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“Κατακερματισμός και επανασύνδεση σε υδατικές μετακοινότητες”
“Fragmentation and reconnection in aquatic metacommunities”

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ABSTRACT

The impacts of fragmentation and connectivity on ecosystem structure and function are central in current ecological research and are directly relevant to ecosystem management efforts concerned with the effects of environmental change. These issues are investigated within the framework of metacommunity ecology, which is directly applicable to coastal aquatic ecosystems, such as interconnected lagoons, salt marshes and rock pools affected by pulsed nutrient inputs. As water levels vary and patches become periodically isolated and reconnected, the structure and function of an aquatic metacommunity should not solely depend on connectivity among local communities but also on their previous state during fragmentation. The aim of the present study was, thus, to assess the effect of fragmentation and connectivity on different community attributes (i.e. species composition, diversity and productivity) in aquatic metacommunities characterized by environmental heterogeneity. Towards this aim both experimental and modeling approaches were employed. Experiments consisted of fragmented natural phytoplankton assemblages that self-organized under different nutrient pulsing frequencies. This part of the experiment, together with numerical modeling, was used to address the role of fragmentation and resource heterogeneity on community structure (paper 2).These steady-state assemblages were then employed to represent the local patches that were connected at a dispersal continuum forming metacommunities, to assess the role of spatial averaging (i.e. homogenization towards the average conditions by the species best adapted to them) on species richness and productivity (paper 3). During fragmentation, nutrient pulses of decreasing pulsing frequencies resulted in experimental assemblages of increasing functional diversity and richness. These findings were also validated by numerical simulations which revealed the importance of both growth rate and competitive ability in assembly processes under pulsed resource inflows. Moreover, these environmental conditions led to the dominance of a species at the intermediate pulsing frequency, which, during connectivity, dominated the metacommunity. Therefore, the pivotal role of spatial averaging described in the present study, is directly linked to the conditions that gave rise to the isolated local assemblages. The present thesis addressed questions that have important implications for the management of coastal habitats. In particular this study revealed that optimal ecosystem function (maximal richness, functional diversity, and productivity) in heterogeneous aquatic metacommunities can be achieved when the interval between resource pulses exceeds the generation times of constituent species, and when patches are connected at intermediate connectivity levels.

ΑΝΑΛΥΤΙΚΗ ΠΕΡΙΛΗΨΗ

ΕΡΕΥΝΗΤΙΚΟ ΥΠΟΒΑΘΡΟ

Η επίδραση του κατακερματισμού και της συνδεσιμότητας στη δομή και τη λειτουργία των οικοσυστημάτων είναι πρωταρχικής σημασίας στη σύγχρονη οικολογική έρευνα (Sawyer et al., 2011). Θεωρώντας τα τοπία ως δίκτυα απομονωμένων τοπικών κοινοτήτων που συνδέονται μέσω της διασποράς ατόμων και ειδών, δημιουργήθηκε η έννοια της μετακοινότητας (metacommunity). Η διερεύνηση οικολογικών διεργασιών μέσα σ'αυτό το πλαίσιο, πέρα από τις συμβατικές προσεγγίσεις, βασισμένες μόνο στη μελέτη απομονωμένων τοπικών κοινοτήτων, μπορεί να διευρύνει την οικολογική έρευνα. Την τελευταία δεκαετία, θεωρητικές και πειραματικές μελέτες, αλλά και μελέτες πεδίου, έχουν μελετήσει τα χερσαία οικοσυστήματα υπό το πρίσμα της θεωρίας των μετακοινοτήτων. Εντούτοις, τα υδατικά οικοσυστήματα δεν έχουν μελετηθεί αρκετά στο θεωρητικό αυτό πλαίσιο (Matthiessen and Hillebrand, 2006).

Η θεωρία των μετακοινοτήτων έχει άμεση εφαρμογή στα υδατικά οικοσυστήματα, ιδιαίτερα στην παράκτια ζώνη, όπως σε συστήματα λιμνοθαλασσών, αλμυρά τέλματα και βραχώδεις λημνίσκους (rock pools). Τα μεταβατικά αυτά οικοσυστήματα συνεισφέρουν σε μεγάλο ποσοστό στην παγκόσμια πρωτογενή παραγωγικότητα (Cloern et al., 2013). Αυτή τροφοδοτεί τη δευτερογενή παραγωγικότητα και συμβάλει σε πολλαπλές οικοσυστημικές υπηρεσίες, συμπεριλαμβανομένου του κύκλου των θρεπτικών, του άνθρακα και των ιχνοστοιχείων, την αλιεία, τις οστρακοκαλλιέργειες, την αντιστάθμιση της ρύπανσης και την αναψυχή (Costanza et al., 1997). Αυτά τα συστήματα είναι ευάλωτα στην άνοδο της στάθμης της θάλασσας λόγω της κλιματικής αλλαγής (Cyrus et al., 2010) αλλά και στον κατακερματισμό από ανθρωπογενείς παρεμβάσεις (Kang and King, 2013). Η μελέτη της συνδεσιμότητας και του κατακερματισμού στη δομή τους (σύνθεση των ειδών και ποικιλότητα) και τη λειτουργία τους (παραγωγικότητα) είναι απαραίτητη για τις προσπάθειες αποκατάστασης των ενδιαιτημάτων (Januchowski-Hartley et al., 2013).

Οι παράκτιες υδατικές μετακοινότητες έχουν δύο ιδιαίτερα χαρακτηριστικά. Πρώτον, η υδρολογική συνδεσιμότητα μεταξύ των κατατμημάτων (patches) είναι προϋπόθεση για την αναπλήρωση των θρεπτικών συστατικών και των ειδών. Η τοπική αλλά και χωρική ποικιλότητα και παραγωγικότητα μπορεί να αλλάξει σημαντικά ανάλογα με το επίπεδο συνδεσιμότητας. Παρόλο που η σχέση του πλούτου ειδών με τη συνδεσιμότητα έχει μελετηθεί αρκετά τόσο σε θεωρητικές όσο και σε πειραματικές μελέτες (π.χ.Mouquet and Loreau, 2003; Cadotte, 2006), η επίδραση της συνδεσιμότητας στην παραγωγικότητα – ένα κοινό μέτρο της οικοσυστημικής λειτουργίας- είναι λιγότερο μελετημένη. Σύμφωνα με θεωρητικές μελέτες (Loreau et al., 2003) η παραγωγικότητα εξαρτάται από δύο μηχανισμούς, τον πλούτο ειδών και την ομογενοποίηση προς ενδιάμεσες περιβαλλοντικές συνθήκες (spatial averaging). Ο δεύτερος μηχανισμός θεωρείται πιο σημαντικός σε υψηλά επίπεδα συνδεσιμότητας, αντισταθμίζοντας σε κάποιο βαθμό τη μείωση της παραγωγικότητας λόγω μείωσης του πλούτου ειδών. Ενώ αρκετές μελέτες έχουν αποδείξει την επίδραση του πλούτου ειδών στην παραγωγικότητα (π.χ.Matthiessen and Hillebrand, 2006), ο ρόλος της ομογενοποίησης προς τις ενδιάμεσες συνθήκες δεν έχει διερευνηθεί.

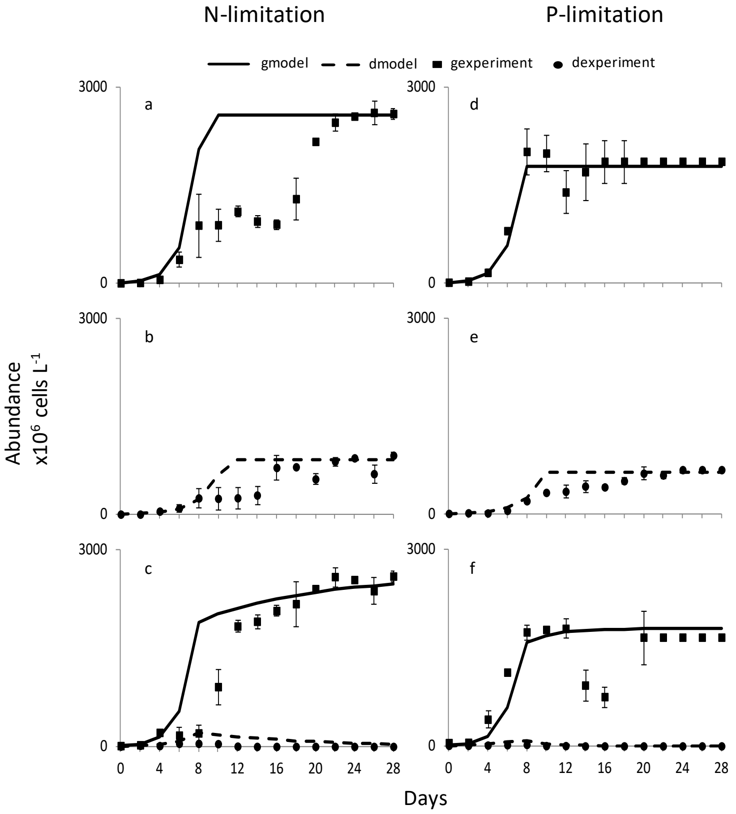
Δεύτερον, οι παράκτιες υδατικές μετακοινότητες χαρακτηρίζονται από υψηλή περιβαλλοντική ετερογένεια, κυρίως εξαιτίας των διαφορετικών εισροών θρεπτικών στο κάθε κατάτμημα (patch). Για παράδειγμα, η ετήσια ή μικρότερης χρονικής κλίμακας διακύμανση των εισροών γλυκού και θαλάσσιου ύδατος στο σύστημα, οδηγεί σε διαφορετικούς και συχνά παλμικούς εμπλουτισμούς θρεπτικών στοιχείων (Buyukates and Roelke, 2005; Spatharis et al., 2007). Οι παλμοί αυτοί μπορούν αν χαρακτηριστούν ως εξωτερικές διαταραχές, προκαλώντας απώλειες πληθυσμού μέσω υδρολογικής μετατόπισης αλλά και αύξηση της ανάπτυξης λόγω του θρεπτικού φορτίου που εισάγουν. Η διαφορετική εισροή θρεπτικών σ'ένα σύστημα μέσω υδρολογικά συνδεδεμένων περιοχών μπορεί να οδηγήσει σε ένα ετερογενές περιβάλλον. Η ετερογένεια εξαρτάται επιπλέον από την απόσταση κάθε περιοχής από τις εισροές γλυκών υδάτων ή θαλάσσιων ζωνών άντλησης. Η διαβάθμιση στη συχνότητα των παλμών των θρεπτικών παρουσιάζει μία σύνθετη σχέση με τον πλούτο ειδών, σύμφωνα με την υπόθεση της ενδιάμεσης διαταραχής (Padisak, 1993; Sommer, 1995). Παρόλο που αυτή η σχέση έχει μελετηθεί αρκετά, άλλα χαρακτηριστικά των βιοκοινοτήτων, όπως η σύνθεση και η δομή τους, δεν έχουν διερευνηθεί. Είναι πιθανό, μια διαβάθμιση στη συχνότητα των παλμικών εισροών να οδηγήσει όχι μόνο σε διαφορετικό πλούτο και ποικιλότητα ειδών, όπως έχει μέχρι τώρα δειχθεί, αλλά και σε διαφορετική σύνθεση, όσον αφορά την ταυτότητα των ειδών και τα λειτουργικά τους χαρακτηριστικά. Η ικανότητα να προσδιορίσουμε αυτά τα χαρακτηριστικά χρησιμοποιώντας προσομοιωμένες συναθροίσεις μπορεί να αποκαλύψει τους πιθανούς μηχανισμούς για τον τρόπο που τα είδη συναθροίζονται.

Η περιβαλλοντική ετερογένεια συναντάται συχνά στα φυσικά οικοσυστήματα και για το λόγω αυτό είναι σημαντικό να λαμβάνεται υπόψη στις πειραματικές και αριθμητικές προσεγγίσεις που διερευνούν τις σχέσεις των διαφορετικών επιπέδων συνδεσιμότητας με τα χαρακτηριστικά των βιοκοινοτήτων. Όμως μια μετακοινότητα δεν επηρεάζεται μόνο από τη συνδεσιμότητα αλλά και από τα χαρακτηριστικά των απομονωμένων τοπικών βιοκοινοτήτων που προήλθαν από τον κατακερματισμό. Αυτή η προσέγγιση σχετίζεται άμεσα με τα παράκτια οικοσυστήματα, καθώς τα επίπεδα του νερού αυξομειώνονται και τα κατατμήματα μπορούν να είναι κατά καιρούς απομονωμένα ή συνδεδεμένα. Μέχρι στιγμής, δεν υπάρχει κάποια μελέτη που να προσπαθεί να συνδέσει τις δύο αυτές διεργασίες του κατακερματισμού και της επανασύνδεσης, με σκοπό να εξηγήσει τους μηχανισμούς που επιδρούν στη δομή και λειτουργία των βιοκοινοτήτων.

ΕΡΕΥΝΗΤΙΚΑ ΕΡΩΤΗΜΑΤΑ

Ο σκοπός της παρούσας διδακτορικής διατριβής είναι η μελέτη της επίδρασης του κατακερματισμού και της συνδεσιμότητας πάνω σε διαφορετικά χαρακτηριστικά των βιοκοινοτήτων (όπως η σύνθεση των ειδών, η ποικιλότητα και η παραγωγικότητα) σε υδατικές μετακοινότητες που χαρακτηρίζονται από περιβαλλοντική ετερογένεια. Για την επίτευξη αυτού του σκοπού έθεσα επιμέρους στόχους και χρησιμοποίησα ένα συνδυασμό πειραματικών και αριθμητικών προσεγγίσεων, χρησιμοποιώντας ως οργανισμό-μοντέλο το φυτοπλαγκτόν.

Στόχος 1: Ανάπτυξη μεθοδολογικής πλατφόρμας για την αριθμητική μοντελοποίηση της διατριβής.
Για την προσέγγιση των βασικών στόχων (2 και 3) και την ανάπτυξη της μεθοδολογίας που σχετίζεται με το τμήμα της αριθμητικής μοντελοποίησης της διατριβής ήταν σημαντικό να εφαρμοστεί μία απλή μελέτη περίπτωσης δύο ειδών μικροφυκών. Χρησιμοποίησα δύο είδη του γένους *Pseudo-nitzschia,* απομονωμένα από το Αιγαίο, για τον υπολογισμό των λειτουργικών χαρακτηριστικών τους (π.χ. τάχος αύξησης, σταθερές ημικορεσμού). Οι παράμετροι αυτές χρησιμοποιήθηκαν για την παραμετροποίηση του μοντέλου ανταγωνισμού για διαθέσιμους πόρους (Tilman, 1982), που εφαρμόστηκε στο αριθμητικό τμήμα της διατριβής. Το αποτέλεσμα του μοντέλου περιέγραψε επιτυχώς τη δυναμική των πληθυσμών των δύο ειδών υπό περιορισμό αζώτου και φωσφόρου (Εικ.1)(επιστημονικό άρθρο 1). Έτσι το ίδιο μοντέλο επεκτάθηκε για 100 είδη που ανταγωνίζονται για φώσφορο και άζωτο, στην προσπάθεια κατανόησης των χαρακτηριστικών των ειδών που επηρεάζουν τον τρόπο που τα είδη ανταγωνίζονται και οδηγούνται στις συγκεκριμένες αυτό-οργανωμένες συναθροίσεις κάτω από συνθήκες κατακερματισμού.



Εικόνα1. Το μοντέλο ανταγωνισμού που παραμετροποιήθηκε με τα λειτουργικά χαρακτηριστικά των ειδών, προέβλεψε με επιτυχία το αποτέλεσμα της ευσταθούς κατάστασης τόσο στις μονοκαλλιέργειες (a-b, d-e) όσο και σε συνθήκες ανταγωνισμού (c, f) υπό περιορισμό θρεπτικών. Οι γραμμές αντιπροσωπεύουν τις προβλέψεις του μοντέλου ενώ τα σημεία τα πειραματικά αποτελέσματα.

