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RÉPONSES ÉCOLOGIQUES ET ÉVOLUTIVES DE POPULATIONS DE PHYTOPLANCTON SUITE À UNE ACIDIFICATION

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TABLE DES MATIÈRES

LISTE DES FIGURES vii
LISTE DES TABLEAUX viii
RÉSUMÉ ix
INTRODUCTION 1
CHAPITRE I
RAPID ECO-EVOLUTIONARY RESPONSES OF PHYTOPLANKTON LAKE COMMUNITIES TO A PERTURBATION
1.1 Introduction 11
1.1.1 Ecological rescue 12
1.1.2 Evolutionary rescue 13
1.1.3 Genotyping natural populations 15
1.2 Methods 16
1.2.1 Experimental design 16
1.2.2 Sampling 17
1.2.3 Genotyping 18
1.2.4 Statistical analyses 20
1.3 Results 22
1.3.1 Ecological response 22
1.3.2 Genetic response 33
1.4 Discussion 44
1.4.1 Ecological response 44
1.4.2 Genetic response 46
1.4.3 Potential top-down effects 50
1.4.4 Eco or evo? 52
1.5 Conclusion 53
CONCLUSION 55

BIOBLIOGRAPHIE.....60

LISTE DES FIGURES

Figure		Page
1.1	Phytoplankton community total biovolume time series in each mesocosm..	23
1.2	Boxplot showing resilience with total Chl *a* for each of the experimental treatments.	24
1.3	Proportion of each phytoplankton class as a function of total biovolume through time in each mesocosms..	26
1.4	Boxplot showing the community synchrony index, measuring compensatory dynamics, for each treatment based on genus and species level biovolumes	27
1.5	Axis 1 of the PRC analysis (1) including diatoms.	29
1.6	Axis 1 of the PRC analysis (2) excluding diatoms.	32
1.7	Histograms showing the relative abundances of *Desmodesmus cuneatus* OTUs through time across treatments (columns) for each replicate mesocosms (rows).	38
1.8	Histograms showing the absolute abundances of *Desmodesmus cuneatus* OTUs through time across treatments (columns) for each replicate mesocosms (rows).	39
1.9	Histograms showing the relative abundances of *Chlamydomonas* sp.11 OTUs through time across treatments (columns) for each replicate mesocosms (rows).	40
1.1	Histograms showing the absolute abundances of *Chlamydomonas* sp.11 OTUs through time across treatments (columns) for each replicate mesocosms (rows).	41
1.11	Histograms showing the relative abundances of *Desmodesmus cuneatus* OTU36 OTUs (clustered at 97% identity) through time across treatments (columns) for each replicate mesocosms (rows).	42
1.12	Histograms showing the relative abundances of *Desmodesmus cuneatus* OTU38 OTUs (clusterd at 97% identity) through time across treatments (columns) for each replicate mesocosms (rows).	43
1.13	Histograms showing the relative abundances of *Chlamydomonas* sp.11 OTU70 OTUs (clusterd at 97% identity) through time across treatments (columns) for each replicate mesocosms (rows).	44

LISTE DES TABLEAUX

Tableau Page

1.1 Abbreviated and full names of phytoplankton species identified microscopically across all of the experimental communities and their scores associated with the PRC including (Score 1) and without (Score 2) diatoms. 30

1.2 Genera and the number and names of species identified using ITS2 sequencing. The OTUs column shows the total number of OTUs (including singletons and doubletons) as well as the number (in brackets) used to identify changes in genotype frequencies. The last column the number of OTUs detected per species. 36

1.3 Genetic response to perturbation indicating in which of the two treatments each molecularly-identified species was found. 37

RÉSUMÉ

En cette ère de perturbations anthropiques où l'extinction d'espèces vivantes atteint une vitesse record, l'état de la biodiversité est particulièrement préoccupant. Certaines espèces peuvent réussir à éviter l'extinction si elles évoluent assez rapidement pour s'adapter aux nouvelles conditions de leur milieu selon le processus de sauvetage évolutif. Le but de cette étude était d'explorer les mécanismes stabilisateurs responsables de la récupération des communautés de phytoplancton suite à une perturbation. Pour ce faire, nous avons effectué une expérience pendant l'été 2012 dans des mésocosmes installés sur le Lac Hertel en Montérégie dans lesquels nous avons abaissé le pH jusqu'à 5.0 (le pH naturel du lac étant de 8.0) et étudié la récupération des communautés naturelles du Lac Hertel. Cette perturbation a été appliquée de deux façons pour tester l'effet du type de perturbation sur la récupération des communautés i) de manière ponctuelle, c'est-à-dire que le pH n'a été abaissé qu'une seule fois et ii) de manière constante où le pH a été maintenu à 5.0 durant toute l'expérience. Afin d'observer les changements génétiques potentiels dus au sauvetage évolutif, nous avons séquencé l'ITS2, une région non-fonctionnelle de l'ADN ribosomal. Nous avons observé que dans le cas d'une perturbation de type ponctuel, la récupération des communautés se fait via des mécanismes écologiques, plus particulièrement par le biais des dynamiques compensatoires et aucune réponse génétique n'a été détectée dans ces traitements. Par contre, lorsque le pH était maintenu à 5.0, non seulement avons-nous observé les mêmes dynamiques compensatoires que dans le cas d'une perturbation ponctuelle, mais pour deux espèces de chlorophytes, soit *Desmodesmus cuneatus* et une espèce de *Chlamydomonas*, nous avons également pu observer un évènement de sauvetage évolutif via la sélection clonale. Cette sélection s'opérerait à partir d'une variation génétique déjà présente dans le génome et non à partir d'une mutation apparue post-perturbation, puisque nous avons limité le temps et l'immigration. Cette étude démontre que bien que rare, le sauvetage évolutif est possible sur des communautés naturelles gardées dans leurs conditions naturelles lorsque les nouvelles conditions environnementales dues à une perturbation sont maintenues assez longtemps pour enclencher une sélection qui permet à la population de s'adapter.

Mots-clés : Acidification, dynamiques compensatoires, sauvetage évolutif, perturbation, phytoplancton.

INTRODUCTION

Les scientifiques ont récemment nommé «Anthropocène» la période géologique dans laquelle nous vivons (Crutzen 2002) depuis les débuts de l'ère industrielle. En effet, la vaste majorité (si ce n'est la totalité) des écosystèmes a maintenant été altérée par des perturbations anthropiques; augmentation de la température de l'air et de l'eau, introduction d'espèces exotiques, destruction massive d'habitats et fragmentation du territoire, nous modelons notre environnement, et ce jusqu'au niveau géologique. Ces modifications ont, de toute évidence, des impacts majeurs sur les espèces qui doivent développer des stratégies pour éviter l'extinction.

Pour pouvoir résister aux perturbations de plus en plus nombreuses et fréquentes auxquelles elles font face, les communautés sujettes à ces perturbations peuvent répondre écologiquement ou évolutivement. Une réponse dite écologique impliquera un changement dans l'abondance relative des espèces au sein de la communauté, par exemple les dynamiques compensatoires (sur lesquelles nous reviendrons) sont un cas commun de réponse écologique à une perturbation. Par contre, si un changement génétique est observé au sein d'une espèce suite à une perturbation, on peut alors parler de réponse évolutive qui pourrait découler soit de l'action de la sélection naturelle ou de la dérive génique.

Il est important de mentionner que deux grands types de perturbations ont été définis; les perturbations ponctuelles (ou «pulse» en anglais) et les perturbations constantes ou maintenues («press»). Les perturbations ponctuelles sont des perturbations où le système affecté peut retourner à son état initial après la perturbation, tandis qu'avec une perturbation constante, le système ne peut pas retourner à son état initial, il doit trouver une nouvel équilibre dans ses nouvelles conditions (Bender et al. 1984).

Avec l'attention grandissante portée aux changements climatiques, de nombreux auteurs ont tenté de déterminer si les espèces pouvaient évoluer assez rapidement pour survivre à des changements radicaux de leur environnement et dans la plupart des cas (chez les invertébrés du moins) ils ont découvert qu'elles en avaient effectivement la capacité (Thompson 1998; Bradshaw and Holzapfel 2001; Hairston Jr et al. 2005; Fussmann et al. 2007; Kinnison and Hairston 2007). Il est donc maintenant globalement accepté que la distinction entre temps évolutif et temps écologique est artificielle, pour reprendre les mots de Kinnison et Hairston (2007), étant donné que les deux types de processus peuvent avoir lieu simultanément.

Stabilité

La résistance à une perturbation est une des deux composantes définissant la stabilité d'une population, d'une espèce, d'une communauté ou d'un écosystème (Pimm 1984). Pimm (1984) a défini un système comme étant stable seulement si toutes les variables qui le caractérisent retournent à l'équilibre après la perturbation. Toujours selon Pimm, dont la définition a été reprise par à peu près tous les auteurs travaillant sur la stabilité des écosystèmes, la stabilité d'un système se mesure par ses deux composantes, soit la résistance, telle que déjà mentionnée, et la résilience. La résilience réfère au temps que prend une variable pour revenir à son état d'équilibre alors que la résistance est le degré auquel une variable est affectée par la perturbation (Pimm 1984).

Une plus grande diversité spécifique apporte au système un plus grand étendu de réponses à la perturbation et cette diversité dans le type et l'intensité de la réponse permet une plus grande stabilité (Elmqvist and Folke 2003), en effet en réagissant plus ou moins fortement à la perturbation, certaines espèces peuvent éviter l'extinction alors que d'autres non, permettant ainsi à au moins une partie des espèces de survivre alors que si toutes avaient eu la même réponse, toutes auraient pu s'éteindre. Ces réponses asynchrones à la perturbation sont à la base du concept d'effet d'assurance («insurance effect») qui stipule que la redondance fonctionnelle (plusieurs espèces ont le même rôle dans la communauté) donne une certaine forme d'assurance à la communauté contre les perturbations, en ce sens que

malgré la disparition de certaines espèces, d'autres pourront continuer d'assurer la même fonction dans la communauté(Walker 1992; Petchey et al. 1999; Yachi and Loreau 1999).

Cet effet positif de la diversité sur la stabilité trouve sa source dans deux grands mécanismes; l'effet de dominance ou d'échantillonnage et la complémentarité (Tilman 1999; Loreau et al. 2001). L'effet d'échantillonnage («sampling effect») prévoit que plus la richesse en espèces est grande, plus grande est la probabilité pour qu'un compétiteur supérieur domine la communauté, et un meilleur compétiteur, par définition, utilise plus et mieux les ressources disponibles, d'où la plus grande productivité. Par contre, l'effet d'échantillonnage implique que l'habitat soit homogène. La complémentarité prévoit que plusieurs espèces exploitant chacune une niche distincte, peuvent exploiter l'habitat de façon plus optimale une fois rassemblées, puisqu'elles couvrent un plus grand «étendu» de niches que ne le ferait une association d'espèces plus restreinte.

La diversité ne se mesure pas qu'en nombre d'espèces, mais elle se mesure également en nombre d'écosystèmes ou, d'une manière plus pertinente à la présente recherche, en terme de gènes ou de génotypes. En effet, Naeem a été le premier à établir un lien entre la diversité génétique et les processus écosystémiques (Naeem et al. 1994) et (Reusch et al. 2005) ainsi que (Hughes and Stachowicz 2004) ont également montré que la diversité génotypique peut avoir les mêmes effets sur la stabilité que la diversité spécifique. La diversité génétique sous-tend la diversité phénotypique et donc l'asynchronisme des réponses à la perturbation, mais aussi la diversité fonctionnelle qui elle, comme nous venons de le voir, procure une certaine assurance en cas de perturbation. La diversité génétique agit également positivement sur la stabilité en apportant un bassin de mutations plus grand qui fournit donc par le fait même une plus grande chance de trouver des génotypes plus robustes face à la perturbation (Fridley et al. 2007) ce qui peut permettre à une population d'éviter l'extinction comme nous l'avons vu plus haut. Finalement, (Vellend 2006) a montré que la diversité génétique permet une plus grande couverture de l'espace de niches et donc conformément à l'hypothèse de la complémentarité, permettre une plus grande couverture de cet espace et assurer ainsi plus de stabilité au milieu.

Dynamiques compensatoires

Les dynamiques compensatoires sont un des mécanismes par lesquels la diversité permet plus de stabilité à une communauté. Elles promeuvent cette stabilité en augmentant la résilience et la résistance de la communauté suite à une perturbation, en tamponnant les fluctuations dans la densité et la fonction des différentes populations constituant la communauté (McNaughton 1977; Frost et al. 1995; Ives and Cardinale 2004). La stabilité procurée par de telles dynamiques découle donc de l'effet d'assurance, qui lui-même repose sur la redondance fonctionnelle comme nous l'avons vu précidemment. On parle de dynamiques compensatoires dans les situations où la compétition entre deux espèces les fait covarier négativement, c'est-à-dire que lorsque la densité de l'une augmente, celle de l'autre diminue. Donc, dans un contexte de perturbation, si un des deux compétiteurs vient à s'éteindre, l'autre pourra ainsi prendre la niche écologique laissée vacante et assurer le maintien de la fonction préalablement remplie par le compétiteur qui dominait la communauté avant la perturbation. Par contre, une covariance négative n'est pas suffisante pour provoquer des dynamiques compensatoires, les deux espèces doivent montrer des réponses différentes à la perturbation (Klug et al. 2000); une doit être négativement affectée par cette perturbation alors que l'autre l'est positivement ou, à tout le moins si elle est affectée négativement, elle l'est dans une moindre mesure que sa compétitrice. Pour résumer, suite à une perturbation, si l'espèce A, normalement la meilleure compétitrice, réagit négativement à cette perturbation et voit son abondance diminuer et que l'espèce B d'un autre côté profite de la diminution voire de la disparition de l'espèce A pour utiliser les ressources normalement utilisée par cette dernière et ainsi augmenter sa croissane et son abondance, nous sommes devant un cas de dynamique compensatoire entre ces deux espèces.

Sauvetage évolutif

La résistance et la résilience à une perturbation peuvent aussi être le fait d'une adaptation aux nouvelles conditions environnementales. Pour peu qu'elles évoluent rapidement, les espèces peuvent donc être sauvées de l'extinction par l'évolution selon un processus que l'on nomme sauvetage évolutif (mieux connu sous son patronyme anglais d'«evolutionary rescue»). Nous savons maintenant que la distinction longtemps faite entre

temps écologique et temps évolutif était en fait artificielle (Kinnison and Hairston Jr 2007) et que ces deux types de processus peuvent se dérouler concurrement. L'évolution rapide a été démontrée comme étant non seulement possible (Naeem et al. 1994; Bradshaw and Holzapfel 2001), mais en fait plus fréquente qu'on ne le croyait (Hendry and Kinnison 1999). Le sauvetage évolutif est un cas d'évolution rapide qui se produit suite à une perturbation qui fait décliner dramatiquement la population, celle-ci peut éviter l'extinction si elle s'adapte assez rapidement aux nouvelles conditions de son milieu pour éviter de voir son abondance descendre sous un seuil critique (Gomulkiewicz and Holt 1995). Depuis la définition théorique du concept de sauvetage évolutif apporté par Gomulkiewicz et Holt en 1995, plusieurs expériementation en laboratoire sur des cultures d'organismes à temps de génération court ont démontré qu'il était bel et bien possible (Bell 1991, 2013; Costas et al. 2007; Bell and Gonzalez 2009; Collins and de Meaux 2009; Bodbyl Roels and Kelly 2011)

L'abondance de la population affectée par la perturbation doit éviter de descendre sous un certain seuil critique en-deça duquel il n'y a plus de récupération possible (Gomulkiewicz and Holt 1995) pour qu'un sauvetage évolutif soit possible. De plus, une population doit aussi posséder une variation génétique assez importante pour fournir un bassin de mutation assez grand pour potentiellement contenir une mutation bénéfique face à la perturbation (Willi and Hoffmann 2009; Bell and Gonzalez 2009). La dispersion augmente également les chances de voir survenir un évènement de sauvetage évolutif en augmentant la diversité génétique de la population (Bell and Collins 2008; Weese et al. 2011; Bell and Gonzalez 2011). La dispersion et la taille de la population sont deux facteurs contribuant au sauvetage évolutif en augmentant le nombre de mutations sur lequel la sélection naturelle peut agir. La diversité génétique d'une population avant la perturbation est donc un élément clé déterminant sa capacité à s'adapter après la perturbation. Il a été démontré que les mutations permettant une évolution rapide de la population, sont généralement soit neutres voire même légèrement délétères et sont déjà présentes dans le génome avant la perturbation et ne surviennent pas suite à celle-ci (Barrett and Schluter 2008). En effet, une mutation apparue après la perturbation pourrait difficilement se répandre dans la population assez rapidement pour lui éviter l'extinction puisque la population est vraissemblablement déjà près de l'extinction lorsque la mutation survient et est ainsi trop petite pour se reproduire assez

rapidement pour transmettre largement la mutation à la génération suivante (Orr and Unckless 2008).

