used to correlate the parameters to the light history that the organisms had experienced before the experiments. Light history was calculated by averaging the daily sum of the global irradiance over 10 days prior to each measurement. For samples taken from shaded channels, the attenuation of PAR by the nylon mesh to 40 % or 20 % of the global irradiance was considered, whereas for samples taken from shaded sites directly in the stream, an attenuation of PAR to 40 % was assumed.

RESULTS

Macroalgal distribution

In the observed stretch of the river Ilm, *C. glomerata* was the dominant algae, followed by *Audouinella* sp., *Hildenbrandia rivularis* (Liebmann) J. Agardh, *Vaucheria* sp. and

Stigeoclonium sp.. For *C. glomerata, Vaucheria* sp. and *Stigeoclonium* sp., marked seasonal changes were observed whereas others (e.g. *Audouinella* sp.) showed constant coverage values during the observation time (Fig. 1a). For *C. glomerata*, a reduction in cover was observed beginning in mid-May. Coverage values reached a minimum some time before the summer-flood occurred (Figs 1a, c). This reduction in coverage percentage was paralleled by a reduction of thalli length of *C. glomerata* (Fig. 1b).

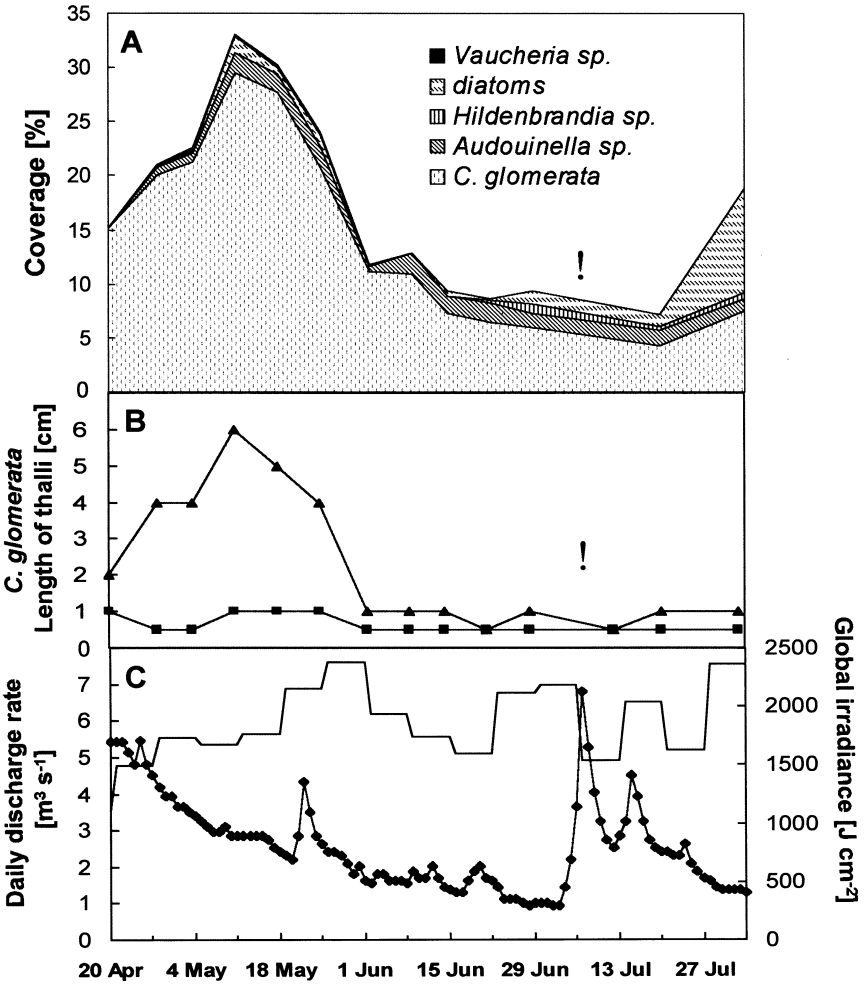

Figure 1 *Coverage values of benthic macroalgae and diatoms relative to sampled area (A); maximum (triangles) and median (squares) length of thalli of* C. glomerata *(B); daily discharge rates (diamonds) and weekly means of daily sums of the global irradiances (line) over the course of the observation period (20 April 1999 until 03 August 1999) (C).* Stigeoclonium *sp. was found on two occasions, 20 July and 03 August 1999, in very low quantities (0.02 and 0.27 % of sampled area, respectively). Expression mark indicates occurrence of summer-flood.*

Bivariate correlations between the algal species revealed a significant positive correlation between *C. glomerata* and diatoms (0.742, $p < 0.01$; Spearman's rank correlation coefficient), and a significant negative correlation between *C. glomerata* and *Auduoinella* sp. (-0.309, $p < 0.01$; Spearman's rank correlation coefficient).

After a preliminary detrended correspondence analysis (DCA) showed a sufficient length of gradient-value, a canonical correspondence analyses (CCA) was chosen in order to detect correlations between species distribution and measured environmental variables (ter Braak 1986). The CCA procedure directly relates data of community composition to the variation in the environmental variables. Results are presented in form of ordination diagrams where the first axis spans the maximal variation, thus having the highest explanatory value. The closer the

measured environmental variables, represented by arrows, come to the first or second axis, the more important is their influence on the community. The arrows start from the origin of the diagram with the grand mean of each environmental variable, they can therefore be extended backwards. By dropping perpendiculars from each species point to the arrow of interest, a ranking of species in regard to the environmental variable can be obtained.