Στόχος 2: Αξιολόγηση του ρόλου του κατακερματισμού και της ετερογένειας, λόγω διαφορετικών εισροών θρεπτικών, στη δομή των βιοκοινοτήτων.

Στο τμήμα αυτό της έρευνάς μου, διερεύνησα τον τρόπο που οι απομονωμένες συναθροίσεις αυτό-οργανώνονται κάτω από διαφορετικές συχνότητες παλμών θρεπτικών χρησιμοποιώντας ένα συνδυασμό πειραμάτων και αριθμητικών μοντέλων. Προκειμένου να εξηγήσουν τα παρατηρούμενα πρότυπα, οι περισσότερες θεωρητικές μελέτες έχουν εστιάσει στα χαρακτηριστικά των ειδών που σχετίζονται με τις ικανότητές τους για την πρόσληψη θρεπτικών (π.χ. Huisman and Weissing, 1999; Roelke and Eldridge, 2008). Στην παρούσα διερεύνηση έλαβα υπόψη μου και έναν μεταβαλλόμενο ρυθμό αύξησης των ειδών. Παρόλο που αυτό το χαρακτηριστικό έχει πολλές φορές παραβλεφθεί (σχόλιο Schippers et al., 2001) μπορεί να είναι πολύ σημαντικό για την ανταγωνιστική ικανότητα των ειδών, ιδίως σε περιβάλλοντα που χαρακτηρίζονται από περιοδική εισροή θρεπτικών και υδρολογική απορροή.

Το πειραματικό σκέλος περιελάμβανε μια φυσική συνάθροιση φυτοπλαγκτού που κατακερματίστηκε και αυτό-οργανώθηκε κάτω από διαφορετικές συχνότητες παλμών θρεπτικών με σκοπό τη διαμόρφωση μιας διαβάθμισης περιβαλλοντικών συνθηκών (ετερογένειας). Οι πειραματικές συνθήκες προσομοιώθηκαν με σκοπό τη διερεύνηση πιθανών μηχανισμών που οδηγούν στις παρατηρούμενες συναθροίσεις. Συγκεκριμένα, αυτή η προσέγγιση βοήθησε στην ταυτοποίηση των χαρακτηριστικών των ειδών αλλά και των μεταξύ τους σχέσεων (π.χ. ρυθμός αύξησης και ικανότητα πρόσληψης θρεπτικών) που επιδρούν στη δομή και σύνθεση των συναθροίσεων. Ο στόχος αυτός προσεγγίζεται στο επιστημονικό άρθρο 2.

Στόχος 3: Αξιολόγηση του ρόλου της αυξανόμενης συνδεσιμότητας ανάμεσα σε τοπικά κατατμήματα που χαρακτηρίζονται από περιβαλλοντική ετερογένεια, στη δομή και την παραγωγικότητα της βιοκοινότητας

Σε μία μετακοινότητα που χαρακτηρίζεται από ετερογένεια και από ένα κυμαινόμενο πόρο, η θεωρία προβλέπει μία μη-μονότονη σχέση μεταξύ πλούτου ειδών και παραγωγικότητας με τη συνδεσιμότητα. Η σχέση αυτή παρουσιάζει δε μέγιστο σε ενδιάμεσα επίπεδα συνδεσιμότητας (Loreau et al., 2003). Ο πλούτος ειδών στις τοπικές κοινότητες είναι χαμηλός σε μικρή συνδεσιμότητα εξαιτίας του ανταγωνιστικού αποκλεισμού και υψηλός σε μεγάλη συνδεσιμότητα εξαιτίας της ομογενοποίησης του συστήματος. Η παραγωγικότητα ακολουθεί το ίδιο πρότυπο, καθώς εξαρτάται κυρίως από τον πλούτο ειδών. Εντούτοις, σε μεγάλη συνδεσιμότητα, το είδος που είναι καλύτερα προσαρμοσμένο στις ενδιάμεσες περιβαλλοντικές συνθήκες έχει τη δυνατότητα να ομογενοποιεί το σύστημα προς τις συνθήκες αυτές και τελικά να κυριαρχεί στη μετακοινότητα. Ο μηχανισμός αυτό ονομάζεται ομογενοποίηση προς τις ενδιάμεσες συνθήκες (spatial averaging) και διατηρεί ελαφρώς αυξημένη την παραγωγικότητα σε μεγάλη συνδεσιμότητα, καθώς παρατηρείται μικρότερη χρονική διακύμανση στη βιομάζα των ειδών που βρίσκονται στη μετακοινότητα.

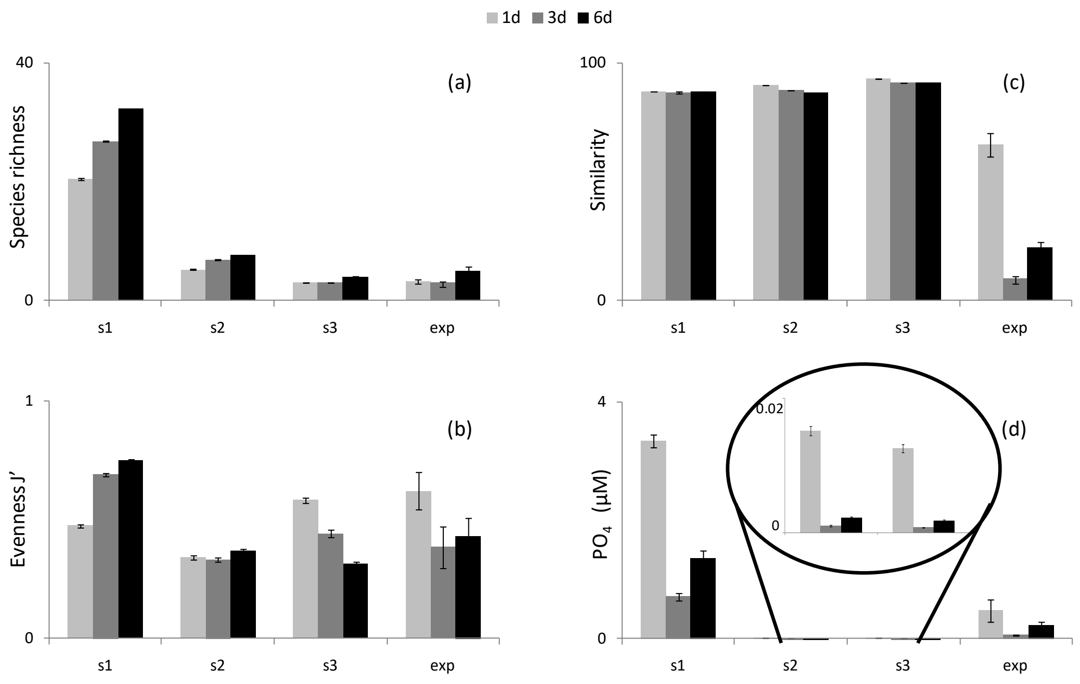
Δεδομένου ότι αυτή η υπόθεση δεν έχει ακόμα εξεταστεί πειραματικά, χρησιμοποίησα συναθροίσεις φυτοπλαγκτού με σκοπό τον προσδιορισμό του ρόλου της ομογενοποίησης προς τις ενδιάμεσες συνθήκες στον πλούτο ειδών και την παραγωγικότητα. Για την αξιολόγηση του μηχανισμού αυτού, χρησιμοποίησα τις κατακερματισμένες συναθροίσεις από τις τρεις διαφορετικές συχνότητες θρεπτικών του προηγούμενου πειράματος (στόχος 2). Αυτές οι συναθροίσεις αντιπροσωπεύουν τα τοπικά κατατμήματα τα οποία επανασυνδέθηκαν σε διαφορετικά επίπεδα συνδεσιμότητας, δημιουργώντας μετακοινότητες. Αυτός ο στόχος προσεγγίζεται στο επιστημονικό άρθρο 3.

Οι φυτοπλαγκτικές συναθροίσεις ως κοινότητες - μοντέλα

Το φυτοπλαγκτόν αποτελεί ιδανικό μοντέλο για τη μελέτη των μηχανισμών που επιδρούν στην ποικιλότητα και την παραγωγικότητα. Καλλιεργείται εύκολα σε εργαστηριακές συνθήκες, παρουσιάζει υψηλό πλούτο ειδών, μικρούς χρόνους γενεών και γρήγορες δια-ειδικές αλληλεπιδράσεις, που επιτρέπουν την παρατήρηση διαδικασιών διαδοχής σε μόλις ένα έτος (Harris, 1986)(η διαδοχή σε άλλες φυτοκοινότητες μπορεί να πάρει πολλά χρόνια, μέχρι και δεκαετίες). Επιπλέον, τα χαρακτηριστικά των ειδών, όπως η ανταγωνιστική τους ικανότητα στην πρόσληψη θρεπτικών, έχουν ποσοτικοποιηθεί πειραματικά, καθιστώντας δυνατή την ευρεία χρήση τους σε μοντέλα ανταγωνισμού για διαθέσιμους πόρους (π.χ. Huisman and Weissing, 1999; Roelke and Eldridge, 2008). Το φυτοπλαγκτόν παίζει επίσης πολύ σημαντικό ρόλο στην οικοσυστημική λειτουργία, καθώς βρίσκεται στη βάση της τροφικής αλυσίδας. Η αυξημένη ποικιλότητά του μπορεί να διατηρήσει υψηλή ποικιλότητα και παραγωγικότητα σε υψηλότερα τροφικά επίπεδα (Striebel et al., 2012) ενώ η αυξημένη παραγωγικότητα μπορεί να ευνοήσει μια ποικιλία οικοσυστημικών αγαθών και υπηρεσιών (Costanza et al., 1997). Επίσης, ευρήματα που βασίζονται στο φυτοπλαγκτόν μπορούν να διευρυνθούν και σε άλλες φυτοκοινότητες όπως αυτές των μακροφυκών και των ανώτερων φυτών, πολλές από τις οποίες βιώνουν εποχική διακύμανση των πόρων, καθώς η δομή των συναθροίσεών τους βασίζεται σε κοινές οικολογικές θεωρίες (π.χ. το μοντέλο Monod).

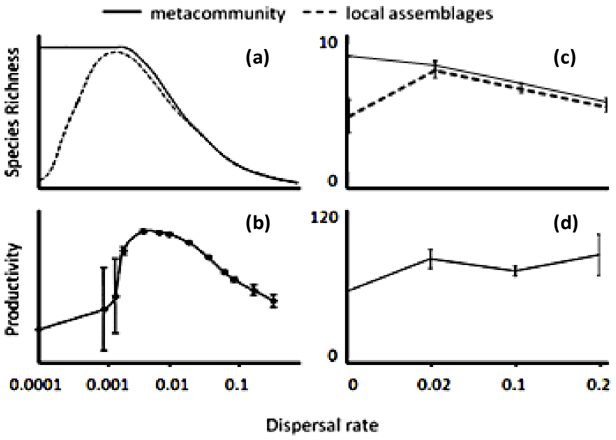
ΚΥΡΙΑ ΕΥΡΗΜΑΤΑ

Αυτή η μελέτη αποκάλυψε ότι ο κατακερματισμός μιας συνάθροισης κάτω από διαφορετικούς παλμούς θρεπτικών (άρθρο 2) μπορεί να παίξει σημαντικό ρόλο στη διαμόρφωση συναθροίσεων διαφορετικής δομής και σύνθεσης. Ωστόσο, η μεταβλητότητα αυτών των χαρακτηριστικών δεν παρατηρείται μόνο ανάμεσα στους διαφορετικούς παλμούς αλλά και ανάμεσα στις επαναλήψεις του ίδιου παλμού. Η μεταβλητότητα μεταξύ των επαναλήψεων ήταν μικρότερη στην υψηλή συχνότητα παλμών θρεπτικών, που παρουσίασε και το μικρότερο πλούτο ειδών, ενώ οι παλμοί χαμηλής συχνότητας εμφάνισαν μεγαλύτερο πλούτο ειδών. Τα αποτελέσματα αυτά αναπαράχθηκαν επιτυχημένα από το μοντέλο, αποκαλύπτοντας ότι το τάχος αύξησης είναι το πιο σημαντικό χαρακτηριστικό κάτω από αυτές τις συνθήκες παλμών, ακολουθούμενο από την ανταγωνιστική ικανότητα για το φώσφορο. Όταν ένα είδος αναπτύσσεται γρήγορα και είναι συγχρόνως καλός ανταγωνιστής για το φώσφορο, τότε έχει τις μεγαλύτερες πιθανότητες να υπερισχύσει στις παραπάνω συναθροίσεις. Σε χαμηλότερες συχνότητες παλμών διαφορετικά χαρακτηριστικά εμφανίζονται, αποκαλύπτοντας υψηλότερη λειτουργική ποικιλότητα μαζί με τον υψηλότερο πλούτο ειδών (Εικ. 2), γεγονός που επιβεβαιώνεται και από τις πειραματικές συναθροίσεις. Ο σημαντικός ρόλος των χαρακτηριστικών των ειδών στη διαμόρφωση της τελικής συνάθροισης κάτω από διαφορετικές συνθήκες παλμού θρεπτικών δείχνει ότι τα παρατηρούμενα πρότυπα των φυτοπλαγκτικών συναθροίσεων δεν ερμηνεύονται από την ουδέτερη θεωρία αλλά από την θεωρία περί οικοθέσης.



Εικόνα2. Πλούτος ειδών (a), ισοκατανομή (b), ομοιότητα Bray-Curtis (c), και συγκέντρωση φωσφορικών στο περιβάλλον (d) για το πείραμα (exp) και τα 3 σενάρια με σχέσεις των χαρακτηριστικών των ειδών - s1 (scenario1), s2 (scenario2), s3 (scenario3), 1d, 3d, 6d οι συχνότητες παλμών κάθε 1,3,6 μέρες.

Όταν οι απομονωμένες συναθροίσεις επανασυνδέθηκαν με αυξανόμενα επίπεδα συνδεσιμότητας (άρθρο 3), ο μεγαλύτερος πλούτος ειδών εμφανίστηκε στο ενδιάμεσο επίπεδο συνδεσιμότητας, σε συμφωνία με τη θεωρία (Εικ.3). Ωστόσο, σε αντίθεση με τη θεωρία, ο μηχανισμός της ομογενοποίησης προς τις ενδιάμεσες συνθήκες (spatial averaging) αποδείχθηκε σημαντικός, ακόμα και σε χαμηλά επίπεδα συνδεσιμότητας, καταφέρνοντας να διατηρήσει αυξημένη παραγωγικότητα σε υψηλή συνδεσιμότητα, παρά τη μείωση του πλούτου των ειδών. Αυτό συνέβη γιατί το κυρίαρχο είδος στη μετακοινότητα ήταν πολύ παραγωγικό, και μπορούσε να αναπτυχθεί σε ετερογενή περιβάλλοντα, ένα χαρακτηριστικό των γενικευμένων ειδών (generalists) (Gravel et al., 2011). Παρόλο που αυτό το είδος δεν μπορούσε να ανταγωνιστεί και να κυριαρχήσει στις ακραίες συνθήκες όταν το σύστημα ήταν κατακερματισμένο, η σύνδεση των κατατμημάτων και η μεταφορά κυττάρων μεταξύ τους ευνόησε τη σταδιακή κυριαρχία του. Τα γενικευμένα είδη ευνοούνται σε υδατικές μετακοινότητες όταν οι συνθήκες μεταβάλλονται στο χώρο και στο χρόνο, καθώς μπορούν να ανθίστανται μεγάλο εύρος περιβαλλοντικών συνθηκών (Costanza et al., 1993).



Εικόνα3. Θεωρητικές προβλέψεις για τον πλούτο ειδών (a) και την παραγωγικότητα (b) κατά μήκος μιας διαβάθμισης συνδεσιμότητας σε μία θεωρητική μετακοινότητα (Mouquet and Loreau, 2003, Loreau et al., 2003) και πειραματικές φυτοπλαγκτικές μετακοινότητες (παρούσα μελέτη)(c, d).