La nature de la perturbation elle-même peut aussi affecter l'issue d'un évènement de sauvetage évolutif. D'un côté, une perturbation plus forte devrait induire une plus forte pression de sélection, provoquant théoriquement une réponse adaptative plus forte (Bradshaw and Holzapfel 2001), mais d'un autre côté une perturbation plus forte signifie aussi un déclin de la population plus rapide et donc un plus grand risque d'extinction. Des études récentes montrent qu'une perturbation plus modeste est plus favorable à l'occurrence d'un évènement de sauvetage évolutif (Bell and Collins 2008; Bell and Gonzalez 2009). De la même façon, une perturbation plus lente donnerait plus de chance à une population de s'adapter et ainsi résister à la perturbation en réduisant les chances d'atteindre une abondance sous le seuil critique (Collins and de Meaux 2009; Lindsey et al. 2013). Une perturbation plus soudaine pourrait réduire le nombre de mutations potentiellement bénéfiques en réduisant la taille de la population (Lindsey et al. 2013). Par contre, une perturbation plus soudaine implique aussi une sélection plus forte et une augmentation de la valeur adaptative (fitness) plus importante (Collins and de Meaux 2009) rendue possible grâce à un plus petit nombre de mutation mais avec des effets plus importants qu'avec une perturbation plus lente, et ces mutations qui ont des effets plus grands son également fixés plus rapidement (Lenski and Travisano 1994). En créant des changements plus importants dans les organismes par des mutations qui ont des effets plus importants, une perturbation plus rapide et soudaine pourrait créer des conditions favorables au sauvetage évolutif (Boeye et al. 2013). Donc, le sauvetage évolutif serait plus commun dans des cas de perturbations plus lentes, mais serait quand même possible lorsque celle-ci est soudaine et rapide.

Finalement, une population d'organismes sexués aura plus de chance de réussir à s'adapter à des nouvelles conditions environnementales suite à une perturbation puisqu'elle possède une plus grande diversité génétique due à la recombinaison. Bell (2013) a démontré expérimentalement que lorsque confronté à une perturbation, les organismes sexués, sexués facultatifs et asexués ne sont pas égaux, les organismes asexués ayant moins de chance de réussir à s'adapter. De plus, les organismes asexués auraient une diversité génétique plus faible due à l'interférence clonale (Bell 2013), processus selon lequel le clone supérieur, le

meilleur compétiteur de la population, tendra à dominer et donc son génome sera sur-représenté. D'un autre côté, Lachapelle et Bell (2012) ont démontré que l'interférence clonale pouvait en fait aider les populations assexuées à faire face à une perturbation en leur permettant une adaptation rapide lorsque la diversité génétique pré-perturbation de cette population est faible. Le meilleur compétiteur des clones après la perturbation peut devenir le dominant plus rapidement grâce à l'interférence clonale. Donc, la reproduction sexuée augmente les chances que l'évolution vienne sauver une population seulement lorsque la diversité génétique de cette population est élevée avant la perturbation (Greig et al. 1998).

Une idée encore assez répandue veut que l'évolution n'est possible que chez des organismes sexués, étant donnée l'absence de recombinaison génétique chez les espèces asexuées. D'ailleurs, Maynard-Smith a postulé en 1978 que la parthénogénèse était un cul-de-sac évolutif (Maynard-Smith 1978), montrant à quel point on estimait la reproduction sexuée comme stérile évolutivement. Par contre, plusieurs autres études ont depuis montré que les organismes asexués (obligatoires ou cycliques) pouvaient répondre à la sélection et ainsi évoluer de manière à pouvoir s'adapter à de nouvelles conditions (Sunnucks et al. 1998; Weeks and Hoffmann 1998; Fagerström et al. 1998; Pfrender and Lynch 2000). Ce sont les mutations qui fournissent la matière première de l'évolution, soit la variation, chez les organismes parthénogénétiques (Wilson et al. 1999), et les populations strictement parthénogénétiques sont très diversifiées (Vorburger 2006). De plus, les différents génotypes d'une espèce n'ont pas nécessairement la même performance dans un milieu donné (Vellend 2006), ils répondent donc de manière différente à une perturbation et cette réponse différenciée entraîne une sélection des génotypes (ou clones) les mieux adaptés aux nouvelles conditions du milieu. On parle donc dans le cas de sélection sur des clones, de sélection clonale.

Dû à l'absence de recombinaison génétique, on croyait que l'évolution était plus lente chez les organismes mitotiques, mais il semble que les changements fonctionnels se produisent significativement plus rapidement qu'on le croyait chez les organismes asexués (Sunnucks et al. 1998). D'ailleurs, les taux de mutations des organismes sexués et asexués sont semblables; de l'ordre de 0,003mutations/divisions cellulaires/génome chez les eucaryotes unicellulaires et de 0,1 à 0,001mutations/génome/génération sexuée chez les

eucaryotes pluricellulaires, mais celui-ci ne serait pas différent du taux des unicellulaires si on considère le génome effectif (qui exclu la fraction du génome où les mutations sont neutres) (Drake et al. 1998).

ITS2

Afin de déterminer si le sauvetage évolutif se produit réellement en milieu naturel et s'il est possible de le détecter en utilisant les méthodes de biologie moléculaire, il faut pouvoir mesurer la diversité génétique de la population ciblée ainsi que d'identifier les différents génotypes. Les eucaryotes possèdent deux espaceurs intergéniques (Internal Transcribed Spacers ou ITS); deux régions non-codantes de l'ADN ribosomal localisés entre les gènes codant pour la petite et la grande sous-unité ribosomale. Le deuxième de ces deux espaceurs, situé entre les gènes du 5.8S (une partie de la grande sous-unité) et celui codant pour la grande sous-unité ribosomal, est déjà utilisé comme code-barres génétique potentiel pour les algues vertes (Hall et al. 2010) et d'autres taxa de plantes et de champignons (Müller et al. 2007), ainsi que comme complément au COI comme code-barre pour les animaux (Chen et al. 2010). Nous avons donc séquencé l'ITS2 sur des échantillons contenant plusieurs espèces mélangées pris sur le terrain pour déterminer la diversité génétique du phytoplancton.

Une base de données accessible par internet (its2.bioapps.biozentrum.uni-wuerzburg.de Dernier accès: 2 juillet 2013) répertorie les séquences d'ITS2 (ainsi que leur structure secondaire) pour plus de 200 000 taxa majoritairement de champignons et d'algues. La séquence de l'ITS2 n'est que peu conservée à des niveaux taxonomiques supérieurs à l'espèces ou au genre, c'est pourquoi pour des analyses à ces niveaux, la structure secondaire de l'ITS2 est utilisée plutôt que sa séquence. En effet, la région de l'ADN ribosomal où est situé l'ITS2 forme certaines boucles variables à des niveaux taxonomiques élevés (Schultz and Wolf 2009) permettant la reconstruction de phylogénies à ces niveaux. La longueur de l'ITS2 est conservée au niveau spécifique, permettant l'identification d'espèces alors que la séquence elle-même est plus variable et permet à la fois des analyses spécifiques et intraspécifiques.

Au niveau de l'espèce, l'ITS2 a surtout été utilisée pour construire des phylognies (Hoef-Emden 2005), identifier des espèces cryptiques (Goetze 2003) ou discriminer des

espèces difficilement identifiables chez plusieurs groupes d'organismes différents comme les acariens (Navajas et al. 1992), les digènes (Noaln and Cribb 2005) et les copépodes calanoïdes (Goetze and Bradford-grieve 2005), mais particulièrement chez les champignons et les algues (O'Donnell 1992; Iwen et al. 2002). Chez les algues, il a été utilisé comme code-barre pour les diatomées (Moniz and Kaczmarska 2010), pour inférer des phylogénies pour les cryptophytes (Hoef-Emden 2005) et les chlamydomonas psychrophilles (Pocock et al. 2004) par exemple. Certains outils ont été développés pour l'identification d'espèces, sans même avoir à obtenir la séquence de l'ITS (1 ou 2); Il s'agit de l'ARISA (Automated Ribosomal Internal Spacer Analysis) qui est tout simplement basé sur la longueur de l'ITS qui est assez variable pour permettre une identification au niveau de l'espèce mais conservée au sein de celle-ci (Fisher and Triplett 1999; Lear et al. 2008; Fechner et al. 2010). Également, l'ITS2 est utile pour la construction de nouvelles phylogénies dans les cas où celles basées sur la morphologie ne sont pas suffisamment précises dû à un haut degré de plasticité entre les espèces, comme ce fut le cas pour les chlorellaceae ou les desmides (Krienitz et al. 2004; Gontcharov and Melkonian 2005).

L'utilisation de l'ITS2 au niveau intraspécifique, pour l'identification de génotypes, de souches ou de cultivars, ne semble pas aussi répandue qu'au niveau spécifique. Par contre, un certain nombre d'études ont constaté de la variation dans l'ITS2 au niveau intraspécifique chez un nombre substantiel d'espèces d'algues telles que *Cladophora* (chlorophyte) (Bakker et al. 1992; Kooistra et al. 1992), *Chordaria flagelliformis* (phaeophyceae, chlorophyte) (Kim and Kawai 2002; Draisma et al. 2012), *Dinophysis* (dinoflagellées) (Edvardsen 2003) et *pseudo-nitzschia delicatissima* (diatomées) (Lundholm et al. 2006). Cette variation intraspécifique pourrait être dépendante du taxon considéré, mais elle a été constaté dans un nombre significatif de taxa et a même utilisée dans certaines études en biogéographie, particulièrement sur des espèces de champignons ou d'algues. Étant donnée que nous travaillons justement sur un groupe d'algues, nous avons choisi d'utiliser le séquençage de l'ITS2 comme marqueur de la variation génétique pour suivre les différents génotypes de certaines espèces de chlorophytes dans le but d'étudier leur récupération suite à une perturbation.

Jusqu'à maintenant, le sauvetage évolutif n'a pas été démontré sur des communautés de plusieurs espèces conservées dans leurs conditions naturelles, toutes les expériementations portant sur le sauvetage évolutif ayant été effectuées sur des monocultures en laboratoires. De plus, on n'avait jamais fait de comparaison des différents types de perturbation tels que définis par Bender, permettant d'identifier quel méchanisme stabilisateur, écologique ou évolutif, est le plus important pour la récupération des communautés selon le type de perturbation. Nous avons effectué cette comparaison sur des communautés naturelles de phytoplancton en utilisant une acidification en guise de perturbation; chaque type de perturbation devrait révéler le mécanisme dominant pour la récupération des communautés, les perturbations ponctuelles devraient enclencher une réponse écologique forte alors qu'une perturbation constante tendrait plutôt à favoriser une réponse évolutive.

CHAPITRE I

RAPID ECO-EVOLUTIONARY RESPONSES OF PHYTOPLANKTON LAKE COMMUNITIES TO A PERTURBATION

1.1 Introduction

In this current era beginning with the industrial revolution and the development of fossil fuels, often referred to as the «Anthropocene» (Crutzen 2002) human activity has become a major geophysical force reshaping the environment (Steffen et al. 2007). A significant proportion of the planet's ecosystems have already been altered by human activities and perturbations, with impacts on organisms making up these ecosystems. There are many ways in which anthropogenic perturbations can manifest, including in terms of their strength and length, but globally, they are considered to be a greater challenge to species than natural perturbations (Hendry et al. 2008). Toxic spills provide a good example of growing anthropogenic perturbation as they are occur suddenly, often with disastrous consequences. Aquatic ecosystems are particularly vulnerable to this type of perturbation because spilled products can usually more easily and rapidly disperse in water and are thus harder to constrain (Giller et al. 2004). The potential for ecological and evolutionary recovery of communities and ecosystems from such sudden perturbations becomes an important question to explore. Given the increasing evidence for rapid evolution (Thompson 1998; Hendry and Kinnison 1999; Kinnison and Hairston Jr 2007), the possibility for an evolutionary rescue response is important to assess in communities that are likely to be impacted by sudden environmental shifts. Using easily manipulable ecosystems such as lake plankton, with fast generation times, it is possible to explore both the ecological and evolutionary response to perturbation, such as a sudded increase in water acidity, *in situ* and with naturally complex communities.

1.1.1 Ecological rescue

Community stability in the face of perturbation can be promoted by compensatory dynamics among the constituent populations by buffering the density and functional fluctuations potentially arising with species' extinction (McNaughton 1977; Frost et al. 1995; Ives and Cardinale 2004). Compensatory dynamics through time occur when two species covary negatively. In the case of a species extinction following perturbation in a two competitor community the other will increase to replace it. In other words, the surviving species can occupy the newly available niche and replace the first species both in terms of density and functional role in the community. Thus, a negative interaction between two species, such as competition is not a sufficient condition for compensatory dynamics; species must also be asymmetrically affected by the perturbation (one negatively and the other positively or at least not as negatively) (Klug et al. 2000). Finally, the stabilizing effect of compensatory dynamics on ecosystem function occurs through the insurance effect, contingent on the presence of a functional redundancy between the two competitors (Petchey et al. 1999; Yachi and Loreau 1999; Descamps-Julien and Gonzalez 2005).

In the case of an acidification perturbation that occurs as a pulse, phytoplankton have an added benefit of a recovery that is favoured by a process of niche construction (Odling-Smee et al. 1996), in addition to compensatory dynamics. Through their direct interaction with the carbon cycle in lakes, phytoplankton growth itself can promote ecosystem recovery following a pH perturbation. Lakes have a property known as alkalinity which buffers pH variation based the quantity of dissolved carbonate (Wetzel and Likens 2000; Kalff 2002). Lake alkalinity varies dependent on the nature of the geology and underlying substrate. Buffering capacity increases mainly with increasing limestone ($CaCO_3$), and functions according to the following chemical equation:

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow 2H^+ + CO_3^-$$

Phytoplankton can play a role and act as niche constructors by influencing this equilibrium: photosynthesis depletes dissolved CO_2 in lake waters, promoting an uptake of free H^+ ions when they are available (or in excess).Thus, phtotsynthetic activity will neutralize acid waters where such a pulse pH perturbation occurs. Note however, that where a perturbation is

maintained such as through a press perturbation (sensu Bender et al. 1984), this buffering capacity by the phytoplankton community will be precluded by continual acidification. Under such conditions, an evolutionary response could permit the continued persistence of the community.