Analysis of our data revealed that at different times of the year, different sets of environmental factors were important in structuring the observed species distribution. Therefore we split the data into subsets comprising shorter periods of time. One subset shown here (Fig. 2A) analyses the distribution as observed in spring (11 May - 25 May 1999) during and after the maximum coverage of *C. glomerata*, the second subset presented here (Fig. 2B) shows the species distribution in summer (20 July - 03 August 1999) during the recolonization phase after a flood disturbance. In spring, 9.1 % of the species distribution could be explained by the measured environmental variables (eigenvalue of first axis 0.126). Among these, water depth and current velocity were the most important factors (relative importance: 28.6 % each).
Toward the summer, the relative importance of light intensity increased and in the second subset, it accounted for 58.5 % of the explainable variance in the species data. This shift in importance of environmental factors was accompanied by a general increase of explanatory values reached with the CCA-procedure: for the summer subset, the eigenvalue of the first axis reached 0.444, and 19 % of the species variation could be explained by the environmental variables. In both subsets, *C. glomerata* is depicted close to the origin, thus showing no clear preference in regard to the measured abiotic variables. *Audouinella* sp. was found at greater depths and higher velocities than *C. glomerata* and *Vaucheria* sp. (Fig. 2A, spring subset). In regard to the light intensity (Fig. 2B, summer subset), *Stigeoclonium* sp. and the diatoms preferred open habitats whereas *H. rivularis* and *Audouinella* sp. were found more often in shaded habitats. *C. glomerata* was found in open as well as in shaded habitats, showing only a weak preference for higher light intensities. Noteworthy is further the changing relationship between the abiotic factors themselves: in spring, light intensity and water temperature were positively correlated, whereas in the summer, water temperature was correlated to the other physical and chemical properties of the water body (conductivity, discharge rate, O_2-saturation) but was independent from the light intensity.

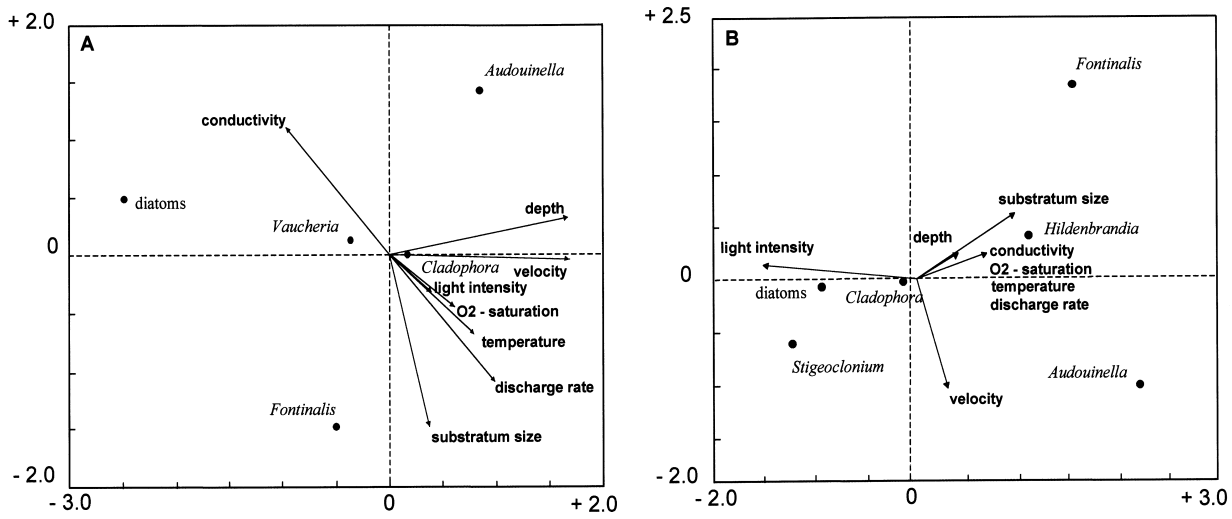

Figure 2 *Canonical correspondence analysis (CCA) diagrams showing the correlation between species distribution and relevant environmental variables during two different periods of the year (A: spring subset, May 1999, and B: summer subset, July/August 1999). Species are represented by points, environmental variables by arrows. For further explanation, see text. All axes were found to be highly significant ($p < 0.01$; Monte Carlo Simulation, 199 permutations). The eigenvalue of the first axis is 0.126 for the spring subset and 0.444 for the summer subset.*

Photosynthetic activity of *C. glomerata* and *Vaucheria* sp.

Mean values and standard errors for the parameters α, $rETR_{max}$, I_k and ω that were derived from the p-i-curves are shown in tables 1 and 2. Samples of *C. glomerata* from HL habitats reached significantly higher $rETR_{max}$- and I_k-values (Table 1) than samples from LL habitats, whereas the values for α and ω did not differ between samples taken from different habitats. Bivariate correlations (Table 3) revealed a significant positive relationship of $rETR_{max}$ and I_k-values to the light history (0.667 and 0.625, respectively; $p < 0.01$; Spearman's rank correlation coefficient). *Vaucheria* sp. (Table 2) reached about the same levels of $rETR_{max}$ as *C. glomerata* from LL habitats. Samples of *Vaucheria* sp. taken from different habitats did not differ in the measured parameters, and no correlation between these parameters and the light history was found (Table 3).