Τα παρατηρούμενα πρότυπα στη δομή και παραγωγικότητα της μετακοινότητας είναι άμεσα συνδεδεμένα με τη διαδικασία του κατακερματισμού που προηγήθηκε. Ο κατακερματισμός κάτω από διαφορετικές περιβαλλοντικές συνθήκες οδηγεί σε διαφορετική σύνθεση ειδών με συγκεκριμένα εγγενή χαρακτηριστικά, γεγονός που επιτρέπει την μελέτη της ομογενοποίησης προς τις ενδιάμεσες συνθήκες. Οι συγκεκριμένες περιβαλλοντικές συνθήκες που δημιουργήθηκαν στην ενδιάμεση συχνότητα παλμού, ευνόησαν την κυριαρχία του είδους που ευθύνεται, κατά τη συνδεσιμότητα, για την ομογενοποίηση του συστήματος προς αυτές. Συνοψίζοντας, φαίνεται ότι η βέλτιστη λειτουργία στις υδατικές μετακοινότητες (πλούτος ειδών, λειτουργική ποικιλότητα, παραγωγικότητα) επιτελείται όταν οι παλμοί θρεπτικών συμβαίνουν ανά διαστήματα μεγαλύτερα του χρόνου γενεάς των ειδών και όταν η συνδεσιμότητα ανάμεσα στις τοπικές βιοκοινότητες είναι σε ενδιάμεσα επίπεδα.

Τα παρόντα πειραματικά αποτελέσματα μπορεί να έχουν ευρύτερες επιπτώσεις, καθώς φαίνεται ότι έρχονται σε αντίθεση με την ευρέως αποδεχτή άποψη ότι αυξημένος πλούτος ειδών είναι απαραίτητος για τη διατήρηση των οικοσυστημικών λειτουργιών (Isbell et al., 2011). Συγκεκριμένα για τα παράκτια οικοσυστήματα, αυτά τα αρχικά αποτελέσματα υπονοούν ότι υψηλή πρωτογενής παραγωγικότητα μπορεί να επιτευχθεί σε όλα τα επίπεδα συνδεσιμότητας, αλλά ο κατακερματισμός τους μπορεί να επιφέρει αρνητικά αποτελέσματα (π.χ. έλλειψη συνδεσιμότητας εξαιτίας των μειωμένων επιπέδων των υδάτων ή ανθρωπογενών κατασκευών). Επιπλέον, ευρήματα από το μοντέλο δείχνουν τη σπουδαιότητα των εγγενών χαρακτηριστικών των ειδών τόσο στη διαμόρφωση των μετακοινοτήτων όσο και στην υψηλή παραγωγικότητα, ανεξάρτητα του πλούτου ειδών.

Εξαιτίας των σημαντικών τους επιδράσεων στην κατανόηση της οικοσυστημικής λειτουργίας αλλά και στις κοινωνικοοικονομικές πτυχές που σχετίζονται με την διαχείριση των παράκτιων ζωνών, είναι απαραίτητη η γενίκευση αυτών των αποτελεσμάτων πέρα από συγκεκριμένες περιβαλλοντικές συνθήκες και είδη. Πρέπει λοιπόν να διερευνήσουμε πόσο μακριά μπορούν να φτάσουν αυτά τα αποτελέσματα, πέρα από τη θεωρία και τα αρχικά πειραματικά αποτελέσματα. Επίσης, είναι σημαντικό να διερευνηθεί αν η παρατηρούμενη υψηλή παραγωγικότητα σχετίζεται πάντα με την κυριαρχία ενός είδους, κάτι που θα οδηγούσε στην υποβάθμιση των οικοσυστημάτων. Αυτό θα μπορούσε να μελετηθεί χρησιμοποιώντας μετακοινότητες που χαρακτηρίζονται από επιπλέον ρεαλισμό στις περιβαλλοντικές συνθήκες, όπως η σύγχρονη ή ασύγχρονη διακύμανση πόρων και η διαφορετική συνδεσιμότητα ανάμεσα στα κατατμήματα.

Η παρούσα διδακτορική διατριβή ασχολήθηκε με ερωτήματα που έχουν σημαντικές επιδράσεις στη διαχείριση των παράκτιων ενδιαιτημάτων, ιδιαίτερα υπό το πρίσμα της αναμενόμενης κλιματικής αλλαγής. Με σωστή διαχείριση, η πρωτογενής παραγωγικότητα αυτών των οικοσυστημάτων μπορεί να διατηρήσει υψηλή ζωική παραγωγή και πλήθος οικοσυστημικών αγαθών και υπηρεσιών. Αντίθετα, αυξανόμενες ανθρωπογενείς διαταραχές που ξεπερνούν την ανοχή των οικοσυστημάτων αυτών, π.χ. εισροές λιπασμάτων και κατακερματισμός εξαιτίας ανθρωπογενών κατασκευών, μπορεί να οδηγήσουν σε φαινόμενα ευτροφισμού, επιβλαβών ανθίσεων φυκών και σημαντική υποβάθμιση των ενδιαιτημάτων που χρησιμοποιούνται από ψάρια εμπορικού ενδιαφέροντος για την αναπαραγωγή τους, καθώς και από παρυδάτια πτηνά και θαλάσσια θηλαστικά. Αποτελέσματα αυτής της έρευνας μπορούν επιπλέον να βοηθήσουν στην εφαρμογή οδηγιών της Ευρωπαϊκής Ένωσης όπως η Οδηγία Πλαίσιο για τα Ύδατα (2000/EU) που απευθύνεται συγκεκριμένα στην οικολογική ποιότητα και διαχείριση των παράκτιων και μεταβατικών υδάτων.

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EXTENDED ABSTRACT

RESEARCH BACKGROUND

The impact of fragmentation and connectivity on ecosystem structure and function is central in current ecological research (Sawyer et al., 2011). Viewing landscapes and seascapes as networks of local communities connected by dispersal has given rise to the concept of metacommunity. This framework, can offer important insights on ecological processes compared to more conventional approaches based on local communities alone. Theoretical and experimental studies focused on terrestrial ecosystems have been widely applied over the last decade, in the framework of metacommunity theory. However, aquatic ecosystems remain considerably understudied within this framework (Matthiessen and Hillebrand, 2006).

Metacommunity theory can be directly applicable in aquatic systems, particularly in coastal ecosystems such as interconnected lagoons, salt marshes, saline ponds and rock pools. These systems account for a disproportionally large amount of global primary productivity (Cloern et al., 2013). The latter sustains greater secondary productivity and a wide spectrum of important ecosystem services, including cycling of nutrients, carbon, and trace metals, secondary production by herbivores, fish catch, production of cultured shellfish, buffering of contaminants, and recreation (Costanza et al., 1997). These systems are particularly vulnerable to sea level rises due to climate change (Cyrus et al., 2010) and increasing seascape fragmentation due to man-made structures (Kang and King, 2013). Assessing the role of hydraulic connectivity and fragmentation on their structure (i.e. species composition and diversity) and functioning (i.e. productivity) is therefore essential for habitat restoration efforts (Januchowski-Hartley et al., 2013).

Coastal aquatic metacommunities have two unique characteristics. First, hydraulic connectivity between patches is a prerequisite for nutrient and species replenishment. Local and regional species richness and productivity can vary significantly along different levels of connectivity. While the relationship of species richness with dispersal has been extensively addressed in both theoretical and empirical studies (e.g. Mouquet and Loreau, 2003; Cadotte, 2006), the effect of dispersal on productivity – an important ecosystem function - has been largely overlooked. Theoretical evidence on this relationship, known as the spatial insurance hypothesis (Loreau et al., 2003), suggests that productivity is driven by two mechanisms, namely species richness and spatial averaging (i.e. ecosystem homogenization towards intermediate-type environmental conditions). Spatial averaging is thought to be more important at high connectivity levels, buffering to some extent the decrease of productivity due to species loss. Several empirical studies have now validated the importance of species richness in driving metacommunity productivity (e.g. Matthiessen and Hillebrand, 2006); however, the role of spatial averaging in affecting productivity has not yet been explored.

Second, coastal metacommunities can be characterized by high environmental heterogeneity mainly due to differential resource regimes that each patch receives. For instance, variation within annual or shorter cycles in freshwater inflows and ocean mixing, leads to variable and often pulsed nutrient supply regimes (Buyukates and Roelke, 2005; Spatharis et al., 2007). These pulsed inflows can be regarded as external disturbances, causing population losses through hydraulic displacement, and growth stimulation associated with nutrient loading. A variable resource supply into a system of hydraulically connected sites may lead to a heterogeneous environment depending on the proximity of each local patch to freshwater inputs or upwelling zones. A gradient of different pulsing frequencies may present a complex relationship with species richness following the intermediate disturbance hypothesis (Padisak, 1993; Sommer, 1995). Although this relationship has been adequately addressed, other community attributes such as composition and structure have not been investigated. It may be hypothesized that a gradient of differential inflow pulses may not only lead to different richness and diversity as previously shown, but also to distinct compositions regarding the identity of species and their functional traits. Being able to identify these traits, using simulated assemblages, can elucidate the underlying assembly rules.

Environmental heterogeneity is often encountered across natural landscapes and seascapes, and should be thus taken into account in both experimental and modeling approaches trying to establish the effects of different connectivity levels on community attributes. However, a metacommunity is not solely related to connectivity but also to the previously isolated local communities created by fragmentation. This is particularly true in coastal ecosystems, where water levels vary and water patches can become periodically isolated and reconnected. So far, there is no study that relates the two processes of fragmentation and reconnection seeking to explain the underlying mechanisms for the observed community structure and function.

RESEARCH QUESTIONS

The aim of the present study was to assess the effect of fragmentation and connectivity on different community attributes (i.e. species composition, diversity and productivity) in aquatic metacommunities characterized by environmental heterogeneity. Towards this aim I addressed a number of objectives employing a combination of experimental and modeling approaches using phytoplankton as model organisms.

Objective 1: Develop the methodological platform for the modeling part of the thesis.

In order to address the main objectives 2 and 3, it was essential to apply a simplistic case study including two microalgal species in order to develop the methodological procedure related to the numerical part of the thesis. I used two *Pseudo-nitzschia* species isolated from the Aegean Sea to calculate their life-history traits (e.g. growth rate, half saturation constant). These traits were used to parameterize the resource competition model (Tilman, 1982) used in the modeling part of the work. The model outcome successfully described the population dynamics of the two competing species under phosphorus and nitrogen limitation (Fig.1) (paper 1). Therefore the same model was extended to 100 species competing for phosphorus and nitrogen in an effort to understand the life history traits that affect the way species compete and lead to particular self-organized assemblages under fragmented conditions.

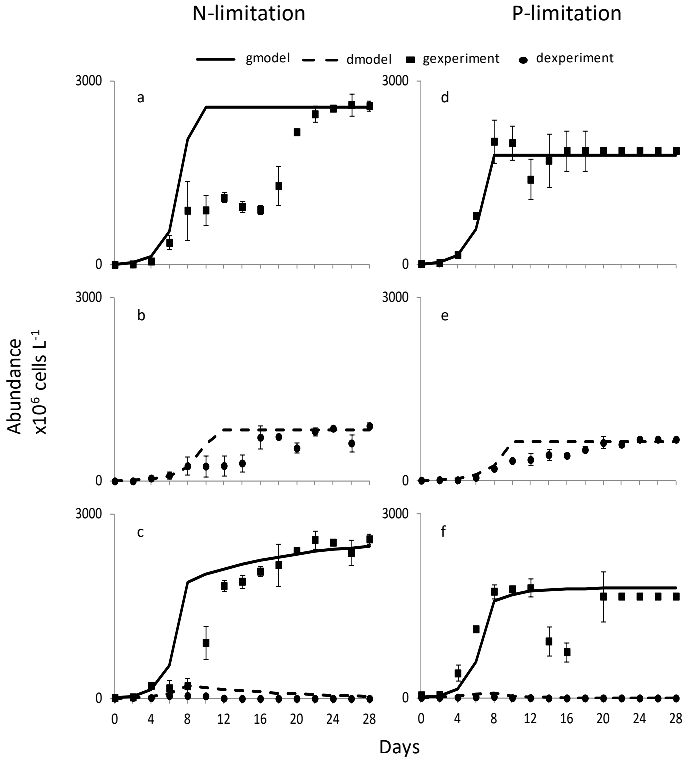

Figure1. The model parameterized with species life history traits, successfully predicted the outcome of steady state both in monocultures (a-b, d-e) and in competition (c, f) under nutrient limitation. Lines are the model predictions and dots the experimental results.

Objective 2: Assess the role of fragmentation and resource heterogeneity on community structure.

In this part of my research I investigated how assemblages self-organize in isolation under different nutrient pulsing regimes employing an experimental and a modeling approach. To explain the observed patterns, most theoretical studies have focused on traits related to the ability of species to acquire nutrients (e.g. Huisman and Weissing, 1999; Roelke and Eldridge, 2008). However, in this investigation, I additionally considered species' growth. Although this latter trait has been often overlooked (see comment in Schippers et al., 2001) it can be also crucial for the competitive ability of a species particularly in environments characterized by periodic nutrient inflows and hydraulic flushing.

The experimental phase involved a natural phytoplankton assemblage that was fragmented and self-organized under different nutrient pulsing frequencies in order to form a gradient of environmental conditions (i.e. heterogeneity). Numerical modeling was additionally employed in order to simulate the experimental conditions and provide insights on the potential mechanisms leading to observed self-organized assemblages. In particular, this approach helped to reveal the species' life history traits and tradeoffs (e.g. growth rate and ability to acquire nutrients) that drive assemblage structure and composition. This objective is addressed in paper 2.

Objective 3: Assess the effect of increasing connectivity between local patches characterized by environmental heterogeneity, on community structure and productivity.

When considering a heterogeneous metacommunity and a fluctuating resource, theory predicts a non-monotonic relationship between both species richness and productivity with connectivity, with a maximum at intermediate dispersal rates (Loreau et al., 2003). Local species richness is low at low dispersal due to competitive exclusion and at high dispersal due to ecosystem homogenization. Productivity is predicted to follow the same trend, driven mainly by species richness. However, at high connectivity, a species best adapted to the intermediate-type environmental conditions can average out heterogeneity and dominate across the metacommunity, an effect called "spatial averaging". This mechanism can retain slightly higher productivity at high connectivity, due to a smaller temporal variability of the biomasses of the species present.

As this hypothesis has not been tested experimentally so far, I employed phytoplankton assemblages to provide empirical evidence on the role of spatial averaging on richness and productivity. To test for this mechanism, I used the steady-state fragmented assemblages characterizing the three different nutrient pulses from the previous experiment (see objective 2). These assemblages now represented my local patches which were reconnected at different connectivity levels, forming metacommunities. This objective is addressed in paper 3.

Phytoplankton as model organisms

Phytoplankton assemblages are ideal as model organisms for the study of mechanisms that drive assemblage diversity and productivity. They grow easily in laboratory conditions, they form species-rich assemblages and they present short generation times and rapid interspecific interactions, that enable observation of successional processes over periods as brief as a single year (Harris, 1986) (succession in most other plant communities take several years or decades). Furthermore, traits of individual species, such as their competitiveness in acquiring resources, have been quantified experimentally, enabling their extensive use in consumer-resource models (e.g. Huisman and Weissing, 1999; Roelke and Eldridge, 2008). Phytoplankton also plays a pivotal role in ecosystem function being at the base of the food chain. Increased phytoplankton diversity can sustain greater diversity and productivity at higher trophic levels (Striebel et al., 2012) whereas increased productivity can benefit a multitude of ecosystem goods and services (Costanza et al., 1997). Moreover, findings based on phytoplankton systems can be extended beyond them, since their assemblage structure follows common ecological theory and the Monod model that seeks to explain their life history traits relationships is applicable to other assemblages including macroalgae and plants, many of which also experience seasonal resource variability.