1.1.2 Evolutionary rescue

Following a perturbation, species within communities may be rescued by evolution if the individuals composing their populations can evolve fast enough to avoid extinction by adaption to the new environmental conditions. We now know that the distinction between ecological and evolutionary timescales is largely artificial (Kinnison and Hairston Jr 2007) and that these two processes can often occur simultaneously. Rapid evolution has been shown not only to be possible (Thompson 1998; Bradshaw and Holzapfel 2001; Schoener 2011), but also to be more frequent than previously thought (Hendry and Kinnison 1999). Evolutionary rescue is a case of rapid evolution that takes place following a perturbation that provokes a serious decline in population abundance, the population might recover if it has been able to adapt fast enough through natural selection to avoid to see its abundance get below a critical threshold (Gomulkiewicz and Holt 1995). Since this early theoretical definition of the process, several lab experiments have demonstrated the possibility of evolutionary rescue in cultures of simple organisms with short generation times, primarly with species-poor communities of yeasts and protists (Bell 1991, 2013; Costas et al. 2007; Bell and Gonzalez 2009; Collins and de Meaux 2009; Bodbyl Roels and Kelly 2011).

For evolutionary rescue to occur, the perturbed populations must remain above the threshold under which extinction is inevitable (Gomulkiewicz and Holt 1995), but also to sustain a large genetic variation upon which selection could act (Willi and Hoffmann 2009; Bell and Gonzalez 2009). Dispersal can further facilitate a population's recovery through evolutionary rescue (Bell and Collins 2008; Weese et al. 2011; Bell and Gonzalez 2011). Both dispersal and population size contribute to evolutionary rescue by providing populations with a bigger mutation pool upon which natural selection can act. Genetic variation within a species prior to perturbation is thus key to its capacity to adapt after a perturbation. It has

been shown that the mutations, whether beneficial or deleterious, responsible for a genotype's selection under natural selection in evolutionary rescue are from the standing genetic variation in the species and not *de novo* mutations acquired after the perturbation (Barrett and Schluter 2008). Indeed, it would be difficult for a mutation appearing post-perturbation to spread widely enough to allow populations to avoid extinction, as perturbed populations are likely to be to small to reproduce fast enough to spread the new mutation (Orr and Unckless 2008).

The nature of the perturbation itself also appears to affect the outcome of an evolutionary rescue event. On the one hand, a stronger perturbation should induce more selection pressure, hence a theoretically greater adaptative response to the perturbation (Bradshaw and Holzapfel 2001); on the other hand, a stronger perturbation also means a faster decline in population abundance and a greater risk of extinction. Recent evidence suggests that it is instead, a more modest perturbation that is most favourable to the occurrence of evolutionary rescue (Bell and Collins 2008; Bell and Gonzalez 2009). In a similar way, a slower perturbation rate would make it easier for a population to adapt and recover as extinction risk is reduced (Collins and de Meaux 2009; Lindsey et al. 2013). A faster perturbation could mean a loss of potentially beneficial mutations as the population size is reduced quickly (Lindsey et al. 2013). Then again, a sudden perturbation implies a stronger selection and a larger increase in overall fitness (Collins and de Meaux 2009). This larger fitness increase is achieved through a smaller number of mutations but these mutations have larger population-level effects than with a slower perturbation, and such larger effect mutations can be rapidly fixed (Lenski and Travisano 1994). By creating bigger changes in the organisms via larger effect mutations, a faster perturbation could create favourable conditions for evolutionary rescue (Boeye et al. 2013). Thus, evolutionary rescue might be more common in cases of slower rate perturbation although they are theoritically still possible in cases of more sudden perturbations.

A final consideration regarding the potential for evolutionary rescue is that sexual populations are more likely to adapt to new environmental conditions following a perturbation as they have a greater genetic diversity potential through recombination. Bell (2013) demonstrated experimentally that amongst obligately sexual, facultatively sexual and

asexual organisms, the latter were the least likely, to survive stress through perturbation. Furthermore asexual organisms should be less genetically diverse because of clonal interference (Bell 2013), occurring because a superior clone will tend to dominate populations through clonal competition. On the other hand, Lachapelle and Bell (Lachapelle and Bell 2012) demonstrated that clonal interference might actually aid asexual populations facing a perturbation when starting genetic diversity is already low. The clone that emerges as the new superior clone after the perturbation can become dominant faster because of clonal interference. Thus, sex helps recovery through evolution only when genetic diversity is high to begin with (Greig et al. 1998).

To date, there have been no demonstrations of evolutionary rescue occurring in multi-species communities maintained under natural conditions in the field. Neither has there been a comparison of different types of perturbation to which an ecological (compensatory dynamics) and an evolutionary (rescue) stabilizing mechanism might be revealed as more critical to recovery. Within this framework, a comparison of pulse and press perturbations (*sensu* Bender et al. 1984) can be done as well. This is because, with respect to lake phytoplankton exposed to acidification in particular, each perturbation type should tend to reveal different dominant stabilizing mechanisms: pulse perturbations should incur a strong ecological response while an evolutionary one would be more likely with a press perturbation.

1.1.3 Genotyping natural populations

To assess whether evolutionary rescue is occurring in natural ecosystems, it is necessary to find a way to determine genetic diversity and to identify genotypes within a mixed community. The eukaryotes second Internally Transcribed Spacer (ITS2) is a potential barcode for green algae (Hall et al. 2010) and other taxa of plants and fungi (Müller et al. 2007), as well as a complement to COI for a barcode for animals (Chen et al. 2010). At the species level, ITS2 has been used to build phylogenies (Hoef-Emden 2005), identify cryptic species (Goetze 2003) or discriminate species on numerous groups of organisms such as mites (Navajas et al. 1992), digenean (Noaln and Cribb 2005) and calanoid copepods (Goetze and Bradford-grieve 2005), but especially for fungi (O'Donnell 1992; Iwen et al. 2002). It has been used as a barcode for diatoms (Moniz and Kaczmarska 2010), to infer

phylogenies for cryptophytes (Hoef-Emden 2005), and psychrophyllic chlamydomonads (Pocock et al. 2004) for example. Intraspecific variation in the ITS2 region has been noted in a substantial number of algae groups such as species of *Cladophora* (chlorophyte) (Bakker et al. 1992; Kooistra et al. 1992), *Chordaria flagelliformis* (phaeophyceae, chlorophyte) (Kim and Kawai 2002; Draisma et al. 2012), *Dinophysis* (dinoflagellates) (Edvardsen 2003) and *pseudo-nitzschia delicatissima* (diatom) (Lundholm et al. 2006). It appears that intraspecific variation in ITS2 sequences might depend on the studied taxon but it has been found in many different taxa and it has been used in some biogeography studies, especially with fungi or algae. Thus sequences of the ITS2 region of DNA were the most appropriate to use on mixed species samples from our field study, to assess phytoplankton genetic diversity in an evolutionary rescue context.

We examined experimentally the ecological (between species) and evolutionary (whithin species) responses of phytoplankton communities to different environmental perturbation types. In a mesocosm experiment we acidified waters to pH 5.0 using either a pulse or a press perturbation. The purpose of this experiment was to study recovery strategies utilized by phytoplankton communities under each scenario, determining whether, under the same set of underlying environmental conditions, the same community will respond with evolutionary rescue when a press perturbation is applied and a predominantly ecological one with a pulse perturbation. Our study is thus the first *in situ* examination of evolutionary rescue in a naturally occurring and diverse community.

1.2 Methods

1.2.1 Experimental design

We performed a mesocosm experiment in and using the plankton community of Lake Hertel, which is located on top of Mont St-Hilaire (45° 32`N, 73° 09`W), surrounded by temperate deciduous forest. Mont St-Hilaire is one of the nine monteregian hills, near Montreal, QC, Canada, and is a biosphere reserve, recognized by UNESCO since 1978. Lake Hertel has a mean depth of 6m and a mean surface area of 0.3km^2 (Kalff 1972). It is classified as mesotrophic to eutrophic based on total phosphorus concentrations of around 20µg/l and

the pH of its waters is normally between 7.5 and 8.5 units. The lake is naturally eutrophic as it has always been surrounded by mature forest with no sources of anthropogenic nutrient enrichment.

The mesocosms consisted of 1m diameter x 4.5m deep clear plastic bags that were suspended in the lake from a large floating dock and open only at the lake surface. The bags were filled on May 25th 2012 by pumping water directly from the lake through a 53μm mesh to remove zooplankton but allowing phytoplankton to pass. We subsequently collected zooplankton from the lake with a series of vertical net hauls (53μm mesh) and mixed the caught animals in a large container. The contents of this container were then equally and repeatedly introduced in small aliquots into each of the mesocosms, as described in Beisner and Peres-Neto (2009) to ensure more equally distributed zooplankton communities than is possible by pumping directly. Finally, we added 10μg/L of phosphorus (as $NaPO_4$) and 85mg/L of nitrogen (as KNO_3) (half the normal concentration of Lake Hertel) to ensure that nutrient levels were sufficient to allow the plankton community to persist through an extended experimental period.

Experimental perturbation was accomplished through acidification using 37% HCl added slowly to the treated bags until the pH in each was reduced to 5.0. Acidification was applied on June 5, 2012; two weeks after setup. Treatments were (i) a Press perturbation in which mesocosms were acidified by maintaining pH at 5.0 for the remainder of the experiment, (ii) a Pulse perturbation in which mesocosms were acidified only once with the pH allowed to re-establish itself subsequently through photosynthesis, and (iii) a Control set in which no manipulation was performed. All treatments were replicated with three mesocosms; the exception being for the molecular analyses where only two Control replicates could be utilized because one set of replicate samples was inadvertently lost.

1.2.2 Sampling

The experiment was terminated on July 17th 2012 after a period of seven weeks. For most parameters (exceptions for genetic and zooplankton samples; see text below) we sampled once per week in the two weeks prior to the acidification, every two days during the two weeks immediately following the perturbation, and then twice weekly (every 3 and 4

days) for the remaining five weeks. Phytoplankton communities were sampled using an integrated 3m long PVC tube sampler. Whole water aliquots (125ml) were preserved with Lugol's solution in amber glass bottles to prevent light degradation. Zooplankton communities were sampled weekly via vertical net hauls from 3m to the surface using a 30µm mesh net (20cm diameter, 59cm long). Animals were anesthetized prior to preservation in 70% ethanol. Oxygen concentration, pH and temperature were measured using a YSI multisonde (6600). For the Press treatment, if pH exceeded 5.5 we added HCl until pH returned to around 5.0.

In the lab, chlorophyll *a* (Chl*a*) levels were measured using ethanol extraction (Nusch 1980) and spectrophotometry with HCl addition (Wintermans and DeMots 1965). Phytoplankton samples were identified microscopically at 480X magnification to the species level whenever possible; or alternately to the genus level. The Utermöhl method (Lund et al. 1958) was used with an inverted microscope (480Xmagnification; Diavert, Leica) after sedimentation of Lugol's preserved samples in 10ml (6 hour minimum sedimentation) or 25ml (18 hour minimum sedimentation) sedimentation columns. At least 400 cells in total and a minimum of 200 cells of the most abundant species were counted per sample and ten individuals of each species were measured for biovolume calculations based on geometric equations (Hillebrand and Dürselen 1999).

1.2.3 Genotyping

Samples were collected from each mesocosm using a dark 1L narrow-mouth Nalgene bottle at 0.5m depth on May 25, June 5, June 26 and July 10. A 400ml (150ml for the May 25 samples) subsample of this 1L bottle was then filtered onto a 0.22µm nitrocellulose membrane (0.22 µm GSWP from Millipore) to retain all phytoplankton and bacteria. The filter was folded closed and kept in foil and in a -20°C freezer until DNA extraction (<7 months).

To extract DNA, each thawed filter was unfolded into a 50ml Falcon tube to which 2ml of lysis buffer was added, followed by 100µl of lysozyme (125mg/ml) and 20µl of RNase A (10µg/ml). The tubes were then sealed with parafilm and placed in an incubator at 37°C for 1 hour. 100µl of Proteinase K (10mg/ml) and 100µl of SDS (20%) were then added

before another incubation at 55°C for 2 hours. We then removed protein using protein precipitation reagent (MPC) and centrifugation at 10 000G at 4°C for 10 minutes. DNA was precipitated in isopropanol, washed with 70% ethanol, and then re-suspended in 10mM Tris buffer.

We designed primers targeting the ITS2 of *Scenedesmus* spp. as this taxon was identified microscopically as one of the most responsive chlorophyte genera, driving the ecological recovery of the communities following acidification. To design our primers, we aligned all sequences of *Scenedesmus* ITS2 retrieved from the ITS2 Database (Schultz et al. 2006) using the Geneious software (Drummond et al. 2012). The forward (5'-catgtctgcctcagcgtcg-3') and the reverse (5'-ggtagccttgcctgagctca-3') primers were located in the conserved 5.8S and 28S rDNA regions, respectively. A 10-11bp unique barcode for each sample was added to the forward primers, and an Ion Torrent sequencing adaptor was added to both the forward and reverse primers.

We performed PCR in 25µl reaction mixtures containing (for each reaction): 16µl of MilliQ water, 5 µl of 5X PCR buffer (Phire), 1.25µl of each primer, 0.5µl of dNTPs, 0.5µl of polymerase (Phire) added just prior to the addition of 0.5µl of DNA and tagged forward primers. PCR was conducted on a BioRad C1000 Touch Thermal Cycler with the following cycle: three minutes at 98°C, five secondes at 98°C, five seconds at 50°C, ten seconds at 72°C and finally, one minute at 72°C. Amplification products were subsequently purified by gel extraction (using Qiagen gel extraction kit) of bands not exceeding 400bp. Finally, we pooled together 1ng of DNA across all samples that were to be sequenced together on the same chip and sequenced them using the 200bp sequencing kit and the 316 chip on an Ion Torrent Personal Genome Machine. Samples were sequenced in two runs on two different chips for each runs.

Raw sequences were processed using Mothur (Schloss et al. 2009): we trimmed and chopped sequences at a length of 148bp, thereby keeping only sequences with this precise length. We then clustered these sequences using CD-HIT-EST algorithm on the CD-HIT server (Li and Godzik 2006) and a representative sequence of each of the 298 identifed clusters was searched against the NCBI nucleotide database using nucleotide BLAST

(Altschul et al. 1997) in order to identify and group clusters by genus. Sequences from each identified genus were then seperatly aligned with MUSCLE v3.8.31 (Edgar 2004). Note that when there were more than 1500 sequences to align for a genus (it was the case for *Chlamydomonas*, *Coelastrum*, *Desmodesmus*, *Oedogonium* and *Yamagishiella*), we limited MUSCLE to 1 iteration. We then reclustered the sequences within each genus and assigned them to their original sample. Finally, we grouped similar sequences with a 90% identity into Operational Taxonomic Units (OTUs) using Mothur.

We used these OTUs (removing singletons and doubletons as well as all OTUs associated with less then five sequences) to build trees along with reference sequences extracted from the ITS2 Database and aligned with Geneious. Maximum-likelihood trees were built in PhyML (Guindon et al. 2010) using Generalised Time Reversible (GTR) substitution model with Among Site Rate Variation (using a gamma distribution), and we obtained bootstrap values from the same program.

1.2.4 Statistical analyses

We used Principal Response Curve (PRC) (Van den Brink and Ter Braak 1999) analysis to assess the treatment effect on phytoplankton communities through time using the *vegan* package in R (Oksanen et al. 2012). PRCs are based on Redundancy Analysis (RDA) but modified to produce a more intuitive visual showing the time-dependent effect of applied treatments (Van den Brink and Ter Braak 1999) in the form of a principal response curve. As in RDA, the PRC produces more then one axis of variation, but unlike the RDA it displays them individually. We focused on the first axis, which explains the most variation in the data, testing the significance of this axis using a permutation test with 1000 permutations. PRCs express the treatment effect as variation from the Control, i.e. the Control treatment is represented as a flat line and the further the line representing a treatment is from that flat line, the more important the treatment effect.

PRCs also give information at the taxon level by providing a score for each group, indicating the strength and the directionality (positive or negative) of the score in response to the treatment. When both the principal response curve and the taxon score are negative, their multiplication gives a positive specific response curve, indicating overall, a positive response

of that taxon to the treatment. Overall, the PRC encapsulates the effect of each treatment through time on both the overall community, as well as the individual taxa.