Table 1 C. glomerata*, parameters derived from P-I-curves. Given is the number of samples (N) and the mean ± standard error of the following parameters: α, the initial slope of the P-I-curve; $rETR_{max}$, maximum relative electron transport rate; I_k, characteristic light intensity; ω, photoinhibition. Significant differences between the samples from different habitats (HL, high light habitats, and LL, low light habitats) as detected by one-way ANOVA are indicated as follows: ** significant on 0.01 level; * significant on 0.05 level.*

Habitat	N	α	$rETR_{max}$	I_k		ω
HL	9	0.297 ± 0.024	40.723 ± 6.22 **	134.616 ± 12.98	**	0.332 ± 0.329
LL (40% of HL)	11	0.279 ± 0.015	18.769 ± 0.709 **	69.336 ± 5.058	**	0.963 ± 0.438

Table 2 Vaucheria *sp., parameters derived from P-I-curves. For explanation, see table 1.*

Habitat	N	α	$rETR_{max}$	I_k	ω
HL	15	0.203 ± 0.018	18.799 ± 1.660	113.900 ± 21.950	1.320 ± 0.749
LL (40 % of HL)	10	0.181 ± 0.021	20.268 ± 1.659	120.164 ± 13.095	-0.202 ± 0.299
LL (20 % of HL)	5	0.221 ± 0.038	17.720 ± 3.061	88.655 ± 15.594	1.544 ± 0.914

Table 3 *Spearman's rank correlation coefficients for the correlations between the parameters α, $rETR_{max}$, I_k and ω as given in tables 1 and 2 and the light history comprising 10 days prior to each measurement. Significant correlations are indicated as follows: ** significant on 0.01 level; * significant on 0.05 level.*

	N	α	$rETR_{max}$		I_k		ω
Light history of *C. glomerata*	20	-0.176	0.670	**	0.619	**	-0.212
Light history of *Vaucheria* sp.	30	-1.49	0.136		0.083		0.133

The diurnal measurements also revealed marked differences between the two algae: *C. glomerata* kept a higher level of $\Delta F/F_m'$ throughout the day but showed decreased F_v/F_m-values during times of high incident PAR (Fig. 3A). *Vaucheria* sp. under the same conditions retained a relatively high level of F_v/F_m regardless of extremely low $\Delta F/F_m'$ -values (Fig. 3B). For *C. glomerata*, a distinction between the samples from different habitats could be made (Fig. 4). *C. glomerata* from HL habitats kept a higher level of $\Delta F/F_m'$ during the day as compared to samples from LL habitats (Fig. 4A). However, both reached the same level of $\Delta F/F_m'$ in the evening hours.

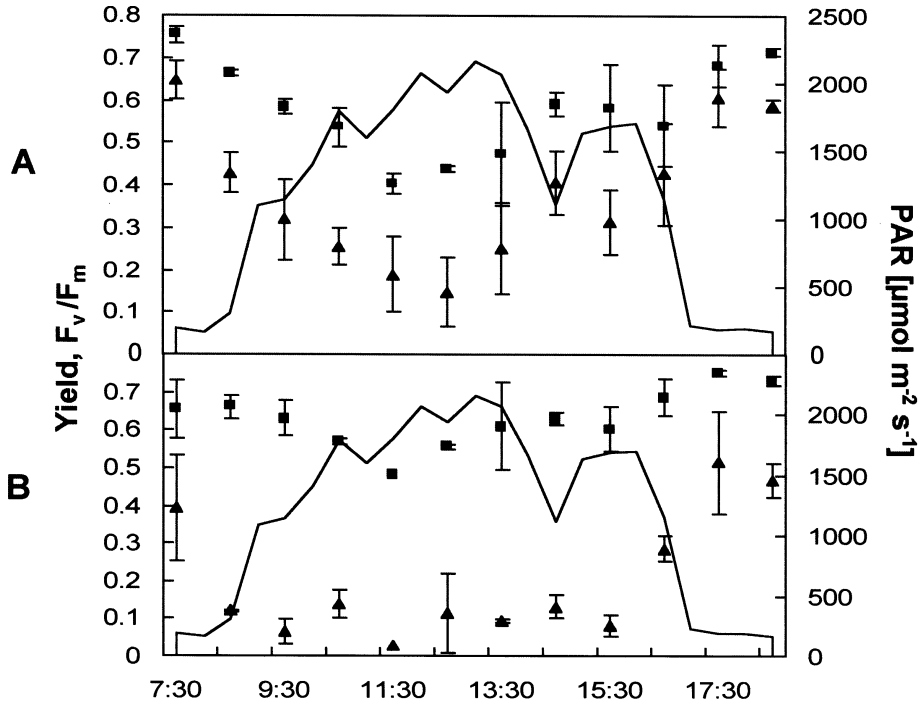

Figure 3 *Daily course of photosynthesis in (A)* C. glomerata *(N = 3) and (B)* Vaucheria *sp. (N = 2) as measured on 01.06.1999. Squares indicate the optimal quantum yield, F_v/F_m, after 5 min. of darkness and 20 s of far red. Triangles represent the effective quantum yield $\Delta F/F_m'$ measured under actinic light* in situ*. The incident photosynthetic active radiation (PAR) is given as a line in the background.*