MAIN FINDINGS

This study revealed that fragmentation of an assemblage under different nutrient pulsing regimes (paper 2) can be of paramount importance in forming assemblages of different structure and composition. However, variability in these assemblage attributes was not only observed among the different pulsing regimes but also among the replicates of the same regime. The among-replicate variability was less pronounced in the high-frequency nutrient pulses, which also presented low species richness whereas low-frequency pulses yielded higher species richness. These results were successfully reproduced by numerical modeling, revealing that growth rate is the most important life history trait under the applied pulsed regimes, followed by the ability to compete for phosphorus. The combination of these life history traits (grow fast and be competitive for phosphorus) in a single species is what primarily shapes the above assemblages. At lower frequencies species with different traits start to appear, revealing a higher functional diversity along with higher species richness, also observed in experimental assemblages (Fig.2).The prominent role of species traits in shaping the final assemblages under different pulsing regimes suggests that niche-based rather than neutral processes shape phytoplankton assemblages.

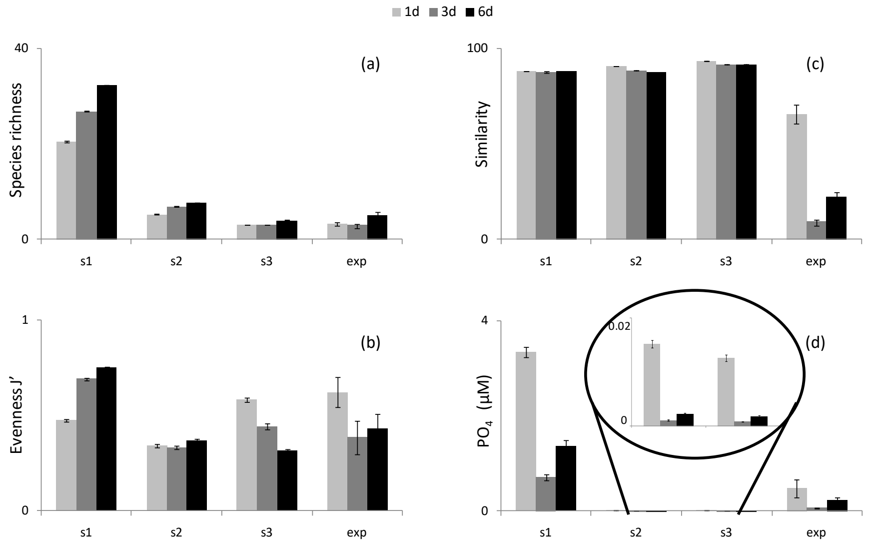

Figure2.Species richness (a), evenness (b), Bray-Curtis similarity (c), and ambient phosphate concentration (μM) (d) for the experiment (exp) and the tree scenarios, when species initial relative abundances vary - s1 (scenario1), s2 (scenario2), s3 (scenario3), 1d, 3d, 6d the pulsing frequencies of 1,3,6 days.

When isolated assemblages were connected at increasing levels of dispersal (paper 3), species richness was higher at the intermediate connectivity levels, in agreement to theory (Fig.3). However, in contrast to theory, the mechanism of spatial averaging was prominent even when dispersal rates were low, and was also able to maintain increased productivity at high connectivity despite the decreased species richness. The latter occurred because the dominant species across the metacommunity was also highly productive being able to exploit heterogeneous environments, a characteristic of generalist species (Gravel et al., 2011). Although this productive generalist was unable to compete in isolation in the extreme environmental conditions, with the onset of hydraulic connectivity among patches, recurrent transfer of cells and nutrients enables it to gradually dominate regionally. Generalist species are favored in aquatic metacommunities where conditions vary in time and space, since they can tolerate a wide range of environmental conditions (Costanza et al., 1993).

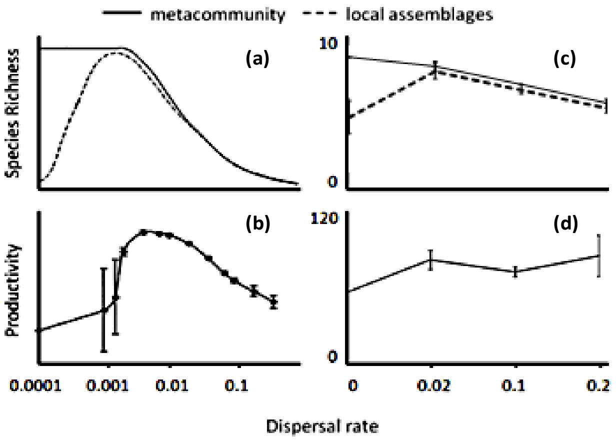

Figure3. Theoretical species richness (a) and productivity (b) along a gradient of dispersal in a theoretical metacommunity (Mouquet and Loreau, 2003, Loreau et al., 2003) and experimental laboratory phytoplankton metacommunities (c, d).

The observed patterns of metacommunity structure and productivity are directly linked to the preceded fragmentation process. Fragmentation under different environmental conditions leads to the persistence in each pulsing regime of species with particular life history traits, enabling to test for spatial averaging. Environmental conditions at the intermediate pulsing frequency led to the dominance of a species, which, during connectivity, was responsible for the system homogenization towards them. It seems that optimal ecosystem function (maximal richness, functional diversity, and productivity) in heterogeneous aquatic metacommunities can be achieved when the interval between resource pulses exceeds the generation times of constituent species, and when patches are connected at intermediate connectivity levels.

The present experimental findings might have important broader implications as they seem to contradict the generally established principle that species richness is essential to maintain ecosystem function (Isbell et al., 2011). Considering coastal aquatic communities in particular, these preliminary results would suggest that high primary productivity can be sustained at any connectivity level, however negative effects are expected when the system becomes fragmented (e.g. no connectivity due to reduced water levels, or man-made barriers). Moreover, findings from the modeling part of this work, underline the importance of species life history traits in forming metacommunities and sustaining high productivity, independently of species richness.

Due to their crucial implications for both our understanding of ecosystem functioning and socioeconomic aspects related to coastal zone management, it is essential to generalize these findings beyond particular environmental conditions and beyond particular species. It is thus essential to explore how far-reaching these results might be, beyond the basic assumptions of theory and these preliminary experimental findings. Furthermore, it is important to establish if this high productivity is always associated with dominance of one species, which could lead to ecosystem degradation. This could be addressed with the use of metacommunities characterized by environmentally plausible conditions such as multiple fluctuating resources and differential between-patch connectivity.

The present thesis addressed questions that are certain to have important implications for the management of coastal habitats, especially under the anticipated effects of climate change. If managed appropriately, primary productivity in these systems may sustain greater animal productivity and a multitude of ecosystem goods and services to our society, including fisheries, aquaculture, and absorption of contaminants. In contrast, increasing anthropogenic disturbances beyond the tolerance of these systems, e.g. fertilizer inflows and landscape fragmentation due to man-made structures may lead to eutrophication, harmful algal blooms and severe degradation of the habitats used as breeding grounds by commercial fish species and iconic seabirds or marine mammals. Results from this research can inform the implementation of EU policies such as the Water Framework and Marine Strategy Directives, which implicitly address the ecological quality and management of coastal and transitional waters.

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COMPETITION BETWEEN TWO GLOBALLY IMPORTANT *PSEUDO-NITZSCHIA* SPECIES UNDER N- AND P- LIMITATION (PAPER 1)

ABSTRACT

To promote the understanding of mechanisms leading to coexistence and exclusion, especially as it relates to incidence of some harmful algal blooms (HABs), it is essential to establish information related to the nutritional needs of species for macronutrients, mainly nitrogen (N) and phosphorus (P). In this research, we focused on *Pseudo-nitzschia* species, namely *P. delicatissima* and *P. galaxiae*, two frequently coexisting diatom species capable of producing the toxin domoic acid and forming blooms. We employed monoculture experiments to determine various growth kinetic parameters important for understanding resource use (i.e. maximum specific growth rate (μ_{max}), half-saturation coefficients of growth (K_S) and cell quotas (c)), mixed-culture experiments to determine outcomes of resource competition, and numerical modeling to explore the role of exploitative resource competition relative to unknown factors. Experimental results showed that both species had a high requirement for N and low requirement for P, which is consistent with field observations of *Pseudo-nitzschia* blooms in N-rich conditions. Mixed culture experiments showed that *P. galaxiae* outcompeted *P. delicatissima* quicker than predicted by our numerical model that considered only exploitive resource competition. This suggests that other interactions, i.e., some type of interference, between *P. galaxiae* and *P. delicatissima* are important. The consistent competitive exclusion of *P. delicatissima* by *P. galaxiae* in our laboratory experiments also underscores the importance of other factors in the natural environment, not related to resource exploitation, which might enable coexistence of these species. These processes likely include differential sinking rates and grazer feeding preferences, both greater for *P. galaxiae* compared to *P. delicatissima.*

Keywords: growth kinetic parameters, Monod model, population dynamics, *P. delicatissima, P. galaxiae,* resource competition

INTRODUCTION

Coastal ecosystems around the globe are increasingly subject to eutrophication phenomena due to terrestrial nutrient loadings (Anderson et al., 2002; Yeager et al., 2005; Spatharis et al., 2007; Spatharis et al., 2009). These loadings, often rich in nitrogen (N) and phosphorus (P), can significantly modify water chemistry, community composition and diversity with subsequent alterations in the food web (Spatharis et al., 2007). Harmful algal blooms (HABs) are a result of such phenomena (Anderson et al., 2002; 2008; Heisler et al., 2008) having important consequences on ecosystem stability but also on local economies and public health (Scholin et al., 2000; Hallegraeff, 2010; Klein et al., 2010). To promote the understanding of mechanisms leading to HABs in environments affected by resource fluctuations, it is essential to establish information related to their nutritional requirements and competitive abilities on multiple resources.

The genus *Pseudo-nitzschia* is cosmopolitan and most species can cause Amnesic Shellfish Poisoning (ASP) symptoms, due to the production of domoic acid which has an impact on different levels of the food web and human health (cf., review by Bates 1998). The species *Pseudo-nitzschia delicatissima* in particular, has received increased attention due to its widespread occurrence and HABs around the globe including North America (Kaczmarska et al., 2007; Quijano-Schecggia et al., 2008), East Atlantic Coasts (Fehling et al., 2005; Klein et al., 2010), the Mediterranean Sea (Carropo et al., 2005; Cerino et al., 2005), and New Zealand (Trainer et al., 2012). *P. galaxiae*, is also receiving increased attention due to its widespread occurrence and late discovery on its ability to produce domoic acid (Cerino et al., 2005). This species has been frequently reported in multi-species blooms with *P. delicatissima*, in North America (Lundholm et al., 2006), Australia and New Zealand (Lundholm and Moestrup, 2002), and the Mediterranean Sea (Cerino et al., 2005; Moschandreou and Nikolaidis, 2010; Zapata et al., 2011; Trainer et al., 2012).

A plethora of studies suggest that *Pseudo-nitzschia* blooms are associated with N-rich freshwater inflows related to anthopogenic activities in the watershed (Turner and Rabalais, 1991; Dortch et al., 1997; Trainer et al., 2000; Caroppo et al., 2005; Loureiro et al., 2005; Spatharis et al., 2007). Indeed, the significance of N in *Pseudo-nitzschia* nutrition cannot be overstated as it is required for its growth and domoic acid production (Bates, 1998; Cochlan et al., 2008). Despite the importance of quantifying the requirements of HAB species for macronutrients to elucidate their occurrence, dynamics, bloom formation and/or toxin production, studies on the growth kinetics of *Pseudo-nitzschia* spp. are still scarce (Cochlan et al., 2008; Loureiro et al., 2009; Thessen et al., 2009). The quantification of life-history traits of naturally coexisting *Pseudo-nitzschia* spp. would seem paramount considering that HABs of this genus often comprise many abundant populations (Kaczmarska et al., 2007; Thessen and Stoecker, 2008; Leblad et al., 2013), possibly suggesting similar growth requirements of the species involved.

Coexistence of multiple species in the field may be attributed to different mechanisms. Resource-ratio theory (Tilman, 1982) is a useful conceptual and computational framework illustrating one such mechanism, exploitative competition for resources. This framework enables comparison of species' competitive abilities for multiple resources that are defined by common life history traits. This in turn enables prediction of competitive outcomes under various nutrient loading conditions (Tilman and Kilham, 1976; Tilman et al., 1982; Huisman and Weissing, 2001; Huisman, 2002). This theory postulates that coexistence is possible at resource supply ratios situated in an area of the resource trade-off space somewhere between the resource consumption vectors of the competing species. When resource supply ratios are closer to the resource consumption vector for one species, competitive exclusion results. Resource-ratio theory can also be used to explain the effect of variable environmental conditions in regards to the resource ratio in the supply. Here, environmental fluctuations enable coexistence of a number of species much greater than the number of limiting resources (see Tilman, 1977; 1981; Sommer, 1984; 1985; Grover, 1989a).

There are many other biodiversity sustaining mechanisms acting in plankton environments that Resource-ratio theory does not describe. For example, vertical mixing and pulsed inflows differentially affect the different groups of organisms so when these vary in time, coexistence is promoted (Richerson et al., 1970; Roelke et al., 1997; 1999). Similarly, incomplete horizontal mixing leads to niche differentiation and dispersal of the species which in turn retains a high number of species due to rescue effects, thereby promoting coexistence (Richerson et al., 1970; Bracco et al., 2000). Selective grazing or higher vulnerability to pathogens for species that are superior competitors for resources enable persistence of competitively inferior species, again promoting coexistence (e.g.Sterner, 1989; Brussaard, 2004; Roelke et al., 2004). Finally, differential sinking rates that lead to elevated losses of the best competitor is another mechanism promoting coexistence (Smayda, 1970; Grover, 1989b; Litchman and Klausmeier, 2008).

An approach to evaluating the relative importance's of these various biodiversity-sustaining mechanisms is to compare the predictions of conceptual and computational models, such as Resource-ratio theory, to empirical observations. In this case, the importance of exploitative competition, as predicted by Resource-ratio theory, can be compared to the net effect of other mechanisms. To accomplish this, however, knowledge of common life-history traits for the species being compared must be known. To apply Resource-ratio theory, knowledge of the maximum specific growth rate (μ_{max}) and the half-saturation coefficient (K_S) must be known. While these life-history traits have been measured for many freshwater species (Tilman and Kihlam, 1976; Tilman, 1981; Tilman et al., 1982) they are less frequently measured for marine species (Sommer, 1986).

The present study provides the first estimation of μ_{max} and K_S for inorganic N and P for two globally important HAB species, *P. delicatissima* (Cleve) Heiden and *P. galaxiae* Lundholm and Moestrup, that have been isolated from a coastal bay where they naturally coexist. We focus on these two important nutrients for algal growth as they are associated with freshwater inflows and HAB phenomena in coastal environments. We employed experiments using batch and continuous cultures to determine μ_{max} and K_S for N and P. These life-history traits were then used to parameterize a numerical model simulating the outcome of resource competition between these two species. Through comparison of model simulations with empirical observations from competition experiments, we evaluate the likely importance of exploitative resource completion relative to other factors that might influence coexistence between these species.

METHODS

Pseudo-nitzschia species

Experiments were carried out with two *Pseudo-nitzschia* species, namely *P. delicatissima* and *P. galaxiae*. Both species were isolated from Kavouri Attiki, Greece where they coexisted at abundances of 3.0×10^4 cells/L for *P. delicatissima* and 1.1×10^4cells/L for *P. galaxiae*. Taxonomic identification of the species was carried out in the Laboratory of Ecology and Taxonomy, University of Athens using Transmission Electron Microscopy (TEM). *P. delicatissima* cells were narrow and linear, 46.8 – 87.1 μm long (mean ± S.D.: 58.2 ± 10.7, *n*= 31), and 1.4 – 3.1 μm wide (mean ± S.D.: 2.04 ± 0.45, n= 20) forming stepped chains. *P. galaxiae* was 23.7-32.3 μm long (mean ± S.D.: 27.9 ± 2.03, *n*= 31), and 2.5-6.3 μm wide (mean ± S.D.: 3.6 ± 0.9, n= 31) lanceolate to needle-shaped, with a swelling in their central part, and two chloroplasts in the central area. Cell biovolume, cell surface area, and surface area-to-volume ratio were estimated using cellular dimensions corresponding to representative geometrical shapes (Hillebrand et al., 1999).