To assess community stability, we measured resilience of each mesocosm community following acidification and compared the resilience parameters between treatments using one-way ANOVA. We defined resilience as the time taken by the community to return to its pre-perturbed state. We thus used the length of time defined by the date of the acidification as time t=0 and the date when phytoplankton biomass (Chl *a*) first returned to or surpassed 100% of the t=0 biomass. In some mesocosms, 100% biomass was never re-achieved, in which case we used the last date of the experiment as the recovery date. This result is a conservative indicator for resilience in this case, as it occurred prior to the recovery of 100% biomass. Resilience was defined as the number of days between these two time points, with a smaller value indicating greater resilience.

To measure the degree of compensatory dynamics, we used a metric developed by Loreau and De Mazancourt (2008) that quantifies community synchrony. This metric takes into account covariances between species and the periodicity of these covariances over time, giving a result between 0 and 1. It is based on stochastic population dynamics theory and is based on a neutral model that assumes equivalence between individuals in a stochastic community (Gonzalez and Loreau 2009). However, as stated by Loreau and De Mazancourt « ... the covariances or synchrony of species per capita population growth rates predicted by the neutral model should serve as the proper null hypothesis to test for nonneutral, deterministic asynchrony driven by niche differences between species» (Loreau and de Mazancourt 2008). The formula used is as follows:

$$\varphi\rho = \frac{var(C)}{(\sum_{i=1}^{n}\sqrt{var(P_i)})^2}$$

where C is the variance of community size and P_i is the variance of individual populations (species) (Loreau and de Mazancourt 2008). A value of 0 indicates complete community asynchrony and 1 indicates complete synchrony. The more a community is asynchronous, the more species covary negatively over time and the more compensatory dynamics there are.

This metric was calculated using all sampling dates in the experiment and using biovolumes for all taxa identified either to genus or species.

To assess zooplankton community response to perturbation and consequently to infer top-down effects on phytoplankton, we performed four two-way repeated measures ANOVAs (using the *Anova* function of the *Car* package in R) for the three dates on which we enumerated zooplankton abundances: June 5th (just before acidification), June 26th and July 10th. We tested for effects within (i) the whole community with all zooplankton taxa included, (ii) only copepods (total, adults only and nauplii only), (iii) and all pelagic cladocerans (i.e. no chydorids), and (iv) chydorids only. Chydorids were analysed separately from other cladocera as they have a particular feeding strategy adapted to littoral environment, crawling on and scraping food from surfaces (Dodson and Frey 2001), including the surface of mesocosm bags. Where an interaction between the two factors was significant, we performed a simple ANOVA and a Tukey-HSD test seperately on all levels of the significant factor in a one-way repated measures ANOVA to determine which group at which time point or in which treatment was different from the others. Note that, due to small sample sizes, data were not distributed normally and it was not possible to attain normality through transformations.

All statistical analyses were performed using the open-source software R (R Development Core Team 2008).

1.3 Results

1.3.1 Ecological response

1.3.1.1 Total biomass

Time series of the total biovolume of each mesocosms showed an important peak on the first or second sampling dates (Fig. 1.1), likely owing to the nutrients added when setting up the mesocosms.To avoid this transient, we considered the date just prior to the HCl addition (Julian day 156) as the «initial» baseline condition of the communities. While community biovolumes were very stable in the three Control mesocosms (Fig. 1.1 a,b,c)

recovery following acidification was noticeable in the Pulse (Fig, 1.1, d,e,f) and to some degree, in the Press mesocosms (Fig. 1.1, g,h,i).

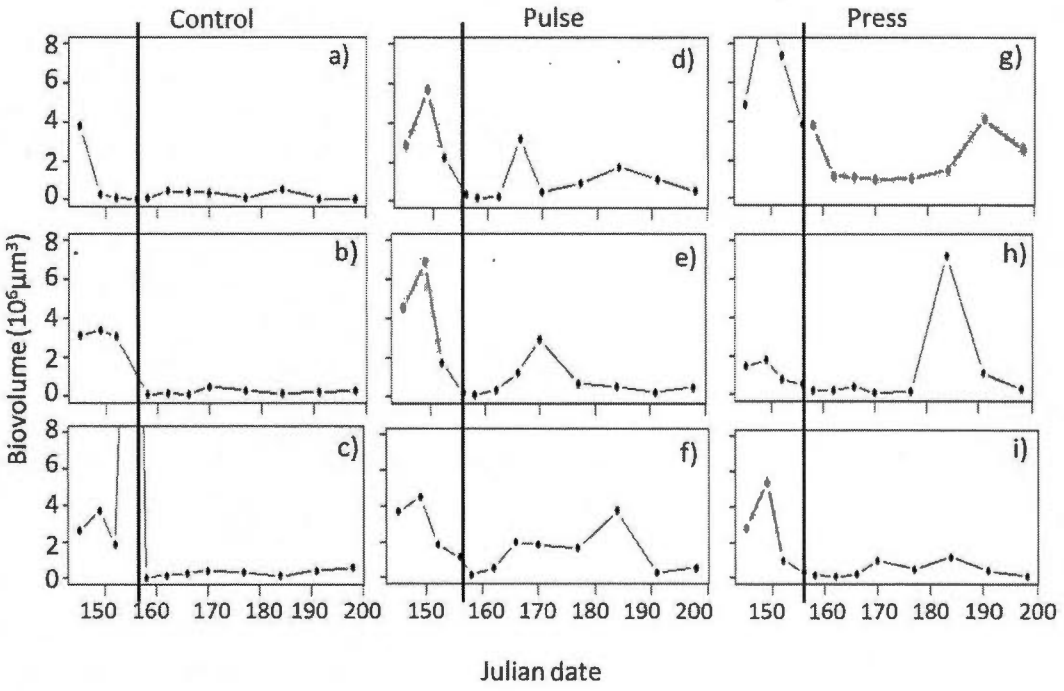

Figure 1.1 Phytoplankton community total biovolume time series in each mesocosm. Panels a, b and c are Control replicates; d, e and f are Pulse perturbation replicates; and g, h and i are Press perturbation replicates. The date of acidification is indicated by vertical lines. The y-axes maxima were limited for clarity of the entire time series; for those peaks that are not visible, maximum biovolumes are $3.21X10^6$ µm^3 in panel c and $10.5X10^6$µm^3 in panel g.

Community stability

Resilience calculated with Chl *a* as an estimate for phytoplankton biomass showed a significant difference between treatments ($p = 0.00255$; Fig. 1.2). Specifically, this difference was between the two perturbed treatments and the Control (Tukey-HSD results: Press-Control: $p = 0.00215$. Pulse-Control: $p = 0.0207$). Time between t=0 and recovery date was greater in the Press and Pulse treatment, indicating less resilience with perturbation.

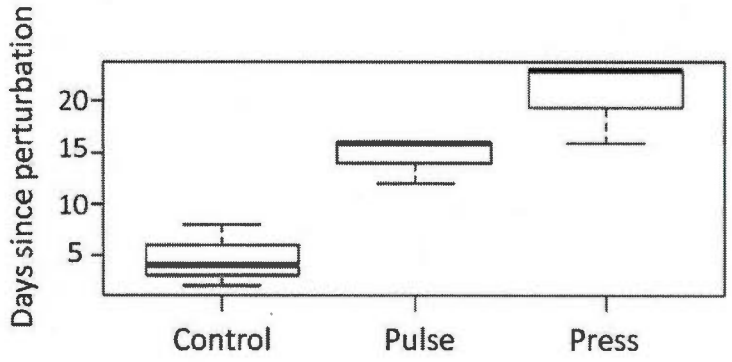

Figure 1.2 Boxplot showing resilience with total Chl *a* for each of the experimental treatments.

1.3.1.2 Community composition

Relative biomass of classes

The peaks in total phytoplankton biovolume observed at the beginning of the experiment (Fig 1.1) were primarly a result of diatom increases. Meanwhile, while recovery in the Press and Pulse mesocosms was mainly a result of chlorophytes, at the expense of cryptophytes (Fig. 1.3). Cyanobacteria, chrysophytes and dinoflagellates contributed only marginally to all communities (Fig. 1.3).

Compensatory dynamics

The community synchrony test showed the greatest means (0.63) for the Control treatment, intermediate for the Press treatment (0.42) and the smallest for the Pulse treatment (0.23) (Fig. 1.4). All values differed significantly from each other (ANOVA $p=0.00147$, Tukey HSD).

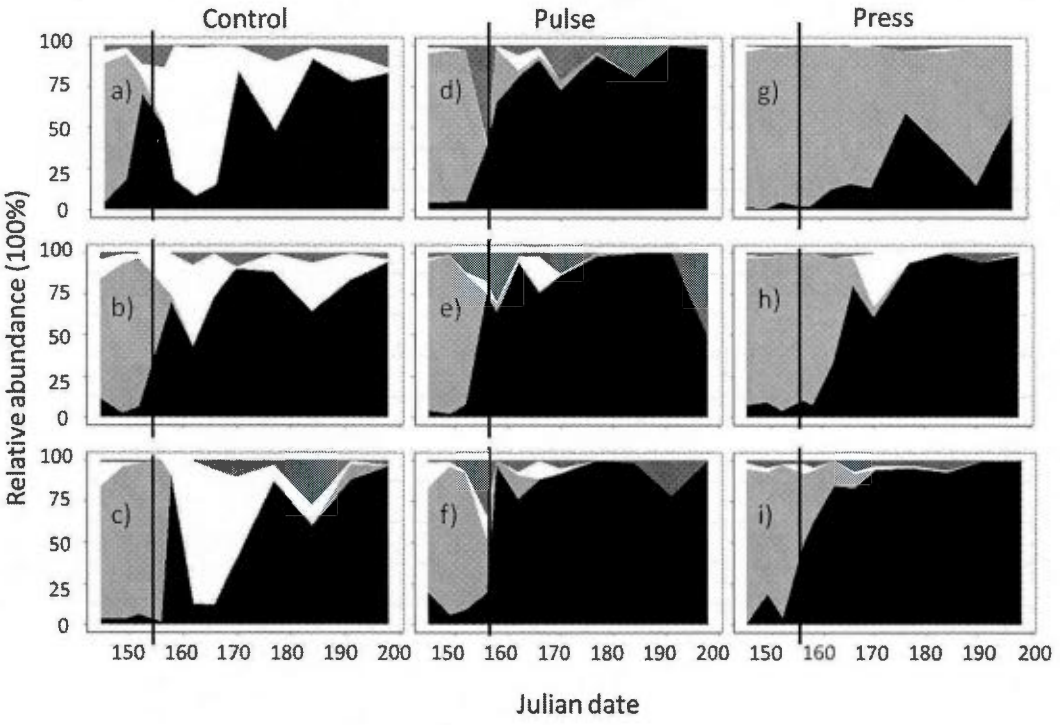

Figure 1.3 Proportion of each phytoplankton class as a function of total biovolume through time in each mesocosms. Panels a, b and c are Control replicates; d, e and f are Pulse replicates; and g, h and i are Press replicates. The date of acidification is indicated by vertical lines. Classes are indicated by the following colours: chlorophytes (black), diatoms (pale grey), cryptophytes (white), others (chrysophytes + cyanobacteria + dinoflagellates; dark grey).

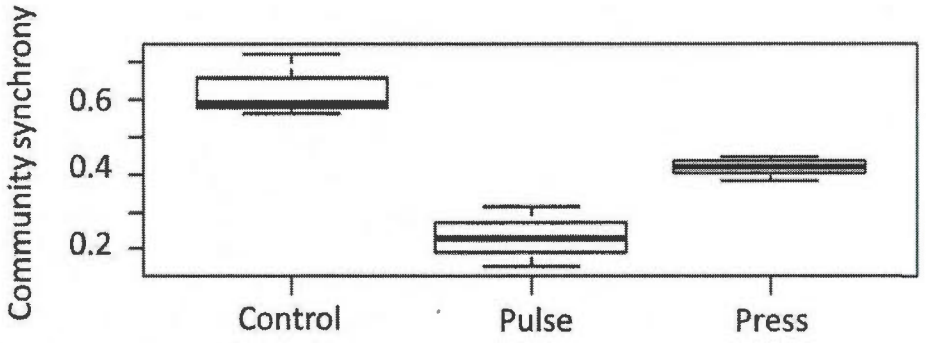

Figure 1.4 Boxplot showing the community synchrony index, measuring compensatory dynamics, for each treatment based on genus and species level biovolumes

Species composition changes

In terms of community taxonomic composition, *Synedra* sp. and *Cyclotella glomerata* were the dominant diatom species and both persisted the longest in all treatments. Chlorophyte replacement following perturbation resulted mainly from growth of *Scenedesmus* spp., and while they were present before acidification, they became dominant and more diverse following acidification. Of the ten *Scenedesmus* species, *Scenedesmus ecornis*, followed by *Scenedesmus incrassatulus*, were dominant. Other chlorophyte species that had been rare or undectable prior to perturbation became more important following it, including the two filamentous taxa of *Mougeotia* sp. and *Ulothrix* sp.

It is important to note that the dominant chlorophyte, *Scenedesmus* spp. was identified using microscopic identification and taxonomic keys based on morphology (Prescott 1964, 1982; Whitford and Schumacher 1984). Subsequent molecular analyses classified them as *Desmodesmus* spp. There were two exceptions in which a *Scenedesmus* species was also detected by molecular methods, although they could not be identified to species because their ITS2 region had not previously been sequenced. *Desmodesmus* was considered to be a subgenus of *Scenedesmus* until the late 1990's when studies sequencing ITS2 determined that they should be classified as a separate genus (Kessler et al. 1997; An et al. 1999). These

two genera are very morphologically similar and also highly plastic (Lürling 2003; Johnson et al. 2007), further complicating their identification through microscopy.

The Principal Response Curve analysis (PRC) shows the composition of the treated communities relative to the Controls. When all species were included (Fig. 1.5) the first axis of the PRC was significant ($p=0.005$). That is, while there was little variation between the treatments before perturbation, about two days following acidification a clear differentiation of the two perturbed treatments from the Control clearly occured, while a differentiation between the two perturbed treatments began later, after about one week. Also, the Press treatment diverged more from the Control than did the Pulse treatment following acidification.

Only species with a species score greater than +/- 0.5 in a PRC are considered to be responsive to the treatments (Van den Brink and Ter Braak 1999). Some species responded negatively to our treatments, including all cryptophytes, indicating that their abundance diminished in the treated mesocosms relative to the Controls. Most species that reacted positively showed a mild reaction (score <1), and most were chlorophytes. Also, some diatom species appeared to respond positively, despite the fact that diatoms as a group largely disappeared following acidification (Fig. 1.3). For example, the diatom *Tabellaria floculosa* showed the strongest positive response in the PRC, likely because it remained in some of the Press perturbation replicates. This species is known to tolerate a wide range of environments, including acidic waters (Patrick and Reimer 1966), explaining this reaction to our treatments. The diatoms *Cyclotella glomerata* and *Synedra* sp. also appeared to react positively to the perturbation in the PRC, despite the fact that both disappeared completely in the time series after acidification (data not shown). We suspect that this is a statistical artefact arising because these species also disappeared completely from the Control treatment, making their dynamics similar across all treatments. As a result, we conducted a second PRC without diatoms included (see Fig. 1.6).