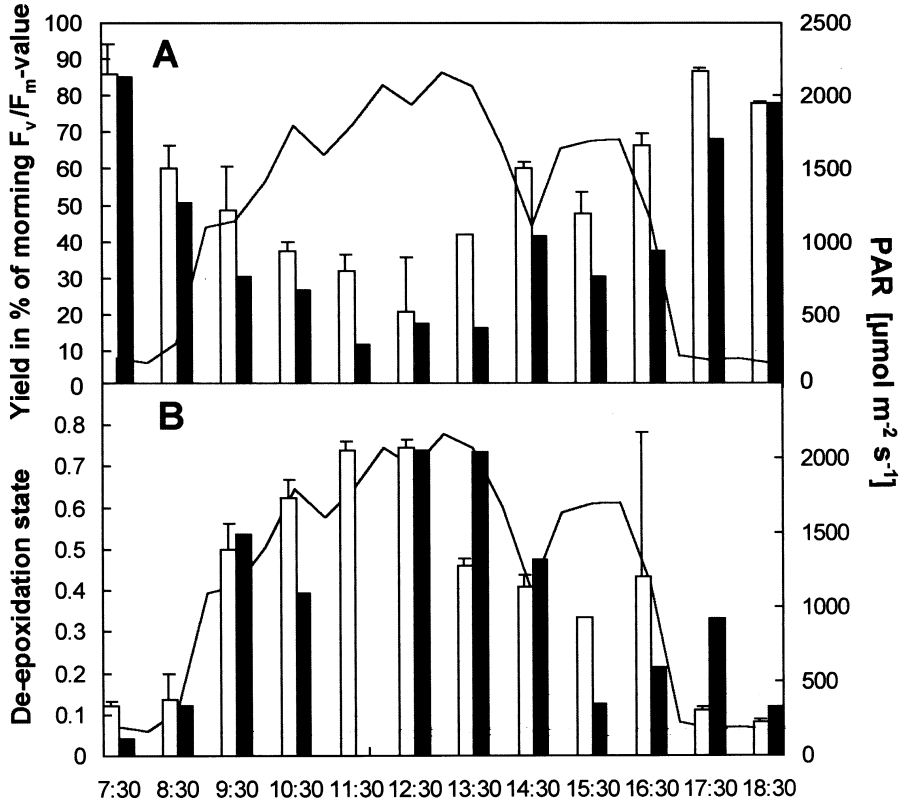

Figure 4 *Diurnal course of $\Delta F/F_m'$-values, presented here in percent of the F_v/F_m-value measured at 07.30 h in the morning; diurnal course of de-epoxidation state of samples of* C. glomerata *from HL (white bars, N = 2) and LL (dark bars, N = 1) habitats during exposure to HL conditions. The incident photosynthetic active radiation (PAR) is given as a line in the background (B).*

Pigment analysis

The results obtained from the pigment analysis for samples of *C. glomerata* and *Vaucheria* sp. are shown in tables 4 and 5, respectively. Samples of *C. glomerata* from HL habitats showed higher Chl a/Chl b-ratios and contained more VAZ/Chl a as compared to samples from LL habitats (Table 4). For *C. glomerata*, positive correlations between the light history and the Chl a/Chl b ratio (0.530; $p < 0.01$; Spearman's rank correlation coefficient) as well as between the light history and the amount of xanthophyll-cycle pigments per Chl a (0.425; $p < 0.01$; Spearman's rank correlation coefficient) were found (Table 6). Samples of *Vaucheria* sp. taken from different habitats did not show clear differences regarding their pigment content (Table 5). Consequently, no correlations with the light history could be established. Parallel to the measurement of diurnal patterns of photosynthesis, activity of the xanthophyll-cycle and subsequent changes in the de-epoxidation state (DEPS) were measured on samples of *C. glomerata*. The DEPS (Fig. 4B) showed pronounced changes over the course of the day, reaching a maximum during periods of high incident PAR. Samples from LL habitats started with a lower de-epoxidation state. Nevertheless, they reached the same maximum level as samples taken from HL habitats. Samples taken from LL habitats retained a high level of DEPS for a longer time in the afternoon and still showed a slightly higher DEPS in the evening.

Table 4 *Pigment content of samples of* C. glomerata *taken from HL and LL habitats. Given is the number of samples (N) and the mean ± standard error for the following parameters: Chl a/Chl b, ratio of Chl a to Chl b; VAZ/Chl a, the amount of xanthophylls that belong to the VAZ-xanthophyll-cycle (i.e. violaxanthin, antherxanthin and zeaxanthin) per Chl a. Significant differences between the samples from different habitats as detected by one-way ANOVA are indicated as in table 1.*

Habitat	N	Chl a/Chl b		VAZ/Chl a	
HL	20	1.795 ± 0.050	**	0.163 ± 0.007	**
LL (40% of HL)	16	1.519 ± 0.049	**	0.132 ± 0.006	**

Table 5 *Pigment content of samples of* Vaucheria *sp. taken from HL and LL habitats. Given is the number of samples (N) and the mean ± standard error for the following parameters: Chl a/Chl c, ratio of Chl a to Chl c; (Ddx+Dtx)/Chl a, the amount of xanthophylls that belong to the Ddx-xanthophyllcycle (i.e. diadinoxanthin and diatoxanthin) per Chl a. Significant differences between the samples from different habitats as detected by one-way ANOVA are indicated as in table 1.*