Estimation of growth kinetic parameters

Both batch and continuous cultures (chemostats) were employed for the estimation of growth kinetic parameters. The maximum growth rate ($\mu_{i\,max}$) of the two species was estimated using batch cultures with no nutrient limitation, replicated three times. In the present study, $\mu_{i\,max}$ was evaluated from the slope of a plot of ln (N) versus time (t) in the exponential growth period, based on the equation:

$$N(t) = N_0 e^{(\mu i_{max} t)}$$

Where N_o is the number of cells at the beginning of the exponential growth phase and $N(t)$ the number of cells at time t (Hill and Robinson, 1974).

Parameter R^* (the resource availability at steady-state) and cell quota, c, for N and P were measured in flow-through vessels (chemostats). These consisted of vertical glass tubes (volume of 360ml) containing the culture positioned within an outer glass tube where water was circulated for temperature control. The volume of the inner tube was held constant by a continuous inflow of nutrients through a peristaltic pump and a continuous outflow of nutrients and cells through an overflow system. The flushing rate was constant (D=0.4 d^{-1}) throughout the experiment. Stirring and aeration was conducted by an air pump that sent a big bubble in the inner tube once every minute to avoid cell sedimentation and clump formation. Experiments were performed in a climate-controlled chamber, where temperature was held constant at 20°C and photoperiod at a 12-h light:dark cycle. Cool white fluorescent bulbs were used as a light source and irradiance was 65 μmol m^{-2} s^{-1} according to Moschandreou and Nikolaidis (2010).

Experimentations for each species consisted of two treatments with two replicates each. The first treatment involved nutrients supplied that were N-deficient, eventually leading to N-limitation, and the second treatment involved nutrients supplied that were P-deficient, eventually leading to P-limitation. The total number of continuous cultures was thus 8 (2 species x 2 treatments x 2 replicates). The condition for N-deficiency was achieved by a five-fold reduction in nitrate concentration and for P-deficiency by a five-fold reduction in phosphate concentration over the initial f/2 medium concentration (Guillard and Ryther, 1962; Guillard, 1975).

Daily, 6 mL of each culture were collected and *in-vivo* chlorophyll-a (Chl a) fluorescence was measured using a Turner fluorometer. Each sample was fixed with Lugol's iodine solution and sonication was used to break the clumps apart. Cell counting was performed using a Palmer-Maloney nanoplankton chamber (0.1mL^3) and a Motic AE31 inverted microscope at 400x. When biomass was invariable over a period of 5 days, cultures were considered at steady-state. Then, a 50 mL sample of each culture was filtered and filters were kept in the freezer for measurement of chlorophyll-a, and intracellular TP and TN (Bachmann and Canfield Jr, 1996), which when divided by the cell density at steady state yields the cell quota. Filtered water was analyzed for ambient nitrate and phosphate concentrations according to Parsons et al. (1984). Two samplings were conducted during the steady-state period (thus 2 replicates x 2 samplings, n=4).

The concentration of the ambient limiting nutrient of a species *i* measured at steady-state corresponds to the R_i*. The half saturation constant (K_{si}), which summarizes, in part, the competitive ability of the species for a resource when it is at low availability, was calculated using the $R*$ equation (Tilman, 1982) solved for K_S:

$$K_{S_i} = R_i^* \left(\frac{\mu_{max_i} - D}{D} \right)$$

Where μ_{max_i} is the maximum growth rate of the species *i* and *D* is the dilution rate (0.4 d^{-1}).

Competition experiments

To test the outcome of competition for N and P between these two species and compare it to model predictions, the two *Pseudo-nitzschia* species were put together in a chemostat culture where supplied nutrients were N-deficient (2 replicates) and where supplied nutrients were P-deficient (2 replicates). Mixing of the two species populations was conducted using equal biovolumes, a common approach to phytoplankton mix cultures (Schmidtke et al., 2010; Schabhüttl et al., 2013).

Numerical model

For modeling population dynamics of two species competing for one resource, we employed a widely used mathematical model (Tilman 1982) that describes changes in phytoplankton cell density (N_i) and nutrient concentration (R_j) following the equations:

$$\frac{dN_i}{dt} = N_i \left(\frac{\mu_{max_i} R_j}{K_{ji} + R_j} - m_i \right) \quad , i = 1,2$$

$$\frac{dR_j}{dt} = D(S_j - R_j) - \sum_{i=1}^{2} c_{ji}\, \mu_i(R_1, R_2) N_i \quad , j = 1,2$$

N_i denotes the abundance of species i, and R_j the concentration of resource j; μ_{max_i} is the maximum specific growth rate of species I and K_{ji} is the half-saturation constant for resource j of species i, as expressed in the Monod model of growth; m_i is the specific mortality rate of species i;

D is the dilution rate; S_j is the supply concentration of resource j; and c_{ji} is the cell quota of resource j in species i.

To test the role of exploitation competition relative to other interactions that might be occurring between the two diatom species, we compared simulation results from the model after parameterization with the growth kinetic parameters estimated from the laboratory experiments (which only accounted for exploitative competition) with the empirical results from the N- and P-limited competition experiments.

The numerical model and simulations of population dynamics of the two species were carried out using the VisSim software.

RESULTS

Surface area-to-volume ratio was greater for *P. galaxiae* (3.43±0.44) compared to *P. delicatissima* (0.75±0.57), as expected by its smaller size. However, the μ_{max} estimated from batch cultures were similar for the two *Pseudo-nitschia* species (Table 1), although significantly higher for *P. galaxiae* ($p>0.05$). This was also consistent with the observation of chemostat monocultures, where *P. galaxiae* approached steady-state faster than *P. delicatissima*.

The measured R* values for both N and P were lower for *P. galaxiae* than *P. delicatissima*, indicating the higher competitive ability of the former species for both resources at the dilution rate used. When the R* were considered along with the μ_{max} values, the Ks values were also lower for *P. galaxiae* than *P. delicatissima.* The cell quotas for N and P were approximately an order of magnitude less for *P.galaxiae* compared to *P. delicatissima*, allowing *P.galaxiae* to sustain higher cell densities under N and P limitation. Parameters measured by culturing techniques are summarized in Table 1.

Table 1. Growth kinetic parameters under N- and P- limitation for *P. delicatissima* and *P. galaxiae* (mean ± S.D., n=4). Flushing rate (*D)* was set at 0.4 d^{-1} and resource limitation for N and P were a five-fold reduction over the initial f/2 medium concentration.

	Parameter	*P. delicatissima*	*P. galaxiae*
N-limitation	Maximum growth rate μ_{max} (d^{-1})	0.92 ± 0.05	1.11 ± 0.03
	Maximum growth rate for nitrogen μ_{maxN} (d^{-1})	0.70	0.68
	Cell no. at steady-state (cells L^{-1})	8.31×10^8	25.58×10^8
	C (μM N $cell^{-1}$) measured	2.12×10^{-7} $\pm 0.306 \times 10^{-7}$	7.32×10^{-8} $\pm 0.383 \times 10^{-8}$
	Resource requirement -N R* (μM N)	2.602 ± 0.362	1.604 ± 0.102
	Half saturation constant -N Ks (μM)	3.383 ± 0.559	2.847 ± 0.128
P-limitation	C (μM P $cell^{-1}$) measured	1.33×10^{-8} $\pm 0.665 \times 10^{-8}$	3.97×10^{-9} $\pm 1.23 \times 10^{-9}$
	Cell no. at steady-state	6.39×10^8	17.85×10^8
	Resource requirement - P R* (μM P)	0.042 ± 0.019	0.026 ± 0.009
	Half saturation constant - P Ks (μM)	0.055 ±0.030	0.046 ±0.012

Competition experiments showed the superior competitive ability of *P. galaxiae* under N- and P-limitation (Fig.1c,f), confirming predictions based on the R* conceptual model. *P. delicatissima* grew up to a significantly lower population compared to *P. galaxiae* during the first 8 days of the competition experiment, but was outcompeted thereafter. In the absence of a competitor, *P. galaxiae* cell density increased to its monoculture yield.

Overall, model predictions of monocultures and mixtures were similar to the experimental data for the P-limitation condition (Fig.1). The rate of increase in cell density and the final cell density for both species were accurately predicted by the model. However, considering the N-limitation condition, the model showed a rate of cell density increase higher than what was observed in the monoculture experiments. Furthermore, in the competition simulation, the model predicted an accumulation of *P. galaxiae* cells quicker than observed in the competition experiments, and the model predicted an initial accumulation of *P. delicatissima* cells to a greater density than observed in the competition experiment.

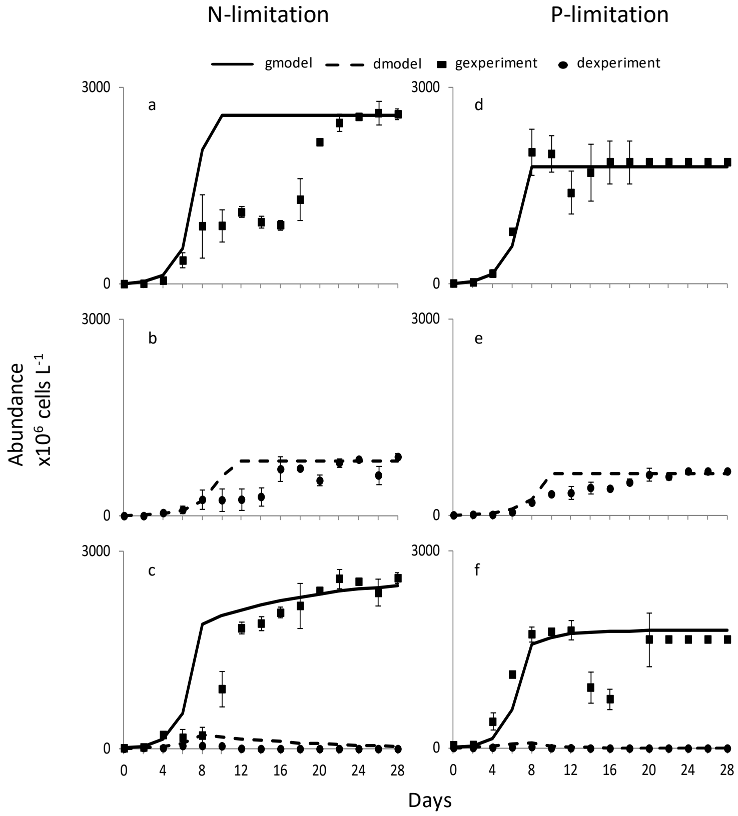

Figure1.Time course of monoculture experiments for *P. galaxiae* (a,d) and *P. delicatissima* (b,e), under N and P limitation and time course of competition experiments for nitrogen limiting conditions (c) and phosphorus limiting conditions (f), parameterizing the model with the same μ_{max}, calculated from the batch cultures. Points indicate experimental means of abundance with standard deviation and lines indicate model predictions of abundance.

To adjust for this, we lowered the μ_{max} for both species under the N-limitation condition. The new maximum specific growth rate for the N-limitation condition (μ_{maxN}) was adjusted to yield the best fit of the model to experimental observations in monocultures (Fig.2a, b), which was found to be nearly equal for both species. The competition simulation under the N-limitation condition using the μ_{maxN} values predicted a slower accumulation of *P. galaxiae* cells than observed in the competition experiments and it did not reach steady state cell densities, at least at the time span tested. Concerning *P. delicatissima,* it predicted an accumulation of cells to a greater density than observed in the competition experiment, and underestimated the rate of exclusion (Fig.2c).

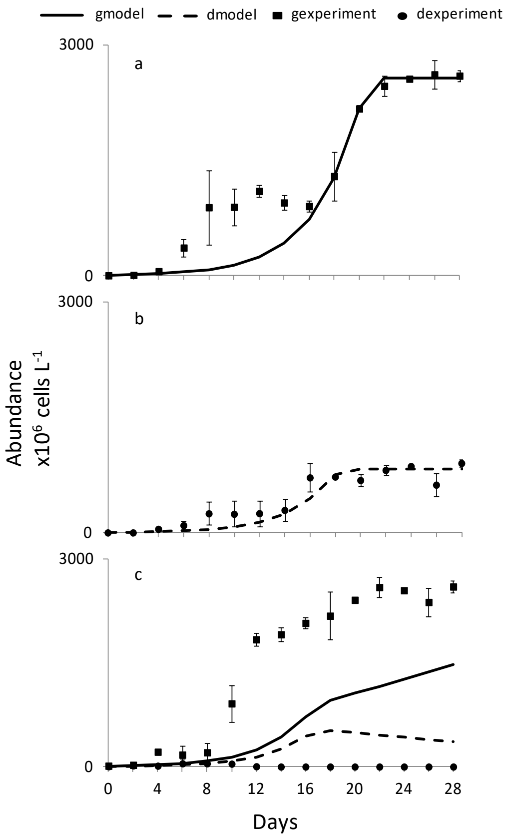

Figure 2.Time course of monoculture experiments for *P. galaxiae* (a) and *P. delicatissima* (b), under N limitation and time course of competition experiments for nitrogen limiting conditions (c), fitting the model μ_{maxN}, to monoculture experiments. Points indicate experimental means of abundance with standard deviation and lines indicate model predictions of abundance.

DISCUSSION

In the present study, we isolated the effect of N and P on the growth kinetics of two *Pseudo-nitzschia* species from the effect of multiple other parameters that are known to affect phytoplankton growth such as salinity, temperature, grazing, hydrodynamics and micronutrients. We chose to focus on macronutrients as their inflow of terrestrial origin into coastal areas often seems to stimulate bloom development (Anderson et al., 2002; Trainer et al., 2012). Our results show that both *P. delicatissima* and *P. galaxiae* were poor competitors for N as evidenced by their moderate μ_{max} and high K_S compared to many phytoplankton species which are known to have μ_{max} values in the range of 0.36 to1.64 d^{-1} and K_S values for N in the range of 0.2 up to 2 μM (Reynolds 2006). This finding is consistent with multiple observations of a strong relationship between ambient nitrate concentrations and abundance of *Pseudo-nitzschia* spp. (e.g. Anderson et al., 2002; Caroppo et al., 2005; Kaczmarska et al., 2007; Spatharis et al., 2007; Trainer et al., 2012). On the other hand, the P requirements of both species were low (~0.05 μmol) compared to reported half-saturation constants of diatom species in the literature ranging from 0.011 to 0.25 μM (Tilman and Kilham, 1976; Tilman, 1977; Holm and Armstrong, 1981; Reynolds, 2006). This indicates that both species are competitive for P when with species of comparable maximum growth rates and are thus able to sustain populations even when the availability of this nutrient in the environment is low (e.g. <0.1 μM).

Many life history traits of phytoplankton species have been associated with their cell size (Richardson, 1998; Sarthou et al., 2005; Edwards et al., 2011). *P. galaxiae*, being the smaller of the two studied species, has a greater surface area-to-volume ratio and presents a higher μ_{max}. A larger surface area also implies that the species is efficient in performing a faster nutrient uptake (Sarthou et al., 2005). Moreover, *P. galaxiae*'s significantly smaller size can explain its lower intracellular nutrient content, in agreement with previous studies on phytoplankton in Florida Bay (Richardson, 1998). These results are consistent with the general observation that the competitive ability of marine phytoplankton for both N and P decreases with increasing cell volume (Edwards et al., 2011).