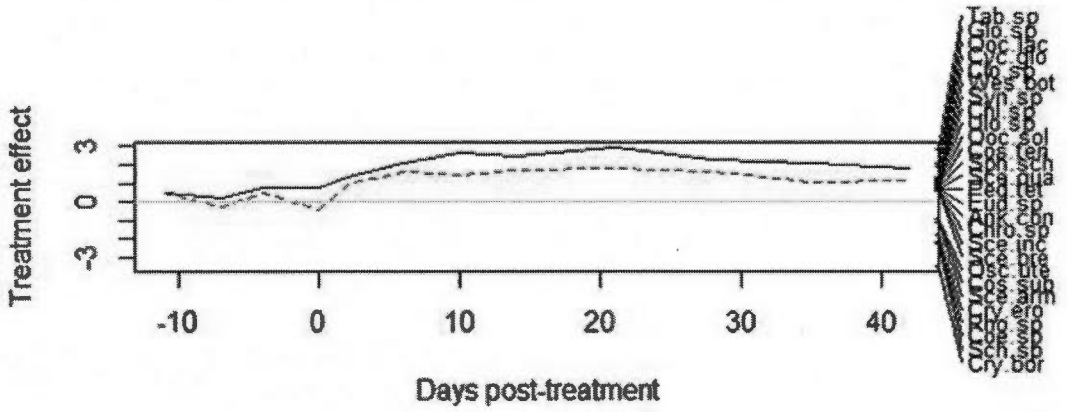

Figure 1.5 Axis 1 of the PRC analysis (1) including diatoms. The grey horizontal line indicates the Control treatment; the dashed line indicates the Pulse treatment and the black line the Press treatment. The response strength of those species with treatment effects beyond the 0.5 cutoff are shown along the right side of the graphic according to the species abbreviations in Table 1.1.

Table 1.1 Abbreviated and full names of phytoplankton species identified microscopically across all of the experimental communities and their scores associated with the PRC including (Score 1) and without (Score 2) diatoms.

Class	Abbreviation	Full name	Score 1 (Fig. 1.5)	Score2 (Fig. 1.6)
Chlorophytes	Ank.con	*Ankistrodesmus convulatus*	0.6117	-0.0428
	Chl.sp	*Chlamydomonas sp.*	1.1760	-1.9820
	Clo.sp	*Closterium sp.*	1.3470	-1.5160
	Coe.sp	*Coelastrum sp.*	-2.1880	3.5030
	Cos.sub	*Cosmarium subalatum*	-0.9695	0.9166
	Cos.ten	*Cosmarium tenue*	0.8575	-0.1801
	Eud.sp	*Eudorina sp.*	0.6692	0.0736
	Glo.sp	*Gloeocystis sp.*	2.0940	-3.1230
	Ooc.lac	*Oocystis lacustris*	1.8580	-1.3840
	Ooc.sol	*Oocystis solitaria*	0.9555	0.0363
	Ped.tet	*Pediastrum tetras*	0.6698	-0.4765
	Sce. arm	*Scenedesmus armatus*	-1.1580	1.9640
	Sce.inc	*Scenedesmus incrassatulus*	0.5933	-0.4970
	Sce.qua	*Scenedesmus quadricauda*	0.7176	0.0336
	Sch.sp	*Schroderia sp.*	-2.2260	1.4640
	Sph.sch	*Sphaerocystis schroeteri*	0.7504	-0.5967
	Ulo.sp	*Ulothrix sp.*	1.0100	-1.5600
	Wes.bot	*Westella botryoides*	1.2690	-1.2790
Diatoms	Cyc.glo	*Cyclotella glomerata*	1.8570	NA
	Syn.sp	*Synedra sp.*	1.2220	NA
	Tab.sp	*Tabellaria floculosa*	2.8670	-3.2850
Cryptophytes	Cry.bor	*Cryptomonas borealis*	-3.4480	2.2650
	Cry.ero	*Cryptomonas erosa*	-1.7920	0.9677
	Rho.sp	*Rhodomonas sp.*	-2.1570	1.5250
Chrysophytes	Chr.sp	*Chromulina sp.*	0.6015	-1.1390
Cyanobacteria	Osc.ute	*Oscillatoria utermoehlii*	-0.8993	0.5863

The second PRC (Fig. 1.6) did not include species that disappeared post-perturbation in both the Control and the treated mesocosms; namely all diatoms except for *Tabellaria* sp., the chlorophytes *Asterococcus* sp., *Dictyosphaerium pulchellum*, *Staurastrum* sp., *Oocystis elliptica*, *Actinastrum* sp. and the cyanobacteria *Aphanothece* sp. They were removed from the analysis because their disappearance from the Control mesocosms indicate more of an effect of being moved from the lake to a mesocosm effect than a treatment effect because the same pattern was observed in all the mesocosms (treated and Control). This second PRC supported the overall interpretations as that with all species included: similar patterns for both treated and Control mesocosms prior to perturbation, a differentiation of both treatments from the Control right after perturbation and a differentiation of the Press from the Pulse treatment one week later. In this analysis however, the Pulse treatment line diverged less from the Control line than it had in the first PRC and to a lesser degree than did the Press treatment line. The same species that reacted strongly to the treatments in the first PRC maintained a strong reaction signal in this analysis as well. The exception was *Mougeotia* sp. which now appears to react positively to the acidification (previously non-significant reaction; Fig. 1.5).

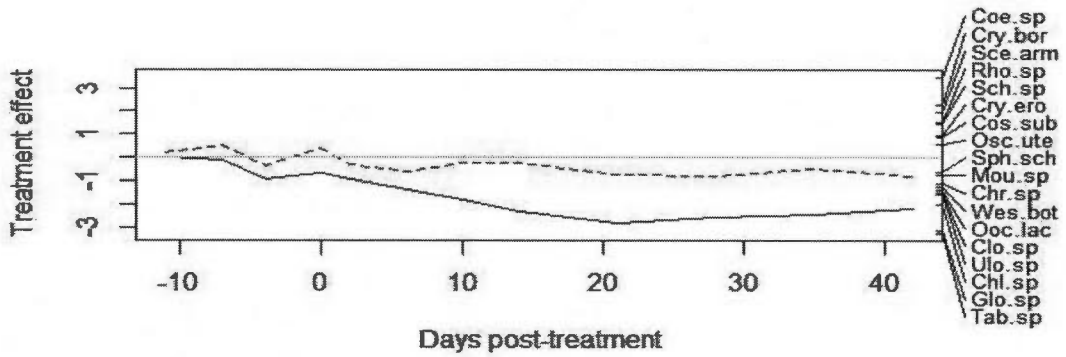

Figure 1.6 Axis 1 of the PRC analysis (2) excluding diatoms.The grey horizontal line indicates the Control treatment; the dashed line indicates the Pulse treatment and the black line the Press treatment. The response strength of those species with treatment effects beyond the 0.5 cutoff are shown along the right side of the graphic according to the species abbreviations in Table 1.1. Because the response curves of our two treatments are negative in this graphic, all the species displayed on the negative side of the y axis have a positive response to the treatment and vice versa.

1.3.1.2 Zooplankton community response

The two-way repeated measures ANOVA showed several significant interactions between treatment and time for all four taxonomic groups. For total community abundances (interaction, p=0.006; two-way ANOVA), perturbation treatment type was the siginificant factor in the one-way repeated measures ANOVA with a significantly lower abundance of zooplankton in the Press mesocosms on June 26th, three weeks after perturbation (p=0.007; one way ANOVA), but nor for the earlier or later dates. There was also an interaction between time and treatments for the copepods (p=0.0007; two-way ANOVA). One-way ANOVAs showed that copepod abundances were significantly lower in the Press treatments than in either the Pulse or the Control on both the post-acidification dates. Additionally, there was a significant abundance peak in copepod abundance in the Pulse mesocosms on June26th (p=0.005; one-way ANOVA). We then performed other ANOVAs separating the two stages of copepods: adults and nauplii. These analyses showed that this peak was a results of an increase in nauplii (p=0.006; one way ANOVA). For adults, there was no significant difference between either treatment or time points.

For pelagic cladocerans (interaction p=0.025; two-way ANOVA), abundances were significantly less on both post-perturbation dates in acidified treatments. Finally, interaction between factors in the chydorids analysis was marginally significant (p=0.0497; two-way ANOVA). The simple ANOVAs showed that there were significantly more chydorids in the Press mesocosms on the first post-acidification date, June 26th (p=0.025; one-way ANOVA).

1.3.2 Genetic response

Our PCR primers were designed to amplify algae from the genus *Scenedesmus*, but as mentioned, they are morphologically cryptic with *Desmodesmus;* with the exception of two of the ten microscopically-identified *Scenedesmus* species confirmed through molecular analyses in our samples (as opposed to the ten species identified visually). These *Desmodesmus* species identified through molecular methods cannot be similarly identified visually with microscopic analyses as they are cryptic. We identified more species than expected (Table 1.2) for a total of 13 genera, all Chlorophyceae, with the exception of *Micractinium* which belongs to the Trebouxiophyceae. Among the 12 genera of

Chlorophyceae, six were Chlamydomonadales, five were Sphaeropleales and only one genus was an Oedogoniales. This shows that the ITS2 of Chlorophycae might be slightly conserved among the class or at minimum at the order level, and that it might be possible to design a PCR primer capable of amplifying the entire class or order.

Table 1.3 shows the treatment responses of all the 44 species sequenced based on molecular data within each genus. The majority of species (33 of 44) did not show any response to perturbation, being present both before and after the acidification, or having a random distribution across time and treatments. Among the 11 species that did respond to the perturbation, four did so negatively: being present in the two time points before the perturbation, but not in the two points after, or being present only in the Controls. Seven species showed a positive treatment response: appearing after or remaining present post-acidification in the treated mesocosms (Table 1.3).

For molecularly-identified species with positive acidification, we determined, using the genotype information, whether that response involved a genetic shift or not. We used relative sequence abundance to compare treatments instead of absolute values because of potential bias in the sequencing methods based on the relative abundance of conspecifics. For example, the largest number of sequences per genotypes for *Chlamydomonas* sp.11 (Fig. 1.8) and *Desmodesmus cuneatus* (Fig. 1.10) occurred in the Press treatment. While there coud be more of these species in the Press mesocosms, the ecological results show less overall biomass in the Press at least versus the Pulse mesocosms. Together, these results suggest that the appearance of more genotypes in the Press treatment is a result of the loss of other species in the Press that remained present in the Pulse and Control treatments. Hence, a larger number of genotypes of these focal species were being sequenced in the Press mesocosms leading to potential interpretation bias when absolute values are used. For this reason, we used relative sequence abundances to compare treatment responses of the genotypes.

To examine the acidification response, we produced histograms of the relative abundance of each genotype within a species at each time point (Figs. 1.7 and 1.9). Note that when an OTU is "absent", it is because it is either present in numbers so small or that its detection was limited by very large abundances of an other species in the sample; such a

species would remain below the detection limit of our sequencing method. Because our mesocosms were mostly closed to immigration from the lake (only open at the top to small amounts of airborne dispersal), all species and genotypes present through time would mostly likely have been present from the beginning of the experiment, although some were too rare to be sequenced, becoming more abundant and detectable only later in the experiment, if and when their abundances increased.

Table 1.2 Genera and the number and names of species identified using ITS2 sequencing. The OTUs column shows the total number of OTUs (including singletons and doubletons) as well as the number (in brackets) used to identify changes in genotype frequencies. The last column the number of OTUs detected per species.

Genus	Number of associated species	OTUs	Species names	Number of genotypes per species
Chlamydocapsa	1	1(1)	NA	NA
Chlamydomonas	12	72(20)	*asymmetrica*	1
			sp.1	1
			sp.2	2
			sp.3	2
			sp.4	1
			sp.5	3
			sp.6	1
			sp.7	1
			sp.8	2
			sp.9	1
			sp.10	2
			sp.11	3
Coelastrum	3	41(6)	*astroideum*	4
			sp.1	1
			sp.2	1
Desmodesmus	12	40(14)	*armatus*	1
			bicellularis	1
			communis	1
			costato-granulatus	1
			cuneatus	2
			denticulatus	1
			elegans	2
			hystrix	1
			opoliensis	1
			ultrasquamata	1
			sp.1	1
			sp.2	1
Gonium	2	11(2)	sp.1	1
			sp.2	1
Micractinium	2	5(3)	sp.1	1
			sp.2	2
Oedogonium	8	51(16)	*angustistomum*	1
			sp.1	1
			sp.2	1
			sp.3	1
			sp.4	6
			sp.5	1
			sp.6	3
			sp.7	2
Pandorina	1	3(2)	*morum*	2
Paulschulzia	undetermined	11(4)	undetermined	NA
Pediastrum	undetermined	4(3)	undetermined	NA
Scenedesmus	2	9(5)	sp.1	3
			sp.2	2
Sorastrum	1	2(2)	sp.1	2
Yamagishiella	1	43(6)	*unicocca*	6

Table 1.3 Genetic response to perturbation indicating in which of the two treatments each molecularly-identified species was found.

Species	Direction of the response to perturbation	Associated treatment	Genotype shift
Chlamydomonas asymmetrica	0		
Chlamydomonas sp.1	0		
Chlamydomonas sp.2	-		No
Chlamydomonas sp.3	0		
Chlamydomonas sp.4	0		
Chlamydomonas sp.5	+	Press	No
Chlamydomonas sp.6	0		
Chlamydomonas sp.7	0		
Chlamydomonas sp.8	-		No
Chlamydomonas sp.9	0		
Chlamydomonas sp.10	0		
Chlamydomonas sp.11	+	Press and Pulse	**Yes**
Coelastrum astroideum	+	Pulse	No
*Coelastrum*sp.1	0		
Coelastrum sp.2	-		No
Desmodesmus armatus	0		
Desmodesmus bicellularis	0		
Desmodesmus communis	0		
Desmodesmus costato-granulatus	0		
Desmodesmus cuneatus	+	Press	**Yes**
Desmodesmus denticulatus	0		
Desmodesmus elegans	0		
Desmodesmus hystrix	0		
Desmodesmus opoliensis	0		
Desmodesmus ultrasquamata	0		
Desmodesmus sp.1	0		
Desmodesmus sp.2	+	Pulse	No
Gonium sp.1	0		
Gonium sp.2	0		
*Micractinium*sp.1	0		
*Micractinium*sp.2	0		
Oedogonium angustistomum	0		
Oedogonium sp.1	-		No
Oedogonium sp.2	0		
Oedogonium sp.3	0		
Oedogonium sp.4	+		No
Oedogonium sp.5	0		
Oedogonium sp.6	0		
Oedogonium sp.7	0		
Pandorina morum	0		
Scenedesmus sp.1	+	Pulse	No
Scenedesmus sp.2	0		
Sorastrum sp.1	0		
Yamagishiella unicocca	0		

Two of the 44 starting species showed a positive genetic response to perturbation (*Chlamydomonas* sp.11 and *Desmodesmus cuneatus*). There were clear genotype (OTU) shift in *Desmodesmus cuneatus*, which reacted positively. OTU36 dominated the two post-perturbation time points in all the Press mesocosms, while it was only marginally present on the dates following perturbation in other replicates (Fig. 1.7). OTU36 was present in one of the Control replicates early on, indicating that it was likely present to begin with to some lesser degree in all mesocosms. It did appear on one post-acidification date in one Pulse mesocosm, but did not persist as a dominant to the last date sampled. Results indicated that OTU36 took the place of OTU38 that otherwise predominated in samples taken prior to acidification, but only in the Press treatment.