Habitat	N	Chl a/Chl c	(Ddx+Dtx)/Chl a
HL	17	27.110 ± 1.419	0.874 ± 0.053
LL (40 % of HL)	7	24.198 ± 2.082	0.790 ± 0.122
LL (20 % of HL)	7	30.657 ± 4.170	1.112 ± 0.105

Table 6 *Spearman's rank correlation coefficient for bivariate correlation between the measured pigment contents of samples of* C. glomerata *(data shown in table 4) and the light history (mean of 10 days prior to measurement). ** significant on 0.01 level.*

	N	Chl a/Chl b		VAZ/Chl a	
light history of *C. glomerata*	36	0.530	**	0.425	**

DISCUSSION

Macroalgal distribution

Marked seasonal changes in macroalgal coverage were observed (Fig. 1A). Especially for *C. glomerata*, a pronounced reduction in coverage values as well as in length of thalli was observed from May onward (Fig. 1B). This reduction could not be accounted for by flood-events (Fig. 1C). Apparently, other processes like biotic control by grazers (Dodds & Gudder 1992; Feminella & Hawkins 1995) or endogenous processes like ageing (Dudley, Cooper & Hemphill 1986; Schönborn 1996) were possibly relevant here and have to be considered in further studies. The role of diatoms might be especially interesting in this context. In our study, a positive relationship was established between the presence of *C. glomerata* and the diatoms. It is a common observation that *C. glomerata* can be used by diatoms as substrate for settlement (Whitton 1970; Dodds & Gudder 1992). However, possible functional dependencies between these two groups have not been examined so far.

Over the course of the observation period, different combinations of the measured abiotic environmental variables accounted for the observed distribution of the benthic algae. Whereas in spring, water depth and current velocity were the most important factors (Fig. 2A), over time light intensity gained importance and finally was the most powerful environmental variable (Fig. 2B). At the same time, the explanatory power of the CCA-model increased, reaching an eigenvalue > 0.4. The shift in the relative importances of the measured environmental variables might reflect the following process: in early spring, all sites received equal amounts of PAR. At sites which were bordered by deciduous trees and bushes, the development of the annual foliage changed the light situation, and shaded habitats were created over time. Due to the general increase of light intensity towards summer, the difference between the developing shaded sites and the open sites became more pronounced over the course of the year and showed the most striking effect on macroalgal distribution in summer. Also, the spring subset analysed the distribution of the macroalgae at a time when *C. glomerata* reached maximum coverage. *C. glomerata* proved to be rather indifferent in regard to the measured abiotic variables over the range tested here. Species with a pronounced preference for certain range of light intensities (e.g. *Stigeoclonium* sp., preferring open habitats or *H. rivularis*, preferring shaded sites) appeared only later in the summer and probably contributed to the higher explanatory power in the summer subset. Secondly, the summer period coincided with the recolonization phase after the summer-flood. Since our study included only abiotic environmental variables, the higher explanatory values reached for this period can be seen as a support of the view that abiotic factors are more relevant for describing a community after a disturbance. Townsend (1989) views disturbances as a reset mechanism for the ecosystem, whereas biotic interactions usually gain importance in periods of relative stability.

Photosynthetic activity of *C. glomerata* and *Vaucheria* sp.

In this study, *C. glomerata* from HL habitats consistently reached higher $\Delta F/F_m'$-values than *Vaucheria* sp., resulting in higher rETR- and $rETR_{max}$-values (sometimes twice as high, see Tables 1 and 2). During times of high incident PAR as it occurred on sunny summer days, *C. glomerata* showed a substantial decline in F_v/F_m-values. A decrease in F_v/F_m-values is seen as an indicator for (dynamic) photoinhibition (Krause et al. 1990; Hanelt 1998). At the same irradiance levels, *Vaucheria* sp. showed hardly any photoinhibition. The high levels of F_v/F_m retained by *Vaucheria* sp. throughout the day in combination with its low $\Delta F/F_m'$-values propose that *Vaucheria* sp. has a higher capacity for harmless energy dissipation. Laboratory experiments (data not shown) support this view. Energy dissipation is correlated to the conversion of epoxidized xanthophylls into their de-epoxidized forms in the xanthophyll-cycle (Demmig-Adams & Adams 1992; Horton et al. 1994; Olaizola & Yamamoto 1994). The

Ddx/Dtx-cycle has faster turnover times than the VAZ-cycle (Olaizola & Yamamoto 1994; Lohr & Wilhelm 1999). This may explain the fast recovery of the measured chlorophyll fluorescence parameters in *Vaucheria* sp.: within only 5 min. of darkness, *Vaucheria* sp. recovered from extremely low $\Delta F/F_m'$ -values and reached a high level of F_v/F_m-values. Whether this fast recovery process is due only to the activities of the xanthophyll-cycle or whether there are other mechanisms which contribute to these fast regulatory processes might be of interest for further experiments.

For samples of *C. glomerata*, the activity of the xanthophyll-cycle was shown by monitoring the diurnal pattern of changes in the de-epoxidation state (DEPS). DEPS increased with increasing PAR. This parallels results obtained for a variety of higher plants (Demmig-Adams & Adams 1992). Samples taken from LL habitats reached the same level of DEPS as samples taken from HL habitats, but seemed to react more slowly to changes in the incident PAR. These differences between samples acclimated to different light intensities should be examined more in detail.