Based on nutrient concentrations at steady state in the monocultures (R*), *P. galaxiae* uses both N and P more efficiently than *P. delicatissima,* and therefore outcompetes it under both N and P limitation. Although a trade-off has been observed by previous studies on the requirements of species for resources such as P and Si, or N and Si (Huissman and Weissing, 2001), there is very few data to support such a relationship for the requirements of specific species on N and P (Edwards et al., 2011). Therefore, the present study contributes to our knowledge on the relationship between N and P requirements, showing the absence of a trade-off when considering these two *Pseudo-nitzschia* species. The relationship between cell quota (c) and R* also remains unclarified. Even though a linear relationship has been observed between c and R* for silicate there is no clear trend for P and still no evidence for N (Huissman and Weissing, 2001). In the present study, *P. galaxiae* is clearly a better competitor for both nutrients which suggests that the species would exclude *P. delicatissima in vitro* if exploitative competition was the only mechanism defining the interaction between the two species.

The outcome of competition was predicted by the model, even in the simulations of N-limitation where the rate of cell density increase of the eventual winner (*P. galaxiae*) was too quick when parameterized with measured μ_{max} (from batch cultures) or was too slow when parameterized with fitted μ_{max} (from continuous cultures). Furthermore, model simulations using both estimations of μ_{max} (measured and fitted), showed a greater accumulation of *P. delicatissima* cells than observed experimentally. This suggests that under N-limitation *P. galaxiae* suppresses *P. delicatissima*'s growth in a way that is more pronounced than explained by exploitative competition only. This could be explained by an unknown form of interference completion, such as allelopathy. However, to our knowledge, this has not been reported previously.

These findings suggest that in environments where N or P is limiting for a substantial period of time, *P. galaxiae* is more likely to dominate an assemblage than *P. delicatissima*. However, the two species have been found to co-exist in the natural environment in densities higher than $1x10^4$cells/L. Field interactions are usually more complex than experimental and differential losses experienced in the field might be one of the reasons explaining the coexistence of the two species in the natural environment. *P.galaxiae* is a smaller species and even though it is highly competitive, it can be more susceptible to predation (Chen and Liu, 2010; Edwards et al., 2011). Furthermore, it tends to form clumps which increase its sinking rates (Spatharis, unpublished data). On the other hand, *P. delicatissima* can remain suspended for substantially longer time periods and due to its larger size it can store more nutrients during brief periods of high supply (Grover, 1991; Edwards et al., 2011). There has also been evidence of the consumption of organic substrates from certain *P. delicatissima* strains, which can explain its persistence in low inorganic nutrients concentration (Loureiro et al., 2009). Moreover, factors other than limiting resources including temperature and salinity have been found to switch the competitive ability of species and change the outcome of their competition (Tilman, 1982). Coexistence of these two *Pseudo-nitzschia* species in the field is consistent with other field observations on *Pseudo-nitzschia* blooms often consisting of multiple species at important densities (Kaczmarska et al., 2007; Thessen and Stoecker, 2008; Leblad et al., 2013).

In order for management schemes to efficiently target HAB species in given areas, it is essential to consider the relationship between the species proliferation to the quantities of incoming nutrients. For this purpose, the combination of experimental results on N and P limitation would enable the study of the relationship of resource requirements for two major nutrients of species. This study provides the first experimental evidence on the high nitrate requirement of two globally important *Pseudo-nitzschia* spp. supporting the importance of the latter for growth and possibly toxin production. Moreover this study indicates that N rather than P in the nutrient-rich freshwater inputs is the factor triggering bloom development, as observed previously (Bates, 1998). To conclude, the present study has shown the importance of combining experimental approaches (batch and continuous cultures) in order to provide an efficient estimation of growth kinetic parameters and parameterize models of species competing for limiting nutrients. The present results can be used in order to explain and predict HAB dynamics and occurrence in disturbed marine ecosystems.

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INCREASING FUNCTIONAL DIVERSITY WITH DECREASING RESOURCE PULSING FREQUENCY FOLLOWING FRAGMENTATION (PAPER 2)

ABSTRACT

Fragmentation in aquatic ecosystems is observed in coastal environments due to water level variation and man-made structures. In each isolated patch, different environmental conditions, mainly driven by differential resource regimes, are expected to host functionally different species. In the present study, we explore the effects of fragmentation and differential nutrient pulsing frequencies in the structure and composition of phytoplankton assemblages. This approach couples experimental assemblages from the Aegean Sea, Greece with a widely applied resource competition model, partially parameterized with experimental findings. Phytoplankton was fragmented and self-organized under different nutrient pulsing frequencies (every 1, 3, and 6 days), resulting in assemblages of different structure and composition. To explore the underlying mechanisms of this behavior, different scenarios of relationships between species life history traits were implemented in the numerical model. Furthermore, scenarios of species initial relative abundances and species stochastic extinctions were used to monitor the difference between the replicates in each pulsing frequency. Experimental results were successfully reproduced by numerical modeling, revealing that growth rate is the most important life history trait under the applied pulsing regimes, followed by the ability to compete for phosphorus. Fragmentation under the tested regimes results in substantial system heterogeneity, which yields higher functional diversity and richness in the low frequency pulse, a trend observed in both experimental and simulated assemblages.

Keywords: phytoplankton, coastal ecosystems, life history traits, resource competition model, self-organized assemblages

INTRODUCTION

Landscape fragmentation can severely impact populations and communities, increasing their extinction risk due to genetic and demographic stochasticity and limiting population movements in response to perturbations (Sawyer et al., 2011). Fragmentation can have important implications on ecosystem function through the loss of functionally dissimilar species (Boersma et al., 2014) resulting in the disruption of important ecosystem goods and services. To predict the effects of habitat alterations on the structure (e.g. diversity) and function (e.g. productivity) of ecosystems and optimize their environmental stewardship, it is essential to gain a deeper understanding of mechanisms controlling species coexistence and dynamics. Although fragmentation and its effects on ecosystem properties have been extensively addressed in terrestrial ecosystems (e.g. Debinski and Holt, 2000), aquatic and particularly coastal environments remain significantly understudied.

Coastal ecosystems such as interconnected coastal lagoons, salt marches, ponds and rock pools usually consist of fragmented local patches. Due to the anticipated effects of climate change (Cyrus et al., 2010) and increasing man-made alterations (Kang and King, 2013), the effect of habitat fragmentation on the structure and function of these seascapes are currently key issues in ecological research and habitat restoration efforts (Januchowski-Hartley et al., 2013). The importance of these coastal systems cannot be overstated, as they are characterised by a high diversity of ecological processes (Costanza et al., 1993) and account for a disproportionally large amount of global productivity (Mann and Lazier, 1991). Despite their importance, so far there have been no studies addressing the effects of fragmentation on primary producers.

Coastal communities can be characterized by high environmental heterogeneity mainly due to differential resource regimes that each patch receives. For instance, variation within annual or shorter cycles in freshwater inflows and ocean mixing, leads to variable and often pulsed nutrient supply regimes (Buyukates and Roelke, 2005; Spatharis et al., 2007). These pulsed inflows can be regarded as external disturbances, causing population losses through hydraulic displacement, and growth stimulation associated with nutrient loading. A variable resource supply into a system of hydraulically connected sites may lead to varied localized conditions. A gradient of different pulsing frequencies presents a complex relationship with species richness that follows the intermediate disturbance hypothesis (Padisak, 1993; Sommer, 1995). Although this relationship has been adequately addressed, other community attributes, such as composition and structure, resulting from the fragmentation of an initial species-rich community have not been investigated.

Differential pulsing frequencies applied in each fragment are expected to favor species with particular life history traits (e.g. ability to acquire nutrients and growth rate), according to niche theory (Vandermeer, 1972).Nutrient pulses of high frequencies and low magnitude, are expected to result in low environmental variability, hindering severe nutrient depletion and thus enabling a few fast growing specialists to persist. As frequencies decrease, the time interval between successive enrichments increases leading to greater variability in nutrient concentrations (reflecting environmental variability) and nutrient depletion. In theory, this should facilitate coexistence of more species with different traits, particularly favoring those with lower nutrient requirements (Sommer, 1989; Reynolds, 1993). Although the identity and number of different species in the self-organized assemblages can be established with experimental approaches, the measurement of their respective traits remains a challenging task. A modeling configuration based on realistic ranges of species traits and trait trade-offs that will simulate community dynamics across environmental gradients (Litchman et al., 2007; Edwards et al., 2013) can provide further insights on how functionally different these species are.

In the present study we aim to investigate mechanisms and life history traits driving species composition and structure under different environmental conditions (i.e. pulsing frequencies) following the fragmentation of an initial phytoplankton assemblage. The current approach can complement previous modeling approaches on functional traits under nutrient pulses (Edwards et al., 2013), since it took into account coexisting species in natural assemblages rather than a compilation of traits from literature. The study involved an experimental part consisting of laboratory microcosms of phytoplankton assemblages self-organizing under different nutrient pulsing frequencies. This was combined with a numerical model, adapted to simulate the above experiments and parameterized with realistic life history traits and trade-offs. This integrated approach, will help us test our hypotheses enabling the quantification of the life history traits of species in the self-organized assemblages.

METHODS

Experimental assemblages

Surface water from the Aegean Sea was collected into 10L Nalgene carboys. Upon arrival to the laboratory, a portion of the water was filtered through 47mm Whatman GF/F glass fiber filters, and used for the preparation of f/2 media (Guillard and Ryther, 1962). Water to be used as inoculum for cultures was pre-filtered through a 100μm mesh-size plankton net to avoid bias from mesozooplankton (Katechakis et al., 2002). Experiments were initiated approximately 10h after water collection.

Microcosms consisted of semi-continuous cultures in 1L vessels. Nutrients were pulsed under three different frequency regimes (once every day, 3 days, and 6 days). The volume displacement and magnitude of nutrient loading over time was the same for all culturing conditions. This was achieved by removing a culture volume of 100ml, 300ml, and 600ml from the respective pulsing frequencies, then replacing the volume removed with fresh f/2 media. Each frequency regime was replicated thrice. Inoculation with the Aegean seawater was consistent with the above pulsing regime. In the cultures receiving a daily pulse, 100ml of f/2 media was added to 900ml of the sea water inoculum, in the ones receiving a pulse every 3rd day, 300ml of f/2 media was added to 700ml of the sea water inoculum, and in the ones receiving a pulse every 6th day, 600ml of f/2 media was added to 400ml of the sea water inoculum. Therefore, although species composition was identical, initial species abundances were different among the three pulsing frequencies.

Cultures were kept in a climate-controlled chamber where temperature was held constant at 20°C and photoperiod at a 12-h light:dark cycle. Cool white fluorescent bulbs were used as the light source and irradiance was $200\mu mol/m^2/s$. This value was in the range of typical light saturated photosynthesis rates for phytoplankton (Kirk, 1994). Sufficient mixing was ensured by constant and gentle stirring using magnetic stirrers.

Cultures were allowed to self-organize and reach steady-state under these stable conditions and pulsed frequency regimes. It was assumed that steady-state was reached when the species composition and relative abundances were constant in each of the nine culturing vessels for a period not less than 15 days.

Every 6 days, 5ml of the culture was fixed with Lugol’s iodine solution for microscopic analysis. Cell counting was performed using inverted light microscopy (Motic AE31, 400X) according to the Utermöhl method (Utermöhl, 1958). Measurements of individual dimensions were used to calculate cell biovolume by approximation to simple geometric shapes according to Hillebrand et al. (1999).

Simulated assemblages

To investigate the underlying mechanisms and species traits driving composition and structure in each assemblage, a well known resource-competition model for phytoplankton growth (Tilman, 1982) was adapted to reflect the conditions applied in the experimental assemblages. This model describes the cell density dynamics of 100 phytoplankton species (*N_i*) competing for two nutrients (*R_j*).

$$\frac{\boldsymbol{dN_i}}{\boldsymbol{dt}} = N_i\left(\min\left(\frac{\mu_{max_i}\times R_1}{K_{1i}+R_1},\frac{\mu_{max_i}\times R_2}{K_{2i}+R_2}\right)-m_i\right) \qquad ,i=1-100$$

$$\frac{\boldsymbol{dR_i}}{\boldsymbol{dt}} = D\left(S_j-R_j\right)-\sum_{i=1}^{n} c_{ji}\times\min\left(\frac{\mu_{max_i}\times R_1}{K_{1i}+R_1},\frac{\mu_{max_i}\times R_2}{K_{2i}+R_2}\right)N_i \qquad ,j=1,2$$

N_i denotes the abundance of species i, and R_j the concentration of resource j; μ_{max_i} is the maximum specific growth rate of species i and K_{ji} is the half-saturation constant for resource j of species i, as expressed in the Monod model of growth; m_i is the specific mortality rate of species i; *D* is the dilution rate; S_j is the supply concentration of resource j; and c_{ji} is the cell quota of resource j in species i.

Model parameterization

The two most important nutrients for phytoplankton growth –nitrogen and phosphorus-- were used as potential limiting resources in the model. Surface water of W. Mediterranean Sea are primarily limited by nitrogen and secondarily by phosphorus (Moore et al., 2013) whereas recent evidence from E. Mediterranean coastal waters suggests alternating cycles of nitrogen and phosphorus limitation within the course of a year (Tamvakis et al., 2012). The concentrations of nitrate and phosphate in the pulsed inflow were set as identical to the f/2 concentrations in the experimental pulses (i.e. 36.2 μM for P and 882 μM for N). Once species traits in the initial pool were allocated, the populations of 100 species self-organized (Roelke and Eldridge, 2008) according to the aforementioned equations under the three nutrient pulsing frequencies applied in the experiment (1-, 3-, 6-day). The self-organization process, that lasted 3000 simulated days, was repeated 100 times, thus producing 100 replicated assemblages. As in the experiments, specific mortality rate was set equal to dilution rate ($D=0.1\text{d}^{-1}$).

Assemblages were generated using a numerical approach that involved simulations from an initial pool of 100 species, approximately equal to the number of species observed in the seawater inoculum. The physiological traits defining the dynamics of the 100 phytoplankton species were the intracellular resource content c, the resource-specific half saturation constant Ks, the minimum resource requirement of a species at steady-state R* and the maximum specific growth rate μ_{max}. Parameterization of these traits was based on information on realistic ranges and trade-offs from species present in the experimental assemblages (Papanikolopoulou unpublished data) and literature sources (Reynolds, 2006). More specifically, c-P was in the range of 0.004-0.055 (10^{-6} μmole P $cell^{-1}$) and c-N was in the range of 0.055-0.244 (10^{-6} μmole N $cell^{-1}$), K_P was in the range of 0.02-0.2 μM and K_N was in the range of 0.2-2 μM.

A tradeoff was considered between the competitive ability of species for N and for P, that is the R*-N and R*-P for the 100 species. Furthermore, we considered a complementary use of resources that should lead to their greater usage by the species and, as a consequence, to supersaturated assemblages. This was achieved by applying a shape function that "bent downward" the line that described the distribution of R*s in the resource trade-off space. The shape function used for creating complementarity within an assemblage followed the form $y=\alpha/x$, where *a* was a coefficient that defined the shape of the curve for the region defined over the intervals $x[0,1]$ and $y[0,1]$. In the present study *a* was set at 0.49. This tradeoff has also been observed in experimental works for Si and N and Si and P (Huisman and Weissing, 2001).

Secondly, we considered that each species consumes more of the nutrient that limits its growth (Tilman, 1982; Huisman and Weissing, 2001) which implies a proportional relationship between the half-saturation constant for growth (Ks) and c. We additionally considered variable μ_{max} in our model, which has been often maintained unrealistically constant in previous population dynamics models (e.g. see comment in Schippers et al., 2001). This parameter can be particularly important for species survival in systems with periodic hydraulic flushing such as the one employed in the present experimental design. Two out of the three scenarios developed in the present study assume different relationships of μ_{max} with the resource-specific half saturation constant Ks or the minimum resource requirement R*.

To identify the conditions under which the simulated phytoplankton assemblages best reproduce the structure and composition of the experimental assemblages, two sets of scenarios were explored: (a) three scenarios that assume different relationships among species' traits in the initial species pool, and (b) 12 scenarios related to the initial relative abundances of species and stochastic extinctions. These are described in detail below.