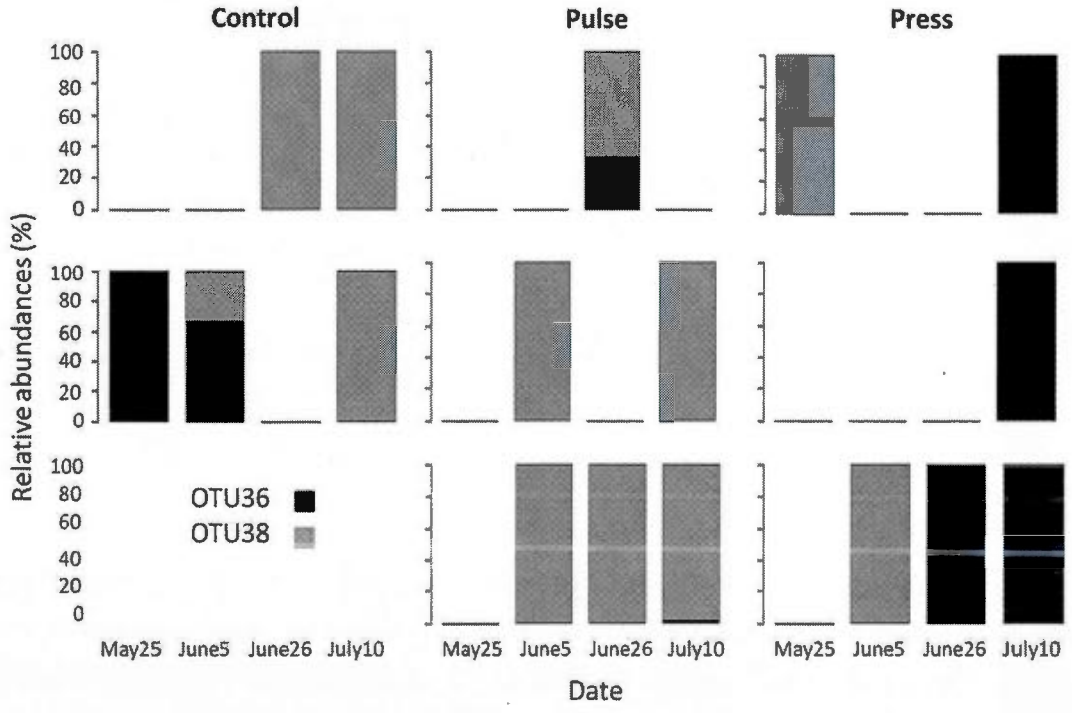

Figure 1.7 Histograms showing the relative abundances of *Desmodesmus cuneatus* OTUs through time across treatments (columns) for each replicate mesocosms (rows). (Note, only two Control replicates could be analysed genetically (see Methods).

For *Chlamydomonas* sp.11, OTU70 dominated the Press mesocosms and at the two dates after perturbation (Fig. 1.9). OTU70 was also present in one of the Pulse mesocosms, but only on the first date post-perturbation (June 26), being fully absent subsequently in this treatment. When *Chlamydomonas* sp.11 was present on the last date in the Pulse and Control treatment, it consisted entirely of OTU49.

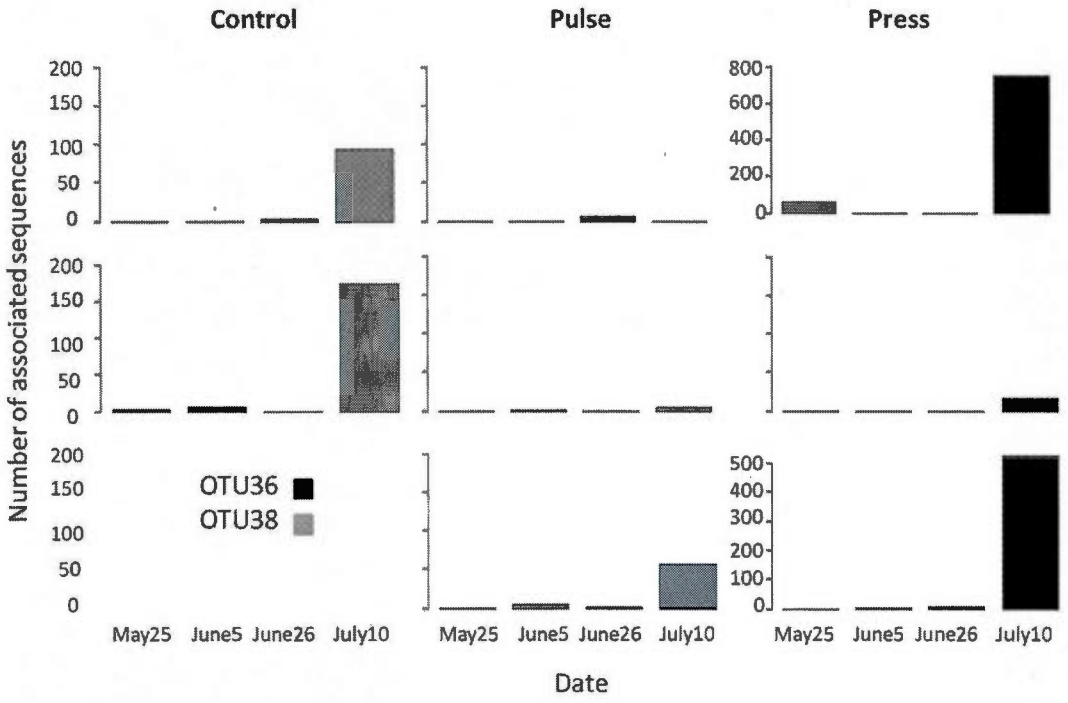

Figure 1.8 Histograms showing the absolute abundances of *Desmodesmus cuneatus* OTUs through time across treatments (columns) for each replicate mesocosms (rows). (Note, only two Control replicates could be analysed genetically (see Methods).

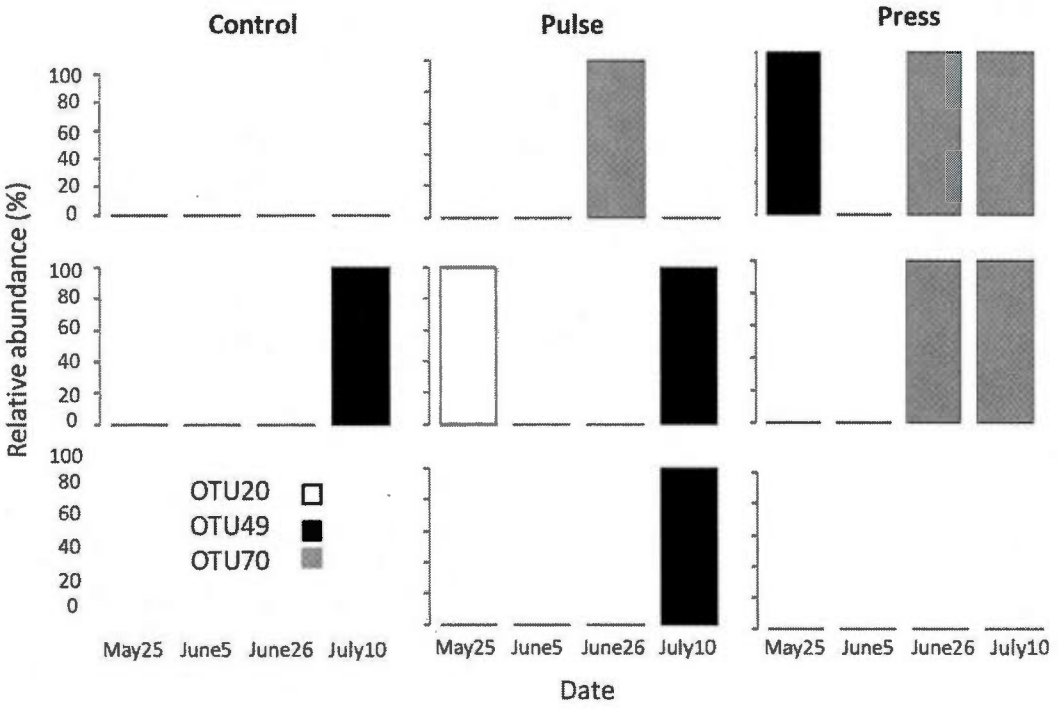

Figure 1.9 Histograms showing the relative abundances of *Chlamydomonas* sp.11 OTUs through time across treatments (columns) for each replicate mesocosms (rows). (Note, only two Control replicates could be analysed genetically (see Methods).

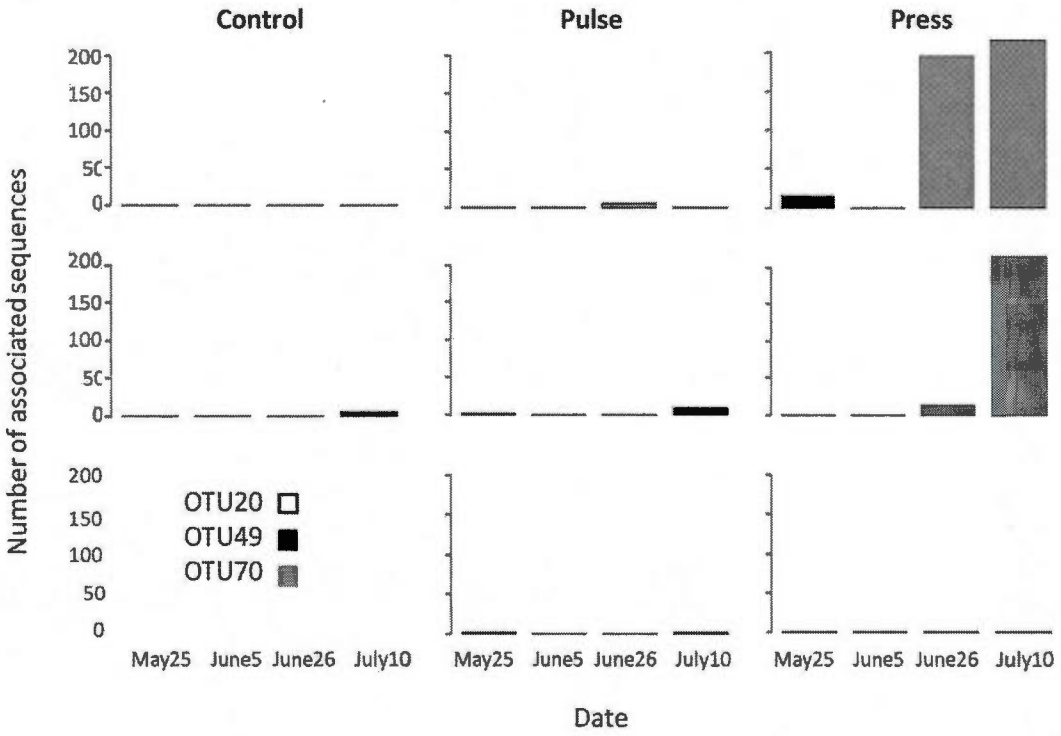

Figure 1.10 Histograms showing the absolute abundances of *Chlamydomonas* sp.11 OTUs through time across treatments (columns) for each replicate mesocosms (rows). (Note, only two Control replicates could be analysed genetically (see Methods).

For both *Chlamydomonas* sp.11 and *Desmodesmus cuneatus*, we performed a deeper analysis of the variation in genetic diversity at a 97% similarity. This is because a clustering at 90% identity is very conservative and may be more representative of species diversity than intra-specific diversity. To better determine intra-specific diversity, we re-clustered representative sequences of each OTUs at 97% identity. Figure 1.11 shows that intra-specific diversity for OTU36 of *Desmodesmus cuneatus*. On the two post-acidification dates, a red OTU (OTU 137) dominated all samples in the Press and Pulse treatments, whereas in the pre-acidification dates in the Control treatment, a different (grey) OTU occurred and the red did not. This grey OTU (OTU136) was also present post-acidification in the treated mesocosms, but was always less abundant than OTU137.

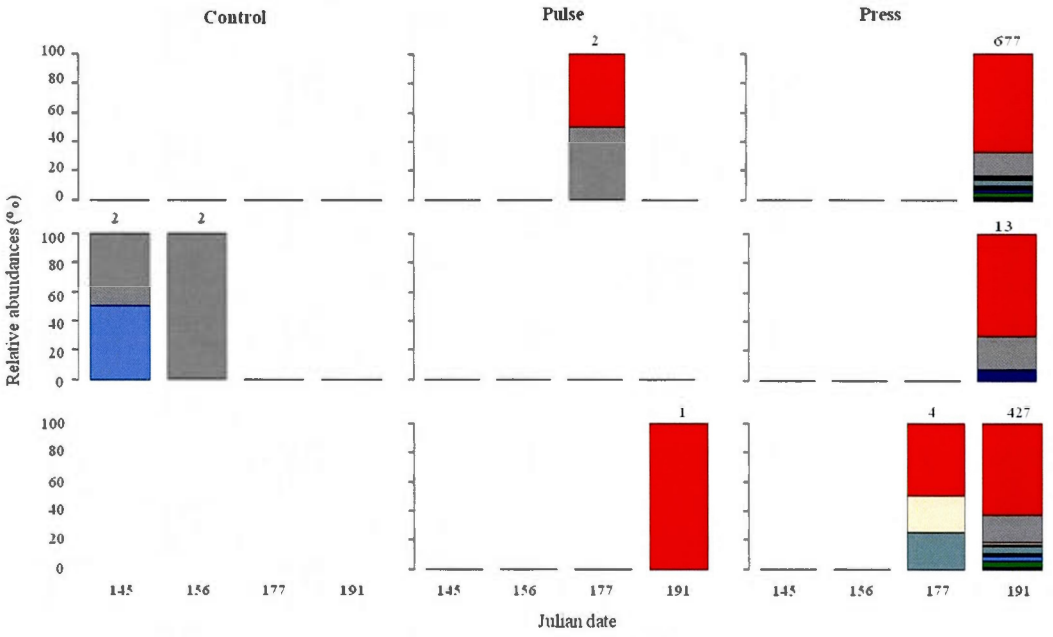

Figure 1.11 Histograms showing the relative abundances of *Desmodesmus cuneatus* OTU36 OTUs (clustered at 97% identity) through time across treatments (columns) for each replicate mesocosms (rows). (Note, only two Control replicates could be analysed genetically (see Methods). Numbers above bars are absolute number of sequences for the entire OTU.

Figure 1.12 shows the relative abundances of OTUs making up OTU 38 of *Desmodesmus cuneatus* with 97% identity clustering. No eveidence for an evolutionary shift were observed with the dominant (green) OTU (OTU77) dominating all samples both pre- and post-acidification.

For *Chlamydomonas* sp.11, OTU 20 and 40 as defined at 90% were each represented by only one OTU at 97% identity. On the other hand, OTU70 was composed of many OTUs at 97% identity, but no distinct patterns was evident in the relative abundances of theses OTUs as shown in Figure 1.13.

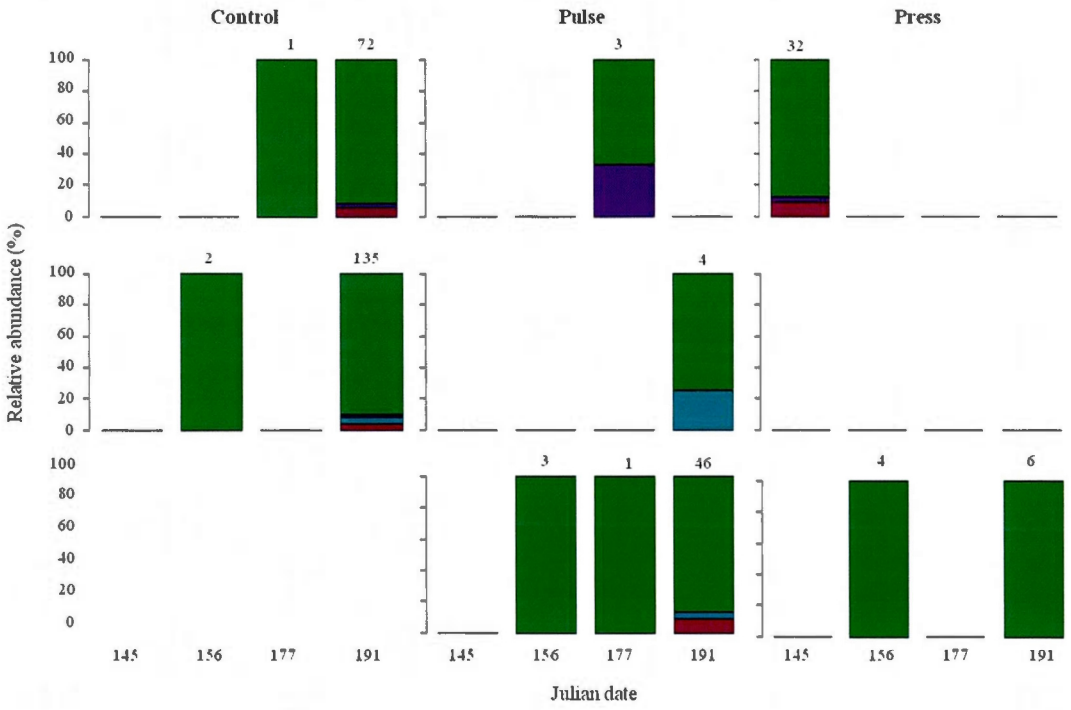

Figure 1.12 Histograms showing the relative abundances of *Desmodesmus cuneatus* OTU38 OTUs (clusterd at 97% identity) through time across treatments (columns) for each replicate mesocosms (rows). (Note, only two Control replicates could be analysed genetically (see Methods). Numbers above bars are absolute number of sequences for the entire OTU.