Acclimation to different light intensities

Acclimation (i.e. dynamic adaptation as allowed by phenotypic plasticity) to different light intensities can be shown by comparing the photosynthetic performances and the pigment contents of samples from different habitats: Organisms adapted to higher light intensities reach higher maximal photosynthetic rates at higher light intensities, whereas shade-adapted organisms can use small amounts of light more efficiently as indicated by higher values of α (Boston & Hill 1991; Osmond 1994). Our results show that in *C. glomerata*, dynamic acclimation to different light intensities takes place. Samples taken from HL habitats reached higher $rETR_{max}$ and I_k-values than samples taken from LL habitats. These findings are consistent with earlier work conducted on *C. glomerata* (Ensminger et al., 1998). Furthermore, we found a significantly positive relationship between $rETR_{max}$- and I_k-values and the light history (covering 10 days prior to each measurement). However, the values for α did not show any significant differences between samples taken from different habitats, and no correlation with the light history was found. The regulation of α may be controlled by other environmental clues as well and maybe operates on a larger time-scale than considered in our study. Silva et al. (1998), testing for acclimation to different light intensities in the marine red alga *Gelidium sesquipedale*, observed the same pattern: samples taken from shallower water reached higher values for $rETR_{max}$ and I_k as opposed to samples from deeper waters, whereas no significant differences in α were detected. In lotic periphyton, Jasper & Bothwell (1986) detected a seasonal variation in α, but found no correlation with temperature or light history.

The results obtained from the pigment analysis support the observation of acclimation to different light intensities in *C. glomerata*: samples from different habitats showed significant differences in their pigment contents. Samples from HL habitats reached higher Chl a/Chl b-ratios and contained more VAZ/Chl a. Again, this was positively correlated to the light history. These results are in agreement with studies conducted on higher plants (Thayer & Björkman 1990; Demmig-Adams & Adams 1992; Gilmore 1997; Brugnoli et al. 1998). *Vaucheria* sp. did not show any sign of acclimation to different light intensities as tested in our experiments, neither on the level of pigment contents nor regarding P-I-characteristics.

Combining the results of the ecophysiological measurements with the results obtained from the analysis of the species distribution, the following conclusions can be drawn:
C. glomerata is able to acclimate to different light intensities, it can flourish in open as well as in shaded habitats. This is supported by the finding that *C. glomerata* did not show any clear preference in regard to the light intensities (Fig. 2). *C. glomerata* grown in HL conditions reached higher rETR-values which indicates that a higher ratio of the incident light energy is used to drive the electron transport chain. This effective energy conversion might constitute an advantage for *C. glomerata* and may possibly be the reason for its dominance at the study site. *Vaucheria* sp. might have an advantage over *C. glomerata* only in high light conditions, due to its fast regulatory mechanisms and reduced photoinhibition. But even during periods of high light stress, *C. glomerata* retained a higher level of rETR than *Vaucheria* sp.. The less effective energy conversion of *Vaucheria* sp. may be one factor contributing to its limited distribution. However, since larger amounts of *Vaucheria* sp. were found at other sites along the Ilm, other factors that limit its distribution might be of relevance here. Especially during periods of relative stability, biotic interactions may have a pronounced effect on the algal communities.

REFERENCES

Biggs B.J.F. (1996): Hydraulic habitat of plants in streams. *Regulated Rivers: Research & Management* **12**, 131-144.

Boston H.L. & Hill W.R. (1991): Photosynthesis-light relations of stream periphyton communities. *Limnology and Oceanography*, **364**, 644-656.

Brugnoli E., Scartazza A., de Tullio M.C., Monteverdi M. C., Lauteri M. & Augusti A. (1998): Zeaxanthin and the non-photochemical quenching in sun and shade leaves of C3 and C4 plants. *Physiologia Plantarum*, **104**, 727-734.

Dau H. (1994): Short-term adaptation of plants to changing light intensities and its relation to PS II photochemistry and fluorescence emission. *Journal of Photochemistry and Photobiology Series B: Biology*, **26**, 3-27.

Demmig-Adams B. & Adams W.W. (1992): Photoprotection and other responses of plants to high light stress. *Annual Review of Plant Physiology and Plant Molecular Biology*, **43**, 599-626.

Dodds W.K. & Gudder D.A. (1992): The ecology of *Cladophora*. *Journal of Phycology*, **28**, 415-427.

Dudley T.L., Cooper S.D. & Hemphill, N. (1986): Effects of macroalgae on a stream invertebrate community. *Journal of the North American Benthological Society*, **5**, 93-106.

Eilers P.H.C. & Peeters J.C.H. (1988): A model for the relationship between light intensity and the rate of photosynthesis in phytoplankton. Ecological Modelling **42**: 199-215.

Ensminger I., Hagen C. & Braune W. (1998): Photosynthetic activity and changes in the pigment pattern of *Cladophora glomerata* in dependence on light intensity in its natural habitat (river Ilm, Thuringia / Germany). *Photosynthesis: III. Mechanisms and Effects* (ed. G. Garab), pp. 2333-2336. Kluwer Academic Publishers, Dorderecht 1998.

Ensminger I., Hagen C. & Braune, W. (2000): Strategies providing success in a variable habitat: II. Ecophysiology of photosynthesis of *Cladophora glomerata*. *Plant Cell and Environment* (in press).