Scenarios of trait relationships in the initial species pool

1st scenario: μ_{max} is constant across all species and equal to 1 d^{-1}. This is a baseline scenario that enables the investigation of competitive abilities of species for nutrients, not influenced by competition related to growth rate.

2ndscenario: There is a three way trade-off between R*- P, R*-N and μ_{max} (ranging between 0.36-1.64 d^{-1}). That is, species with a lower R*-P, not only have a higher R*-N but are also growing slower. Accordingly, species with a lower R*-N have a higher R*-P and a low μ_{max}. Species that are in the middle of the resource trade-off space and don't present a competitive advantage either for N or for P, in order to persist in an assemblage they should have some other competitive advantage by growing faster than the rest (i.e. higher μ_{max}). Therefore, in a species-rich assemblage, each species is never competitively superior regarding more than one trait. A similar three way trade-off was described for the competitive ability for P, and N and the size of phytoplankton species (Edwards et al., 2011), whereas numerous studies have shown the relationship between size and maximum growth rate of phytoplankton species (e.g. Sarthou et al., 2005; Litchman et al., 2007). Trade-off between R*-P and μ_{max} has also been shown in Segura et al. (2013). This scenario enables the investigation of the importance of growth rate, detached from the competitive ability of species in acquiring nutrients.

3rd scenario: pairwise combinations of competitive abilities. Each species is superior for two competitive abilities: (a) Species that are good competitors for both nutrients but slow growers, (b) Species that are good competitors for P but not for N and are fast growers, (c) Species that are good competitors for N but not for P and are fast growers. For example, at high pulsing frequencies, if the system is phosphorus limited we expect species that are both good competitors for P and fast growers to dominate. This could be supported from preliminary measurements of traits of species that were the most abundant in our experimental self-organized assemblages. For example, one of the dominant species (*Nitzschia* sp.) was the best competitor for P (but not for N) as well as one of the most fast growing species (Papanikolopoulou unpublished data) indicating that this scenario may be plausible in natural assemblages. Accordingly, relationships were established between μ_{max} (ranged between 0.36-1.64 d^{-1}), K_N, and K_P (instead of R*). When μ_{max} is constant (1st scenario) relationships between R*-N and R*-P directly reflect relationships between K_N and K_P. However, when considering more complicated relationships, Ks and R* cannot be used interchangeably. While R* is a trait that summarizes the competitive ability of a species at a given resource supply (Tilman, 1990), Ks and μ_{max} are the traits that characterize species.

Scenarios of species' abundances and stochastic extinctions

After allocating the species' traits in the initial pool, different scenarios were applied regarding the initial relative abundances of the 100 species. Initial abundances were either equal among species or they differed up to 4 orders of magnitude. The latter scenario was based on experimental data, where species present at day 0 ranged from very abundant to undetectable. For each of these two scenarios we applied 5 more scenarios of stochastic extinctions of either 1, 2, 3 or 10 species or of the dominant species, at an early day of self-organization.

Data analysis

For consistency, results from both experimental and simulated assemblages were manipulated with the same data analysis methods, when the system reached steady-state. Assemblage structure was expressed as species richness and evenness index J (Pielou, 1975). To define the role of nutrients the ambient concentrations of NO_3 and PO_4 were presented and traits of the surviving species of the model simulations, μ_{max} and R*, were examined. To quantify similarity of assemblage compositions within each pulsing frequency at steady-state (the three of the experiment and the 100 of the simulations) the Bray-Curtis similarity index was applied on log-transformed data. To be able to compare this similarity between the different pulsing frequencies we used as replicates the different days the system was at steady-state.

RESULTS

The simulated assemblages best describing the experimental data were those that self-organized following scenario3 of pairwise competitive abilities (from now on referred to as the model). This was consistent for all measured assemblage attributes (richness, evenness, and similarity among replicates) and nutrient limitations (Fig.1). Regarding the scenarios of differences in initial species' relative abundances and stochastic extinctions, a large differentiation among initial abundances produced more diversification among replicates compared to stochastic extinctions. In most cases, when a species went extinct, either a species already present increased in biomass or an emerging species with similar traits filled its place. The influence of stochastic extinctions was higher when they were considered together with variable initial abundances, especially in the case where the most abundant species was removed from the assemblage. Since stochastic extinctions scenarios did not present any variability, reflecting that observed in the experimental assemblages, only results regarding the variability in species' initial relative abundances are presented.

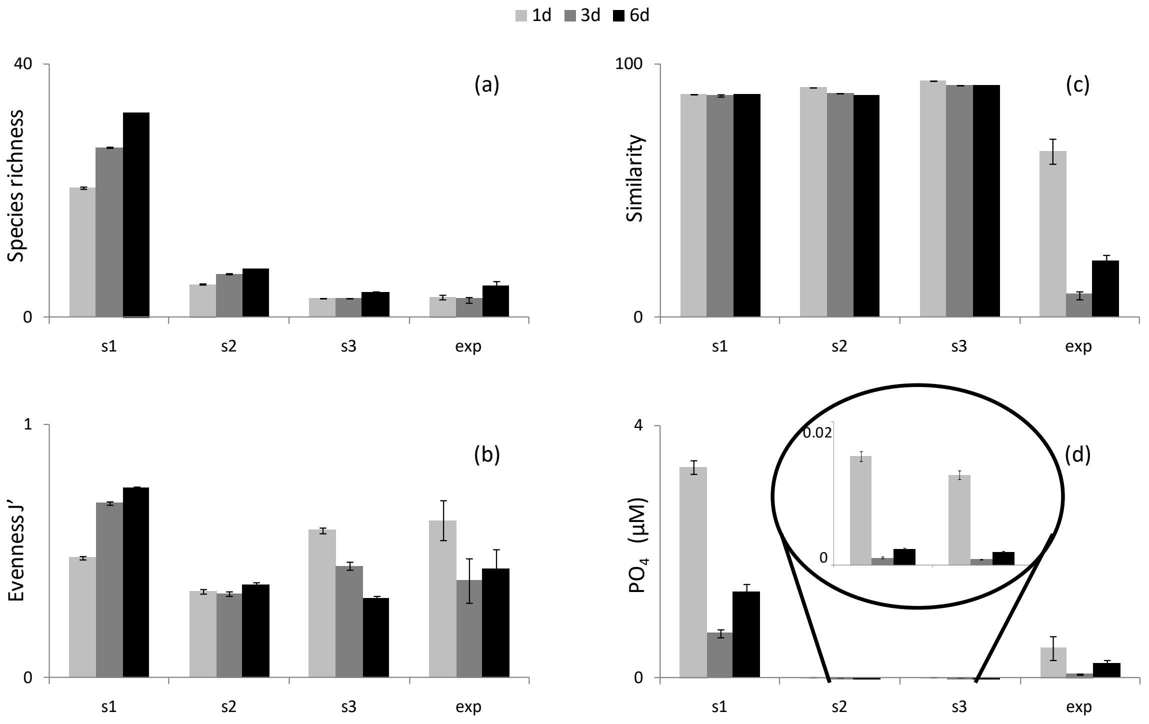

Figure1. Species richness (a), evenness (b), Bray-Curtis similarity(c), and ambient phosphate concentration (µM) (d) for the experiment (exp) and the tree scenarios, when species initial relative abundances vary - s1 (scenario1), s2 (scenario2), s3 (scenario3), 1d, 3d, 6d the pulsing frequencies of 1,3,6 days. Error bars are standard errors. As replicates in order to perform the comparisons in (c) the different days of steady-state were used. Notice the different scale for s2 and s3 in (d).

Assemblages that self-organized under the low frequency pulse (6d) presented the highest species richness at steady-state, whereas evenness was highest at the most frequent pulse both in experimental results and in the model (Fig.1a, b). It is worth noting that more time was needed to reach steady-state in the model than in the experiment (Fig.2). Replicates that self-organized under the same nutrient pulsing frequency were more similar in the model than in the experiments. However, similarity among replicates was significantly higher in the 1d pulsing frequency in both model (ANOVA, p-value<0.01) and experiment (ANOVA, p-value<0.01) (Fig.1c).

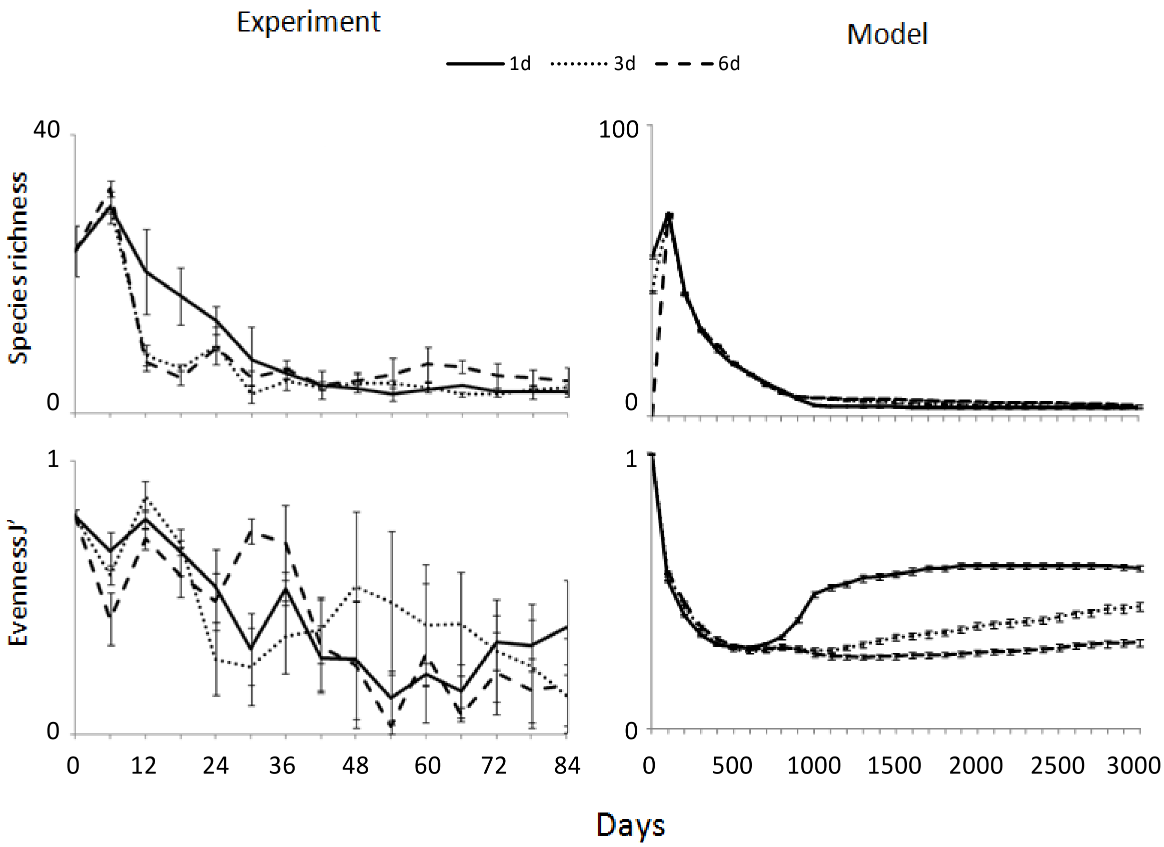

Figure2. Species richness and evenness during the time course of self-organization in both experimental and simulated assemblages. Error bars are standard errors. Notice the different scales.

The model provides extra insights on the species' life history traits present at each pulsing frequency (Fig.3, Fig.4). The traits that seem to drive assemblage composition under these experimental conditions are the R*-P and the maximum growth rate of a species. Surviving species in the scenarios with variable μ_{max} (s2, s3) tend to have high growth rates, whereas in the 1st scenario where μ_{max} is constant and equal to $1d^{-1}$ for all species, the species that survive have the lower R*-P (Fig.3). In the scenario of pairwise comparisons, all the species that survived the self-organization were fast growers. Moreover at steady-state, the species present in the frequent pulse (1d) were those that had a low R*-P and K_P, whereas at lower frequency pulses (6d), some species with a lower R*-N and K_N also emerged. Therefore, the lower frequency (6d) pulse not only presents higher species richness but also higher functional diversity. This was also observed in the experimental assemblages, where in assemblages of the 6d pulsing frequency three functional groups where present (diatoms, green algae and cryptophyceae) whereas in assemblages from the 1d pulsing frequency only diatoms survived. In this scenario, R*-P tended to increase with increasing pulsing frequency (due to the appearance of species at the other end of the resource trade-off curve, Fig. 4), however this change was not significant. In the three way trade-off scenario, R*-P was never very low, since the species with the higher μ_{max} had intermediate R* values for the two nutrients.

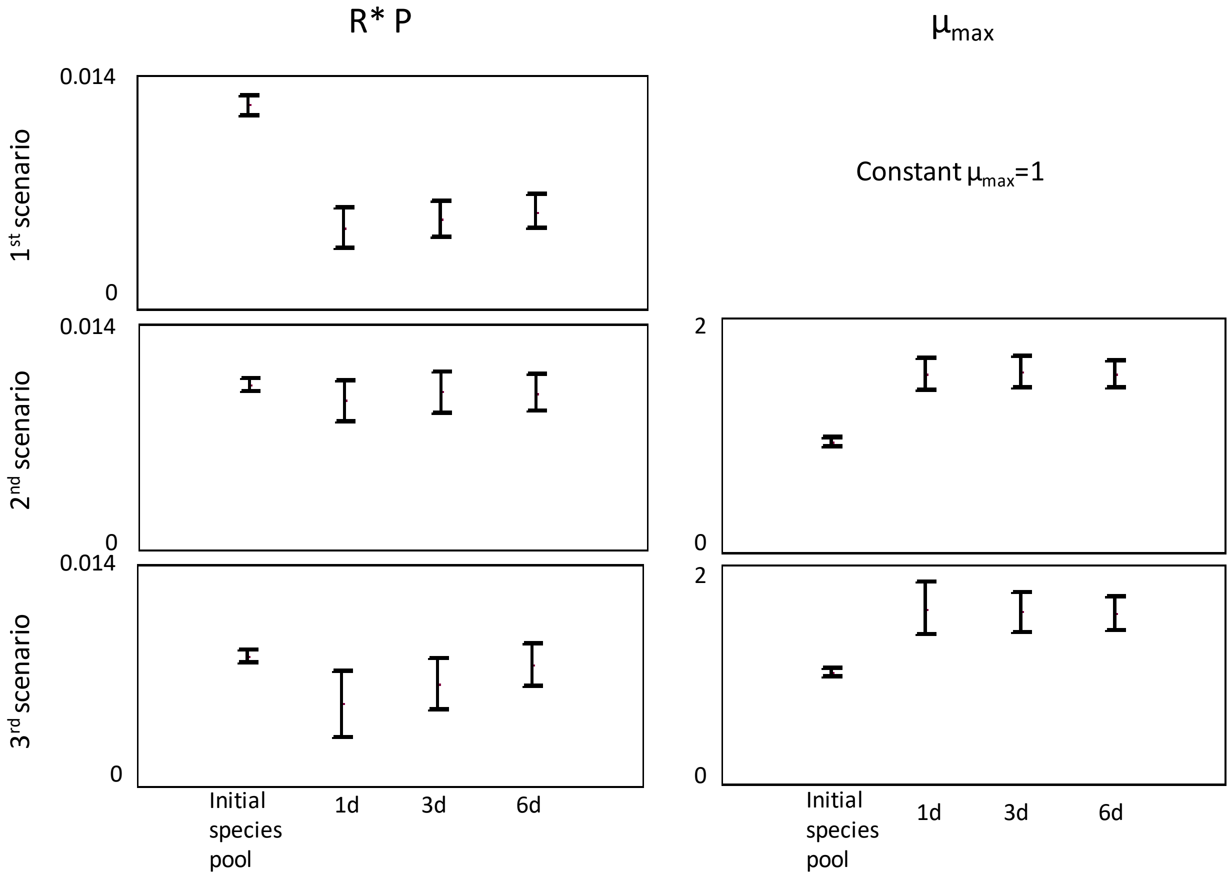

Figure3. Summary of the four scenarios for varied initial abundances for the two life history traits that drive assemblage composition (R* P – R* for phosphorus, and μ_{max} – maximum growth rate), Initial species pool: mean and LSD intervals for the traits of 100 species in the initial species pool, 1d, 3d, 6d: mean and LSD intervals for the traits of the surviving species at steady-state for the 1,3,6 days nutrient pulsing frequencies.