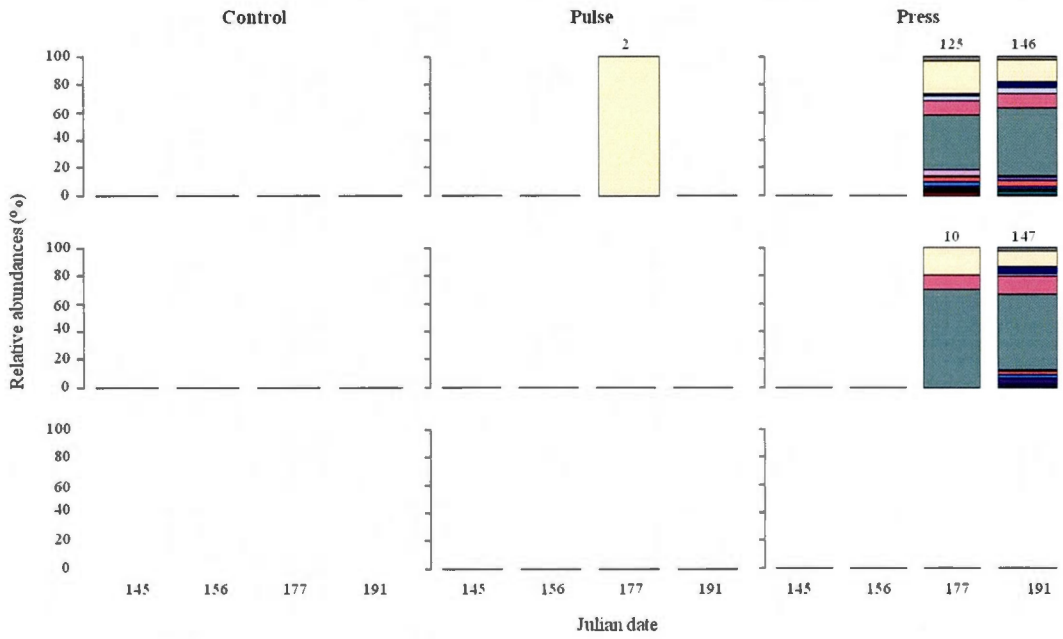

Figure 1.13 Histograms showing the relative abundances of *Chlamydomonas* sp.11 OTU70 OTUs (clusterd at 97% identity) through time across treatments (columns) for each replicate mesocosms (rows). (Note, only two Control replicates could be analysed genetically (see Methods). Numbers above bars are absolute number of sequences for the entire OTU.

1.4 Discussion

1.4.1 Ecological response

A greater biovolume post-perturbation in the two acidified treatments than in the Controls points to a positive effect of the perturbation on phytoplankton. Furthermore, this positive effect can be attributed to enhanced compensatory dynamics between some species forming the communities. We noted that there is a greater biovolume post-perturbation (Fig. 1.1) in the two acidified treatments than in the Control treatment, which seems to indicate an overall positive effect of the perturbation on phytoplankton. Compensatory dynamics can play an important role in community recovery following a perturbation (Vasseur and Gaedke 2007; Gonzalez and Loreau 2009). In phytoplankton communities specifically, they have been previously observed between diatoms and chlorophytes in response to acid perturbation,

in large part because diatoms are less tolerant of acidic conditions (Klug et al. 2000). The dynamics we observed between these two groups do therefore satisfy the condition of two negatively covarying taxa wherein one is more tolerant to a focal perturbation than the other (Klug et al. 2000). The metric used does not allow us to know precisely which species are involved in the compensation, but a quick look at the results show that chlorophytes benefit most from the perturbation (Fig. 1.3). To do so, they could be taking the niche made available by the disappearance of either diatoms, who are dominating prior to acidification, or cryptophytes, which are important in the Control replicates post perturbation, but almost completely absent from the treated mesocosms.

Compensatory dynamics have a global stabilizing effect on communities (Vinebrooke et al. 2003; Frank et al. 2006) mainly via the insurance effect (Descamps-Julien and Gonzalez 2005; Hector et al. 2010). The basis of such insurance effects (which enhances the performance of a community post-perturbation) is in the asynchrony of species' responses (Yachi and Loreau 1999) which allows for the maintenance of the community function, providing that there is some functional redundancy to begin with. Compensatory dynamics were evident in our experiment in the way the communities, particularly in the Pulse treatment, recovered from the perturbation. With a mean synchrony value greater than 0.5, compensatory dynamics were negligible in the Control treatments. Synchrony values were lower in the two acidified treatments, although more so in the Pulse (0.2) treatment than in the Press (0.4) Thus, in the treatment (Pulse) wherein we permitted the natural recovery of initial environmental conditions by ceasing acidification, it is clear that compensatory dynamics play an important role in the recovery of these communities. This role was not as crucial for ecological recovery in the Press treatments, where the synchrony index was almost twice that of the Pulse treatments. Related more specifically to the stability incurred by compensatory dynamics, the Pulse mesocosms also showed the greatest resilience: that is they took less time to recover from the perturbation than did the Press mesocosms.

The PRC analyses provide some information about specific responses to the perturbations, both in terms of the direction and strength of responses. When all taxa were included, it was a species from the normally acid-intolerant diatom group that reacted the most positively to acidification (*Tabellaria floculosa*). Amongst the diatoms however, this

species is known to be tolerant of acidic conditions (Patrick and Reimer 1966). The other taxa that responded positively included mainly Chlorophytes, including *Chlamydomonas* sp., *Gloeocystis* sp., *Closterium* sp. and filamentous species including *Mougeotia* sp. and *Ulothrix* sp. These latter two taxa were so abundant in the mesocosms that they formed floating aggregates that were visible to the naked eye in the field. Generally, some cholorophytes are known to be acid tolerant, even at very low pH (Verb and Vis 2001) like *Chlamydomonas* (Nixdorf et al. 1998; Fyson et al. 1998; Lessmann et al. 2000; Kalin et al. 2006), *Chlorella* (Huss et al. 2002) or *Mougeotia* (Turner et al. 1991), explaining their positive responses to our treatments. Somewhat surprising was the negative response of cryptophyte taxa such as the Cryptomonads and Rhodomonads, which have been found to be acid tolerant in other studies (Vinebrooke et al. 2003). This result suggest therefore that cryptophytes were outcompeted by acid-resistant chlorophytes that otherwise dominated the perturbed mesocosms.

1.4.2 Genetic response

From the ecological responses, we can conclude that compensatory dynamics between species were significantly less important in the Press than in the Pulse treatment. Thus we must search further for another mechanism potentially responsible for the recovery of the Press perturbation communities. Our results indicate that a this mechanism could be an evolutionary one, as per our original hypothesis, involving genotypic rearrangements in one or more species in the Press mesocosms, and thereby allowing recovery of total biomass.

To conduct an analysis on potential evolutionary responses to perturbation, we were able to only focus on a single group of common phytoplankton owing to technical limitations in current molecular techniques. We thus chose a taxonomic group that was identifed as being the most abundant in our microscopic analyses of samples (chlorophytes: *Scenedesmus*, later identified as *Desmodesmus*) to design primers and conduct molecular analysis.

The OTU shift observed in *Desmodesmus cuneatus* was clearly a response to long-term acidification whereby one OTU (OTU36) was replaced by another (OTU38) following

sustained perturbation. The OTU36 completely dominated the Press treatments after perturbation, while the original OTU (OTU38) was absent (Fig 1.9). This contrasted with the relative absence of OTU36 from the Pulse and Control treatments after acidification. Thus OTU36 appears to be outcompeted by OTU38 under more normal pH conditions, while OTU36 thrives in constantly acidic waters at the expense of OTU38. A similar, but less clearly defined shift was observed in the case of *Chlamydomonas* sp.11. In this case OTU70 dominated the two post-perturbation dates in Press treatments (with the exception of one replicate where the species was entirely absent), while in Pulse mesocosms OTU49 dominated. Thus, OTU70 is more tolerant to acidic conditions allowing this genotype to dominate with persistent acidification, being outcompeted by OTU49 under normal pH conditions (Pulse and Control treatments). Note however that there was one Pulse replicate where OTU49 was absent and where OTU70 was present on the first of the two post-perturbation dates. Although this replicate differs from the others, it does not contradict our conclusion: in a mesocosm where OTU49 was apparently absent, OTU70 may have had a competitive advantage during the initially acidified phase in the Pulse treatment. Most likely, OTU70 of *Chlamydomonas* sp. 11 was then outcompeted by another species entirely when a neutral pH was re-established (by July 10).

Clustering OTUs at 90% of identityn is quite a conservative threshold, probably more suitable threshold for genus or even family identification. However, we began with this threshold because the exact value that is critical for intra-specific variation is still unknown for phytoplankton. Since we are interested in intra-specific variation, we also performed some analysis at a less conservative threshold (97%), that may more appropriately represent intra-specific variation. Fig. 1.11 shows that within OTU36 of *Desmodesmus cuneatus*, a similar OTU shift as the one between OTU36 and 38 that occurred at 90% identity observed at the 97% level. The red OTU, OTU137, was absent from the Control treatment but dominated all samples post-acidification, while the grey OTU (OTU136) was still present but in much lower numbers. However, a similar intra-specifi OTU shift was did not occurred within OTU38 when grouped at 97% identity. Overall, for *Desmodesmus cuneatus*, the analysis at 90% identity showed an OTU shift from OTU36 and 38, but only OTU36 showed the same shift with a deeper analysis at 97%. Without further knowledge about the threshold

for species-level variation in these phytoplankton,we can only suggest from these data that the shift observed at 90% identity likely represents a species shift rather then a genotypic shift. This latter shift is thus a stronger signal of evolutionary rescue.

In the case of *Chlamydomonas* only sp.11 showed genetic responses at 97% identity but, as illustrated by Fig. 1.113, no conclusions can be drawn owing to the lack of data with OTU70 present in only 3 samples. Thus, it is hard for us to conclude whether the OTU shift observed at 90% identity represents a shift between genotypes or between species, as no information is available at a finer level of analysis.

This situation outlines the diffculty of defining species and genotypes when dealing with clonal organisms. Species are quite easily identifiable in mammals, other vertebrates and even for many invertebrates. But when dealing with bacteria or protists such as phytoplankton, their ability to reproduce asexually and possible lateral gene transfer complicates the task. Moreover, defining species based only on sequence similarity may not necessarily be the most appropriate way to deal with such organisms, as has been argued for bacteria (Gevers et al. 2005). And bacteria and protists are very similar in some respects: noth groups are unicellular, asexual, and some algal clades (such as *Chlamydomonas* or *Spirogyra*) can perform lateral gene transfer. Thus, it is not possible for us to determine with certainty whether the clades we identified at 90% or 97% of genetic identity best represents genotypes, species or even genera and whether the shift observed in *Chlamydomonas* sp.11 is in fact a species or a genotype shift. On the other hand, it seems fairly safe to affirm that the OTU shift observed in *Desmodesmus cuneatus* at 97% represents a genotype shift and indicative of an evolutionary rescue event.

Our study thus provides evidence for an evolutionary response in at least one, or possibly two, chlorophyte species (depending on the level of genetic identity considered) exposed to sustained habitat alteration (acidification) with demonstrated shifts in dominating genotypes following a perturbation. Without this genotype shift, we can assume that this species would not have recovered from the perturbation, that is to say it has been rescued from extinction by an evolutionary process. Although the marker we chose for genotyping is a neutral marker, thus precluding certainty as to whether this event occurred through natural

selection or genetic drift, the fact that the same genotype arose in all three Press replicates points to a selection process. As already mentioned, this genotype must possess a mutation that makes it more tolerant of low pH than the others. If this mutation arose after the acidification, there would not have been enough time for it to spread and rescue the species (Baos et al. 2002; Barrett and Schluter 2008), as our communities were only exposed to acidic waters for five weeks and mostly closed to immigration. If this were the case, then the mutation would have to have been a part of the standing genetic variation within the species, being either neutral or lightly deleterious, but conferring a notable advantage once environmental conditions changed to more acidic waters.

For evolutionary rescue to happen, a population must be large enough to support a high mutation rate or to possess a great deal of genetic variation (Gomulkiewicz and Holt 1995; Bell and Collins 2008). Genotyping results indicate that the genetic variation in our species was quite low, with a maximum of two and three OTUs (or species) at 90% identity revealed for each group, and between four and 15 genotypes at 97% genetic identity. This is likely a result of the fact that our study focuses on local populations for predominantly clonal phytoplankton wherein one would expect less genetic diversity than in a sexual population wherein recombination occurs regularly. Evolutionary rescue is thought to be more likely to occur in sexual populations (Bell 2013), and it is the least likely to occur in an asexual population, though not imposible. That said, both *Desmodesmus* (Lürling 2003) and *Chlamydomonas* (Pan and Snell 2000) are able to reproduce sexually at times, which could theoretically have increased their genetic variation had this occurred in our experiment. Furthermore, a low level of measured genetic variation might have been a result of our decision to disregard rare OTUs, a fact which might preclude our detection of further genetic variation in our communities. Evolutionary rescue is also facilitated by migration or dispersal (Bell and Gonzalez 2011) as dispersal restocks a perturbed system with greater genetic variation.

Despite the low measured genetic variation in our study and an absence of dispersal, we nevertheless observed events indicative of evolutionary rescue. This result indicates that evolutionary rescue can act even with low genetic diversity. The likely mechanism would be that the mutation allowing for resistance to the perturbation is already present in the genome

of the responsive species. For evolutionary rescue to occur, the range of variation present in a population should include genotypes with positive reactions to the induced perturbation (Bell 2013). This seems to be the case in our study but for only one (possibly two) species present initially in the lake community. The genotype or genotypes responsible for the species recovery in the Press treatment would thus have been pre-adapted to low pH owing to a standing mutation, thereby allowing us to observe evolutionary rescue, even with low genetic variation. This result suggests that even if we know that species can evolve quickly following environmental change (Kinnison and Hairston 2007), rapid evolution of phytoplankton species is rather uncommon and an already favourable type to the new environmental conditions likely needs to be present *a priori* for it to occur.

Evolutionary rescue is often recognized by a U-shape curve (Gomulkiewicz and Holt 1995) of the genotype population abundances over time. We could not test for this U-shape curve in our data as our molecular method does not permit us to compare absolute abundances between dates; DNA sequencing offering only a semi-quantitative method. In particular, the first sampling dates were highly dominated by an unidentifed phytoplankton from the Chlamydomonadaceae family (close to *Chloromonas*; BLAST search), thus precluding us from assessing the presence of other, more rare species present in the samples owing to a saturation of the assessment methods. As this species disappeared with time, the capacity to sequence other species increased.