Feminella J.W. & Hawkins C.P. (1995): Interactions between stream herbivores and periphyton: a quantitative analysis of past experiments. *Journal of the North American Benthological Society*, **14**, 465-509.

Gilmore A.M. (1997): Mechanistic aspects of xanthophyll cycle-dependent photoprotection in higher plant chloroplasts and leaves. *Physiologia Plantarum*, **99**, 197-209.

Hager A. & Holocher K. (1994): Localization of the xanthophyll–cycle enzyme violaxanthin de-epoxidase within the thylakoid lumen and abolition of its mobility by a (light dependent) pH decrease. *Planta*, **152**, 581-589.

Hanelt D. (1998): Capability of dynamic photoinhibition in Arctic macroalgae is related to their depth distribution. *Marine Biology*, **131**, 361-369.

Horton P., Ruban A.V. & Walters R.G. (1994): Regulation of light harvesting in green plants. *Plant Physiology*, **106**, 415-420

Jasper S. & Bothwell, M.L. (1986): Photosynthetic characteristics of lotic periphyton. *Canadian Journal of Fisheries and Aquatic Sciences*, **43**, 1960-1969.

Krause G.H. (1988): Photoinhibition of photosynthesis. An evaluation of damaging and protective mechanisms. *Physiologia Plantarum*, **74**, 566-574.

Krause G.H., Somersalo S., Zumbusch E., Weyers B. & Laasch H. (1990): On the mechanism of photoinhibition in chloroplasts. Relationship between changes in fluorescence and activity of photosystem II. *Journal of Plant Physiology*, **136**, 472-479

Leukart P. & Hanelt D. (1995): Light requirements for photosynthesis and growth in several macroalgae from a small soft-water stream in the Spessart mountains, Germany. *Phycologia*, **34**, 528-532.

Lohr M. & Wilhelm C. (1999): Algae displaying the diadinoxanthin cycle also possess the violaxanthin cycle. *Proceedings of the National Academy of Sciences of the United States of America*, **96**, 8784-8789.

Long S.P., Humphries S. & Falkowski P.G. (1994): Photoinhibition of photosynthesis in nature. *Annual Review of Plant Physiology and Plant Molecular Biology*, **45**, 633-662.

Niyogi K.K. (1999): Photoprotection revisited: genetic and molecular approaches. *Annual Review of Plant Physiology and Plant Molecular Biology*, **50**, 333-359.

Olaizola M. & Yamamoto H.Y. (1994): Short-term response of the diadinoxanthin cycle and fluorescence yield to high irradiance in *Chaetoceros muelleri* (Bacillariophyceae). *Journal of Phycology*, **30**, 606-612.

Osmond C.B. (1994): What is photoinhibition? Some insights from comparisons of shade and sun plants. *Photoinhibition of photosynthesis – from molecular mechanisms to the field*. (eds. Baker N.R. & Bowyer, J.R.), pp. 1-24. Bios Scientific Publishers Oxford.

Power M. & Stewart A.J. (1987): Disturbance and recovery of an algal assemblage following flooding in an Oklahoma stream. *American Midland Naturalist*, **117**, 333-345.

Powles S.B. (1984): Photoinhibition of photosynthesis induced by visible light. *Annual Review of Plant Physiology*, **35**, 15-44.

Schönborn W. (1992): *Fließgewässerbiologie*. Gustav Fischer Verlag

Schönborn W. (1996): Algal aufwuchs on stones, with particular reference to the *Cladophora*-dynamics in a small stream (Ilm, Thuringia, Germany): production, decomposition and ecosystem reorganizer. *Limnologica*, **26**, 375-383.

Silva J., Santos R., Serodio J. & Melo R.A. (1998): Light response curve for *Gelidium sesquipedale* from different depths, determined by two methods: O_2-evolution and chlorophyll fluorescence. *Journal of Applied Phycology*, **10**, 295-301.

Stevenson R.J. (1997): Scale-dependent determinants and consequences of benthic algal heterogeneity. *Journal of the North American Benthological Society*, **16**, 248-262.

ter Braak C.J.F. (1986): Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. *Ecology*, **67**, 1167-1179.

Thayer S.S. & Björkman O. (1990): Leaf xanthophyll content and composition in sun and shade determined by HPLC. *Photosynthesis Research*, **23**, 331-343.

Townsend C.R. (1989): The patch dynamics concept of stream community ecology. *Journal of the North American Benthological Society*, **8**, 36-50.

van Kooten O. & Snel J.F.H. (1990): The use of chlorophyll fluorescence nomenclature in plant stress physiology. *Photosynthesis Research*, **25**, 147-150.

Whitton B.A. (1970): Biology of *Cladophora* in fresh-waters. Water Research, **4**, 457-476.

Xyländer M., Hagen C. & Braune W. (1995): Mercury increases light susceptibility in the green alga *Haematococcus lacustris*. *Botanica Acta*, **109**, 222-228.

BILDTAFEL

Abbildung 1 Cladophora glomerata*, junger Thallus mit typischen Verzweigungen (500x).*

Abbildung 2 Cladophora glomerata*, Thallus mit Zoosporangien, teilweise entleert, sowie epiphytischen Diatomeen (250x).*

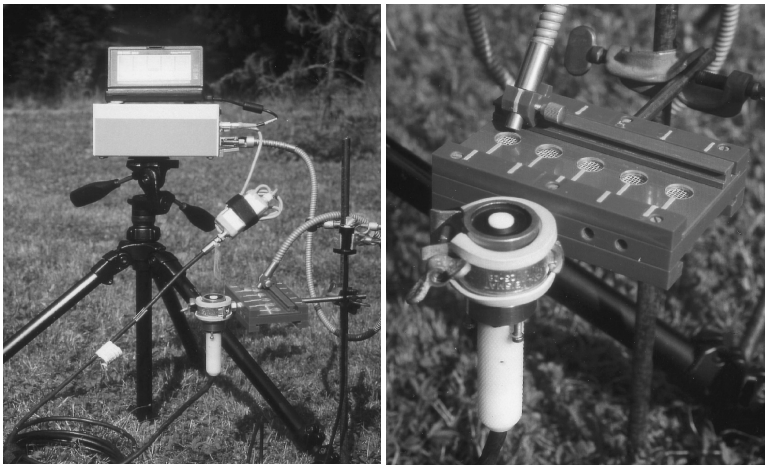

Abbildung 3 *PAM-2000, Anordnung für die* in situ *Messungen in der Ilm. Auf dem Stativ die Grundeinheit des PAM-2000 mit Steuer-computer, über die Fieberoptik erfolgen Anregung und Detektion der Chlorophyll-Fluoreszenz (links). Im Ausschnitt (rechts) ist die Probenhalterung zur Messung der Chlorophyll-Fluoreszenz an fädigen Makroalgen mit 5 getrennten Kammern dargestellt. die Positionierung der Fieberoptik kann über eine Schiene an jder der Kammern erfolgen. Im Vordergrund Li-192 SA Unterwassersensor zur Bestimmung der photosynthetisch aktiven Strahlung.*

DANKSAGUNG

Geschafft! Vorfreude auf die nächsten Etappen („Wir müssen weiter Sal, immer weiter...“). Viele haben auf die eine oder andere Weise zum Gelingen dieser Arbeit beigetragen, wichtig waren aber auch die Rahmenbedingungen: Die kollegiale und freundschaftliche Atmosphäre am Institut für Allgemeine Botanik.

Mein besonderer Dank gilt

Prof. W. Braune - Direktor des Insitituts für Allgemeine Botanik der FSU Jena - für die Möglichkeit, im Rahmen des Graduiertenkollegs „Funktions- und Regenerationsanalyse belasteter Ökosysteme“ ein Thema mit limnologisch/physiologischer Fragestellung bearbeiten zu können sowie für sein großes Interesse am Fortgang der Arbeit;

der Deutschen Forschungsgemeinschaft (DFG), die mir ein dreijähriges Promotionsstipendium gewährte und im Rahmen des Graduiertenkollegs durch die Bereitstellung wesentlicher Mittel die Durchführung meiner Untersuchungen ermöglichte;

Dr. C. Hagen - Institut für Allgemeine Botanik, FSU Jena - „Mr. Photosynthese“ himself, ohne den weder die erste Induktionskinetik noch die erste Pigmentanalyse zustande gekommen wäre;

PD Dr. D. Hanelt - Biologische Anstalt Helgoland - für seine wertvollen Erfahrungen im Umgang mit Makroalgen und dem PAM-2000 und seine stete Diskussionsbereitschaft;

Prof. Dr. B. A. Whitton - Department of Biological Sciences, Durham - gab mir wiederholt Gelegenheit über benthische Algen im allgemeinen und *Cladophora glomerata* im besonderen zu diskutieren;

PD Dr. W. Schönborn - AG Limnologie des Institutes für Ökologie, FSU Jena - für die vor allem in der Anfangszeit unschätzbare Hilfe beim „Kennenlernen“ der Ilm und für sein Interesse am Fortgang der Arbeit;

D. Nohr - Forellengrund Mellingen - der mir freundlicherweise die Möglichkeit gab, einen Teil meiner Freilandexperimente (darunter der Aufbau des künstlichen Fließgerinnes) auf dem Gelände der Fischzuchtanlage durchzuführen;

meinen Kolleginnen und Kollegen am Institut für Allgemeine Botanik, insbesondere J. Foerster, B. Klein, Dr. S. Siegmund und Dr. M. Xyländer;

E. Froneberg - Wien - für die Durchsicht englischsprachiger Manuskripte;

Dr. J. Schumacher - Institut für Ökologie, FSU Jena - für die geduldige Einweisung in verschiedene Verfahren der statistischen Datenanalyse;

Prof. Dr. S. Halle - Sprecher des Graduiertenkollegs und Direktor des Instituts für Ökologie der FSU Jena - sowie Dr. J. Perner, ebenfalls Institut für Ökologie, für die vielfältige Unterstützung meiner Arbeit;

M. Vetter - Institut für Ökologie, FSU Jena - die während vieler gemeinsamer Probenahmetage an und in der Ilm jedem Wetter trotzte (geteiltes Leid ist halbes Leid); ihre Freude zu diskutieren, Meinungen auszutauschen und zusammen Ideen zu entwickeln war eine wertvolle Bereicherung des ersten Untersuchungsjahres;

und *last not least* Beate (und natürlich Yara) für ihre Geduld und ihre moralische Unterstützung.

ERKLÄRUNG

Ich erkläre, daß ich die vorliegende Arbeit selbständig und nur unter Verwendung der angegebenen Hilfsmittel und Literatur angefertigt habe.

Jena, den 27. November 2000

Ingo Ensminger