1.4.3 Potential top-down effects

Given that we wanted to assess the phytoplankton community response to acid perturbation in a way that resembled natural lake conditions as much as possible, we included zooplankton in the mesocosms. As would be expected, zooplankton community structure was also affected to some degree by acidification and this impact may have formed part of the phytoplankton ecological response. However, we expect that differences between treatments in the zooplankton effect on the phytoplankton dynamics would be only slight for a number

of reasons. In part this is because the only difference in total zooplankton abundance was observed on June 26th (the first post-acidification date) and there was no difference between the treatments by the last date of the experiment, indicating that the effect we see in phytoplankton would not result by overall zooplankton abundances. Most differences between treatments in the zooplankton community were a result of some compositional changes. First, there were fewer copepods in the Press treatment and fewer pelagic cladocera post-perturbation in the two acidified treatments. Such declines would have relieved some predation pressure on the phytoplankton community. However, while there were fewer zooplankton, there were also fewer phytoplankton in the Press treatment, indicating a stronger bottom-up perturbation effect than in the Pulse treatment, consistent with our interpretation of phytoplankton dynamics. As for the disappearence of pelagic cladocerans, this could have enabled the compensatory dynamics observed in both Press and Pulse treatments as it might have stimulated phytoplankton growth. Even if indirect, this effect through zooplankton still represents a perturbation effect because this decrease in zooplankton was part of the overall plankton community response to acidification. Our study thus provides information on how a natural plankton community would react to a sudden acidification, informing us about the cumulative ecological impact of both direct and indirect forces of acidification on plankton communities.

As for the evolutionary response to perturbation, we believe that its source was a direct a result of the perturbation and not from predation release. Both drift and evolutionary rescue happen after a significant decline in population abundances, which would not result when predatory pressure is relieved, but rather would occur as a result of the perturbation alone. The genotype shift we observed in *Desmodesmus cuneatus* and *Chlamydomonas* sp.11 could then only be a result of acidification. Moreover, we propose that this shift is caused by the application of a new environmental pressure acting as a selective agent on phytoplankton species. Predation release, on the other hand, would instead liberate phytoplankton from selective predation pressure, thereby not inducing a rapid evolution to adapt to a new predator-free condition, as would a drastically lowered pH. Consequently, while liberation from zooplankton predation might be involved in the ecological response of phytoplankton, it would not for its genetic response.

Finally, there were peaks in chydorids and nauplii on June 26th in the Press and Pulse treatments respectively. These peaks would have had little, to no impact on phytoplankton responses as chydorids graze periphyton growing on the walls of the mesocosms and not the pelagic phytoplankton community with which we were concerned, and nauplii feed preferentially on smaller protozoa than on our response species (Turner 2004).

1.4.4 Eco or evo?

Ecological processes were largely responsible for species recovery in this experiment, while evolutionary ones, while they can be identified as playing a role in some species recovery, overall they did so in a much more marginal way. Compensatory dynamics were particularly important when the perturbation was applied as a Pulse, while they were significantly less important with a press perturbation application. On the other hand, evolutionary process happened only with a press perturbation and with a high degree of certainty in only one a species. Some have suggested that a minimal amount of environmental degradation, without extinction is more favourable to the observation of evolutionary rescue processes (Bell and Gonzalez 2011). While our Press treatment was a more severe perturbation than the Pulse treatment, a lowering of pH to 5.0 is not extremely harsh for lake phytoplankton and commonly experienced by them (Findlay et al. 1999; Baos et al. 2002; Flores-Moya et al. 2005), such perturbation would not be catastrophic for a phytoplankton community. In line with this, other than for most diatoms, very few species went extinct after acidification in our experiment.

As predicted, it was the growth of the phytoplankton biomass itself that restored the original pH of the mesocosm ecosystem in the Pulse treatment: one might consider this "ecosystem rescue" occurring through niche construction (Odling-Smee et al. 1996). Through photosynthesis, phytoplankton rendered their habitat more neutral again. As a result of this ecosystem rescue, phytoplankton species did not need to adapt to new acidic pH conditions as they returned to normal in a few weeks. On the other hand when the ecosystem was pressed and pH maintained at much lower values that those to which the phytoplankton community was accustomed, genotype frequencies of at least one species were modified, through what

was most likely a standing mutation, leading to a what we describe as an adaptation of that species, and thus a process of evolutionary rescue.

1.5 Conclusion

When we applied a Pulse perturbation, phytoplankton species responded to it through an ecological process, namely compensatory dynamics. Compensatory dynamics actually stimulated both the Pulse and Press treatments when compared to a Control treatment, as diatoms disappeared after acidification leaving some niches available for the apparently less competitive, but more pH-resistant chlorophytes. Such dynamics also occurred when the system was pressed but additionally, there were one, possibly two, species that recovered through an evolutionary process similar to evolutionary rescue. These events appear to be rare and would happen only when a system is perturbed in a sustained way because it is only under these conditions that a population needs to adapt. Our results also show that evolutionary rescue can occur even when the genetic variation in the population is small, providing that there is a standing mutation favourable to the perturbation.

This divergence in response type with regard to the length of the perturbation applies to an acidification perturbation, but questions remain. Would it similarly apply to other types of environmental degradation? Would it be the same for a spill of heavy metals or other non-acidic chemicals? We chose a perturbation that the phytoplankton would be able to repair themselves (i.e. ecosystem rescue), but in the case of a chemical or a metal that they can not metabolize, a pulse perturbation may not even be possible. In this case, whether you add a chemical once or in a sustained way, evolutionary rescue may always occur, especially if species already possess a useful mutation or if dispersal provides one. Based on these results, we expect that evolutionary shifts will become a critical mechanism in the case of marine phytoplankton currently undergoing an acidification of their environment, permitting their continued survival. Ocean acidification is a more gradual process than the perturbation we performed here, where pH went from around 9 to 5 in a single day. But a slower perturbation rate has been shown to be even more favourable to evolutionary rescue (Collins and de Meaux 2009; Lindsey et al. 2013), hence giving hope that a greater proportion of species than

the few observed in our experiment will adapt to their new environment and avoid extirpation.

In a context where anthropogenic perturbations are increasing globally, it is crucial to understand whether and how populations recover to provide better management. Our study shows that under relatively natural conditions, with naturally co-occurring and co-evolved species in plankton communities, evolutionary processes can play an important role in the recovery of some of these populations, even in a food web context. It will thus be important for ecologists to integrate these concepts to their analyses of extinction risk. In this context, molecular biology is a powerful tool to observe such processes and ultimately better understand evolutionary processes behind the ecological ones observed as species and communities recovery after a perturbation.

CONCLUSION

La présente étude avait pour but de déterminer quels sont les mécanismes participant à la récupération et au maintien de la stabilité des communautés de phytoplancton face à une perturbation au niveau du pH. Nous cherchions tout particulièrement à démontrer l'importance du processus de sauvetage évolutif, processus selon lequel suite à une perturbation, une évolution rapide permet à la population en question de s'adapter et ainsi éviter l'extinction. La grande majorité, si ce n'est la totalité, des études précédentes portant sur le sauvetage évolutif ayant été effectuées sur des cultures en laboratoire d'un très petit nombre d'espèces, nous avons tenté d'observer le sauvetage évolutif sur une communauté naturelle gardée dans des conditions naturelles.

Pour ce faire, nous avons réalisé une expérimentation en mésocosmes ce qui nous permettait de garder nos communautés dans leurs conditions naturelles tout en évitant d'avoir à manipuler un lac complet. Ces mésocosmes étaient constitués de sacs de plastique transparent accrochés à un grand quai de plastique flottant installé sur le Lac Hertel au Mont St-Hilaire (Qc, Canada). En guise de perturbation, nous avons abaissé le pH dans les mésocosmes jusqu'à 5.0 tandis que le pH naturel du Lac Hertel se situe généralement entre 7.5 et 8.5. Cette perturbation a été appliquée de deux façons, de manière ponctuelle ou constante (ou maintenue), de sorte à pouvoir déterminer si la récupération des populations se fait selon les mêmes mécanismes stabilisateurs selon le type de perturbation. Cette expérience s'est déroulée du 25 mai au 10 juillet 2012, laissant ainsi cinq semaines après la perturbation ce qui offrait un nombre de générations présumément assez important pour permettre une évolution rapide des populations.

Dans le but de suivre la progression de la fréquence de chaque génotype, nous avons séquencé une région non-codante de l'ADN ribosomal connue sous le nom d'ITS2 (Internal Transcribed Spacer 2) et située entre les gènes codant pour les deux parties de la grande sous-unité ribosomale. Ce marqueur est de plus en plus utilisé particulièrement pour

l'identification d'espèces et sert aussi de codebarre, surtout pour des taxa de champignons ou d'algues. Il a également été démontré que l'ITS2 pouvait permettre de détecter une variation intraspécifique, notamment chez un nombre significatif d'espèces d'algues. Les séquences d'ITS2 de plusieurs espèces ont déjà été répertoriées et regroupées sur une base de données web (http://its2.bioapps.biozentrum.uni-wuerzburg.de/ Dernier accès: 2 juillet 2013), facilitant substantiellement la tâche pour le design d'amorces et le séquençage, ce qui en fait un marqueur plus simple et plus rapide à utiliser pour le génotypage que ne le sont les microsatellites (marqueur reconnu pour le génotypage) par exemple. L'ITS2 est également plus simple et rapide à séquencer que ne le sont les microsatellites par exemple, qui sont par contre un marqueur de diversité génétique infraspécifique reconnu. Pour toutes ces raisons, nous avons opté pour l'ITS2 comme marqueur de diversité génétique intraspécifique.

Nous avons d'abord observé une récupération des espèces différenciée selon le type de perturbation auquel chaque communauté a fait face; la récupération était plus rapide et plus importante dans le cas d'une perturbation ponctuelle que lorsque la pH était maintenu à 5.0 pendant toute la durée de l'expérience. De la même manière, les mésocosmes ayant été perturbés de manière ponctuelle ont montré significativement plus de résilience (une composante de la stabilité) que ceux ayant été perturbés de façon constante. Dans le but d'expliquer cette plus grande stabilité des mésocosmes ponctuels, nous avons cherché à établir la présence de possibles dynamiques compensatoires dans nos communautés. Il s'est avéré que des telles dynamiques étaient bel et bien présentes dans nos mésocosmes perturbés, et ce de manière plus importante lorsque la perturbation était ponctuelle. Le dynamiques compensatoires étant un mécanisme reconnu pour apporter une plus grande stabilité à un système face à une perturbation en permettant le maintien des fonctions dans la communauté, nous proposons que la plus grande stabilité en cas de perturbation ponctuelle est due à ces dynamiques stabilisatrices. Par contre, la méthode de mesure de ces dynamiques que nous avons choisie ne permet pas de déterminer entre quelles espèces il y a compensation, mais les analyses de courbes de réponses principales (PRC) nous donnent quelques indications de quelles espèces ont été favorisées ou défavorisées par la perturbation. Une espèce de diatomées reconnue comme étant tolérante au bas pH, soit *Tabellaria floculosa*, a réagit positivement à l'acidification. Les autres espèces réagissant favorablement à la perturbation

sont des chlorophytes telles que *Gloeocystis* sp., *Chlamydomonas* sp. ou *Ulothrix* sp. Pour ne nommer que celles-là. Du côté des algues défavorisées par l'acidification, nous avons été surpris de trouver des cryptophytes telles que des *Crytpomonas* ou *Rhodomonas* sp., car elles ont déjà été identifiées comme tolérantes à l'acide par le passé. Malgré cette capacité à résister à l'acidification, elles auraient fort probablement été désavantagées par la compétition avec une ou plusieurs meilleures compétitrices.

Le séquençage de l'ITS2 nous a permis d'identifier moléculairement 44 espèces de chlorophytes, toutes membres de la famille des chlorophyceae, à l'exception d'une seule espèce, dont deux montrent une réponse au niveau génétique à la perturbation. Dans le cas de *Desmodesmus cuneatus* et d'une espèce de *Chlamydomonas* non-identifiée (*Chlamydomonas* sp.11), un suivi de leur diversité génotypique sur quatre dates réparties sur les neuf semaines d'expérimentation montre un changement dans l'identité du génotype dominant avant et après l'acidification. Ce même suivi, mais effectué avec une similarité génétique plus grande pour délimiter les espèces montre ce même changement dans le génotypes dominant pour *Desmodesmus cuneatus*. Cette espèce est dominée par un génotype particulier dans les premières dates pré-perturbation, et dans les mésocosmes perturbés de manière constante, ce génotype est remplacé par un autre qui domine complètement la population après la perturbation. Ce processus ne se produit que lorsque le pH est maintenu à 5.0 durant toute l'expérience, indiquant que la perturbation doit durer dans le temps pour forcer les espèces à s'y adapter. Pour ce qui est de *Chlamydomonas* sp.11, les résultats sont plus mitigés lorsque l'analyse est effectuée avec une identité génétique plus importante, entre autre par manque de données, mais il n'est pas exclu que le changement de génotype après la perturbation constaté à 90% d'identité génétique soit réel. Nous avons donc observé que certaines populations ont été sauvées de l'extinction par un évènement de sauvetage évolutif comme nous l'avions prédit, bien que le phénomène semble rare.

Il semble donc que le mécanisme stabilisateur responsable de la récupération des communautés dépende entre autre du type de perturbation impliqué. En effet, nos résultats montrent que dans le cas d'une perturbation ponctuelle, le mécanismes principalement responsable de la récupération des communautés soit les dynamiques compensatoire, un processus écologique et dans le cas d'une perturbation maintenue, on observe à la fois ce

processus écologique mais aussi un processus évolutif, soit le sauvetage évolutif, bien qui celui-ci soit très rare. Bref, lorsque la perturbation est circonscrite dans le temps, les espèces en présence n'ont pas besoin de s'adapter, la pression de sélection n'est pas assez forte pour les y pousser et on assiste à des réarrangements dans l'assemblage des espèces, mais rien ne se produit au niveau de leur génome. Par contre, lorsque la perturbation est constante, il y a une plus grande mortalité et les génotypes au sein d'une espèce possédant une mutation les rendant capables de persister et de mieux performer dans les nouvelles conditions du milieu se voient favorisées, on assiste donc non seulement à un réarrangement des espèces mais également à un réarrangement génotypique au sein de certaines d'entre elles due à la pression de sélection.

L'ITS2 étant fiché entre deux gènes bien conservés à des niveaux phylogénétique supérieur à l'espèce, il serait possible de fabriquer des amorces permettant de l'amplifier chez toute une famille, voire même un phylum d'algues. Les chlorophytes sont un groupe d'algues particulièrement large et diversifié, ce qui empêche la fabrication d'amorce pour tout le phylum ou la classe, mais avec les cryptophytes, les chrysophytes voire même les diatomées, il est possible de fabriquer des amorces peu dégénérées et ainsi obtenir de l'information sur tout le phylum ou la classe. On pourrait ainsi assez facilement et rapidement suivre la diversité génotypique d'une très grande partie de la communauté suite à une perturbation. Par contre, ce marqueur est un marqueur neutre, en ce sens qu'il n'est pas soumis à la sélection, ce qui ne nous permet pas de conclure avec certitude que l'évolution rapide que nous avons constaté est réellement dû à la sélection et non à la dérive génique. Le fait que ce soit le même génotype qui est favorisé dans chacun des réplicats et ce pour les deux espèces concernées indique que les probabilités qu'il s'agisse du hasard (comme dans un cas de dérive génique) sont très faibles. Par contre, l'utilisation d'un marqueur sous sélection pourrait être intéressant dans le futur pour confirmer que la sélection naturelle est bel et bien la responsable de ces deux cas de sauvetage évolutif.

Finalement, dans cette ère actuelle de perturbations anthropiques il est important de comprendre comment les espèces réagiront et s'adapteront, si elles le font, à leurs nouveaux milieux. Les outils de biologie moléculaire offrent de nombreuses possibilités pour ce faire et ces outils se développent à une vitesse étonnante et à un coût toujours de plus en plus bas,

augmentant ainsi pratiquement exponentiellement les possibilités offertes. Les analyses génétiques peuvent permettre de répondre à un grand nombre de questions fascinantes en écologie pour peu que les écologistes se les approprient.

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