Regarding nutrient limitation, self-organized assemblages in both simulated and experimental conditions were P-limited. Furthermore, the intermediate pulsing frequency (3d) yielded the lower phosphate ambient concentration. Phosphate concentrations were lower in the model than the actual concentrations measured in the experiment (Fig.1d). Nitrogen was never limiting for species growth, this result being more pronounced in the model, with nitrate concentrations being 10 times greater (500-540 μM-NO_3) than in the experiment (10-50 μM-NO_3).

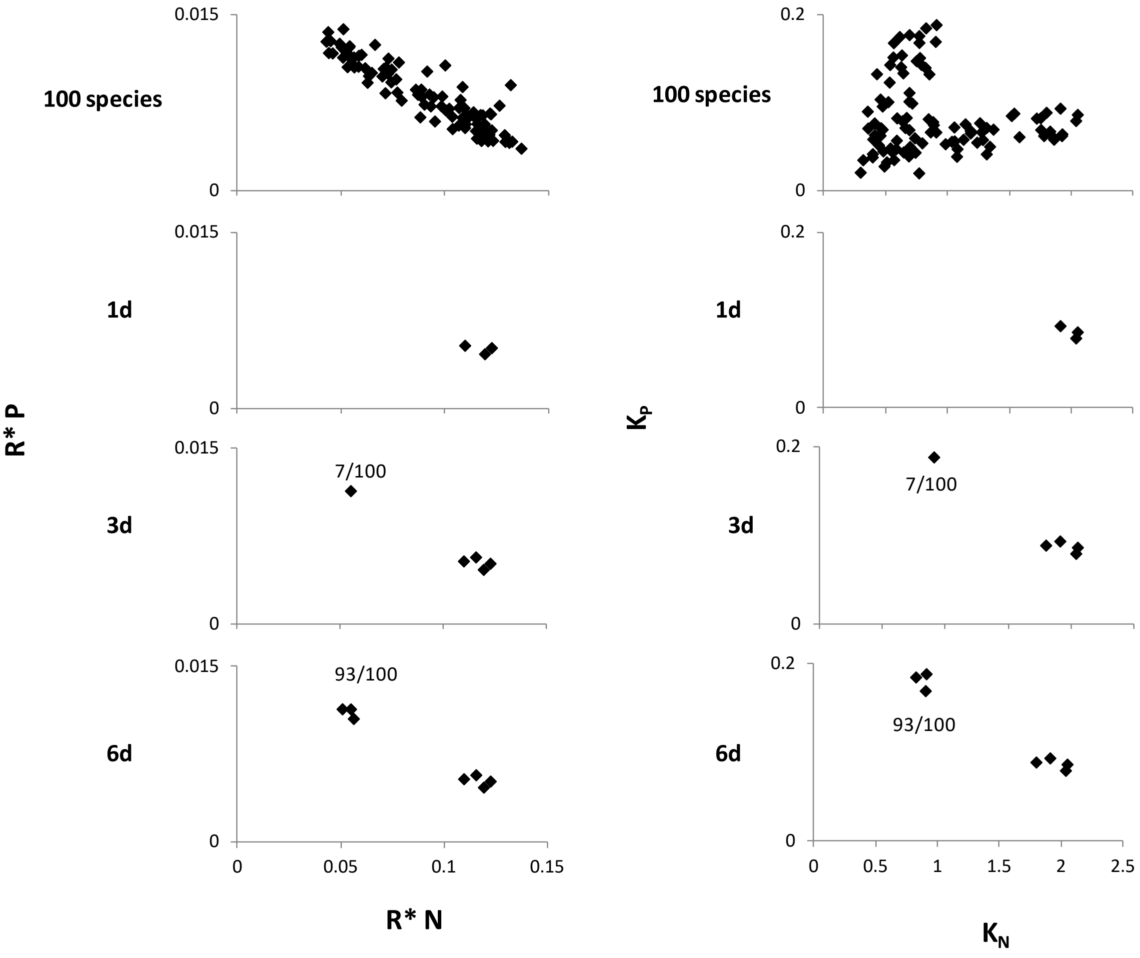

Figure4. R* and Ks relationships of the 100 species present at the initial species pool and the species present at steady-state for the 3 pulsing frequencies. 7/100 and 93/100 denotes the number of replicates in which a species that is a better competitor for N was present.

DISCUSSION

The different assumptions on the relationships between species life history traits provided important insights on the way phytoplankton assemble. When considering only the ability of a species to acquire nutrients (1st scenario), surviving species had low needs for phosphorus (low R*-P). Furthermore, evidence from the model and the experiment showed that the system was P limited. This suggests a stronger P limitation in E. Mediterranean, from where species were collected, in agreement with previous observations (Krom et al., 1991). As species are adapted to P limitation in their natural environment and thus in our initial species pool, this increases the probability to have strong competitors for P at the end of the self-organization process.

However, when considering variable growth rates among species, unrelated to nutrient requirements (2nd scenario), the importance of fast growth under pulsed inflows emerges. Surviving species had higher than average growth rates (Fig. 3), whereas their needs for nutrients were intermediate. When further assuming a relationship between growth and nutrient requirements (3rd scenario), model results became more representative of the observed experimental assemblages. Surviving species' traits under this assumption were both fast growers and strong competitors for P. High frequency pulses are expected to favor fast growing species (Reynolds, 1993), however recent modeling studies have shown the importance of high growth rates under low (>20 days) frequency pulses due to longer periods of phosphorus saturation (Edwards et al., 2013). Our results agree with Reynolds (1993) however we never tested the assemblage attributes under pulsing frequencies as low as those considered in Edwards et al. (2013).

According to resource competition theory, the number of coexisting species cannot exceed the number of limiting resources (Tilman, 1982). However, more species are able to coexist under a variable nutrient supply regime (Tilman, 1982; Sommer, 1989). This variability can result from different pulsing inflows, often referred to as disturbances (Sommer et al., 1993). These can have a stronger positive effect on species richness when the frequency that they occur allows an interval (at least 3 days) greater than one generation time (i.e. pulsing inflows of lower frequency) and when both limiting nutrients are imported simultaneously (Gaedeke and Sommer, 1986; Sommer, 1989). This is in agreement with our results, where higher species richness was observed at the lowest pulsing frequency (6d interval between pulses), in both experiment and model.

Lower pulsing frequencies (3d and 6d) also resulted in a higher functional diversity according to both our experimental and model findings. More functional groups emerged in the experimental assemblages, from only one in the most frequent pulse to three in the least frequent, while two were present in a replicate of the intermediate pulsing frequency. In the model results this is depicted in the resource trade-off curve, since species with different life history traits emerged at the other end of it. This is likely due to the greater variability in resource concentrations between inflow intervals at those pulsing frequencies. These pulsing frequencies are low enough to prevent species extinctions due to hydraulic flushing but high enough to prevent successful competitors from dominating the assemblage and competitively excluding other species (Connell, 1978). Therefore species with different life history (functional) traits are able to coexist. On the other hand, at very high (or very low) frequencies only a few specialists are able to survive (Reynolds, 1993). This could explain the higher similarity between the replicates in the daily pulsing frequency. When fewer possibilities of potentially surviving species are available (specialists are by definition fewer than generalists), the steady-state assemblages would consist of more common between them species.

Even though the model described the trends observed at steady-state, it failed to do so in some occasions. Considering the time of self-organization, simulated assemblages needed more time to reach steady-state than the experimental assemblages. This discrepancy could be related to the initial species abundances or the actual number of species. Even though a total of 100 species were observed during self-organization, not all of them were detected in the initial inoculum, but became abundant during self-organization. This could imply that the initial inoculums could consist of more species which could alter the consumption of nutrients in the experiments compared to the model simulation, resulting in a different time-scale. Discrepancies were also observed at steady-state. Concerning nutrients, phosphate in the model was depleted by nearly a 10-fold compared to cultures, possibly because the species surviving the simulated self-organization were better competitors for phosphorus than the species actually present in the cultures. On the other hand, NO_3 in the cultures was more depleted than in the model simulations, possibly due to its consumption by bacteria present in the cultures, strongly competing phytoplankton (Kirchman, 2000). Furthermore, similarity between replicates was greater in the model simulations than in experimental assemblages, possibly due to the greater number of stochastic processes in the lab experiment over the well - specified conditions in the model.

In aquatic coastal ecosystems, phytoplankton assemblage structure is mainly driven by species life history traits, responsible for species adaptation to given environmental conditions. The present findings seem to suggest that under fragmentation, differential environmental conditions in each patch, represented by a gradient of pulsed nutrient inflows, provides substantial heterogeneity for functionally dissimilar species to emerge. Under the anticipated effects of climate change, where water levels are expected to vary, patches are expected to become either more isolated or connected, forming metacommunities. Connectivity could also have important implications on diversity and ecosystem function (e.g. productivity). However, species in the isolated patches and their life history traits, together with the degree of connectivity, could have a major influence in metacommunity's attributes.

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SPATIAL AVERAGING COMPENSATES FOR SPECIES LOSS IN MAINTAINING PRODUCTIVITY AT HIGH ECOSYSTEM CONNECTIVITY (PAPER 3)

ABSTRACT

Dispersal in heterogeneous ecosystems, such as coastal aquatic environments, is a major force influencing the structure and productivity of metacommunities. According to theory, productivity is influenced by both species richness and spatial averaging - homogenization towards the average conditions by the species best adapted to them -, resulting in a unimodal relationship with dispersal. We experimentally tested the effect of spatial averaging on the productivity of phytoplankton metacommunities along a dispersal continuum. Our experiments involved natural assemblages that self-organized in isolation under conditions that comprised three inflow pulsing frequencies, where hydraulic displacement and nutrient loading influenced assemblage self-organization. After steady-states emerged in these isolated assemblages, they were connected through three levels of dispersal, thus forming three metacommunities of differing connectivity. As dispersal increased, species richness in the metacommunity declined whereas productivity remained high. This latter observation is in contrast to theory, which suggests a respective decrease in productivity. This indicates that at high ecosystem connectivity, the effect of spatial averaging can mask the effect of decreased species richness, thereby maintaining high metacommunity productivity. Our findings are particularly relevant to coastal aquatic environments where patches can be isolated and then connected, being affected by metacommunity dynamics. We emphasize the importance of spatial averaging in maintaining high productivity despite the species loss observed at high ecosystem connectivity. We believe that our observation on the dominance of a highly productive species best adapted to the intermediate environmental conditions is not species specific. It can be applicable to generalist species that tend to inhabit and better exploit heterogeneous environments. Our results can influence management practices since they highlight the pivotal role of dispersal in the functioning of aquatic ecosystems.

Keywords: dispersal, dynamics, coastal aquatic ecosystems, metacommunity, phytoplankton, self-organization, steady-state assemblages

INTRODUCTION

In constantly changing landscapes where local patches are connected and disconnected repeatedly, dispersal of organisms often plays a prominent role in driving ecosystem functions like productivity. Habitats such as estuarine lakes, interconnected lagoons, and salt ponds are typical examples of such spatially heterogeneous systems. Some of these coastal aquatic environments are known to host a high number of species and to contribute significantly to global productivity, partially due to the environmental heterogeneity found therein (Field et al., 1998). Mechanisms maintaining diversity and productivity in these systems are paramount when considering management strategies aimed at preserving ecosystem health (Mouillot, 2007). To-date the relationship of dispersal with productivity has been addressed using modeling approaches (Loreau et al., 2003; Filotas et al., 2010), however experimental evidence to support these findings is scarce (but see Venail et al., 2008; 2010; Gravel et al., 2011).

According to theory, dispersal has a non-monotonic effect on productivity, with maxima at intermediate dispersal rates (Loreau et al., 2003). To elucidate mechanisms underlying this relationship, Loreau et al. (2003) employed a generalized model on consumer-resource dynamics using a gradient of environmental conditions. They suggested that productivity increases up to an intermediate level of dispersal, being influenced by a respective increase in species richness. At even higher levels of dispersal, however, productivity declines due to the decline of species richness. However, productivity at high rates of dispersal is never as low as in the low dispersal condition because a second mechanism, i.e. spatial averaging, becomes more prominent at high dispersal rates, moderating decreases in productivity due to species loss. Spatial averaging takes place when a metacommunity is homogenized to the point that it resembles an assemblage composition comprised of species most fit to the average condition. These two mechanisms form the basis of the spatial insurance hypothesis that addresses the role of dispersal as a diversity-sustaining mechanism that increases stability of ecosystem functions (Gonzalez et al., 2009). Previous experimental studies have focused on species richness as a mechanism driving the productivity-dispersal relationship (Matthiessen and Hillebrand, 2006; Venail et al., 2008; 2010), whereas the role of spatial averaging has not been addressed.

The importance of spatial averaging may be particularly relevant in metacommunities characterized by environmental heterogeneity. Estuarine lakes, systems of interconnected lagoons, salt ponds, marshes and rock pools are typical examples of systems characterized by environmental heterogeneity, especially in regards to nutrients and salinity. Considering nutrients, the magnitude and mode of supply associated with inflows to coastal aquatic environments is known to affect diversity and biomass of primary producers (e.g. phytoplankton) (Roelke, 2000; Buyukates and Roelke, 2005). Moreover, pulsed inflows can be regarded as external disturbances, causing population losses through hydraulic displacement and growth stimulation associated with nutrient loading (Roelke and Eldridge, 2008), thereby presenting a complex relationship with species richness. Greater species richness is promoted at intermediate inflow pulsing frequencies, following the intermediate disturbance hypothesis (Reynolds, 1993; Padisak, 1993; Sommer, 1995). Differential inflow pulses in a heterogeneous environment can thus lead to varied localized conditions and distinct assemblage compositions.

The aim of this study was to assess the role of spatial averaging and species richness on productivity in metacommunities of varied dispersal rates. Towards this end, we incubated a naturally occurring phytoplankton assemblage over a range of inflow pulsing frequencies that resembled different environmental conditions in regards to hydraulic displacement and nutrient loading. Under these varied inflow conditions, processes of self-organization led to distinct phytoplankton assemblages optimized to the various pulsed inflow frequencies. The composition of these assemblages was comprised of species commonly observed in waters of the Aegean Sea, suggesting that no invasive species were a part of the initial condition. After these distinct assemblages emerged through self-organization, we connected them at varied dispersal rates, thereby creating metacommunities of varied connectivity. To elucidate underlying mechanisms of observed relationships, population dynamics in each local assemblage were investigated. We hypothesized that high dispersal, representing high ecosystem connectivity, would lead to decreased species richness and thus lower productivity. Under these conditions, we expected that species adapted to intermediate environmental conditions within the heterogeneous system (i.e. intermediate inflow pulsing frequency) would dominate the metacommunity.

METHODS

Laboratory research employing naturally occurring phytoplankton was carried out in two sequential stages. The first stage involved culturing freshly collected water from the Aegean Sea under different culture conditions that involved three levels of pulsed media inflow and hydraulic displacement. The natural assemblages were maintained under these pulsing conditions until steady-state emerged. These self-organized assemblages, now referred to as isolated local assemblages, were then used in the second stage of the research, where they were connected through culture exchanges of differing volume, thereby creating metacommunities. The volume of culture exchanged was directly related to dispersal rate, since our target organisms were phytoplankton in well-mixed experimental units.

In the experiments described below, dispersal involved an exchange of equivalent culture volumes between vessels. Dispersal did not involve removing any biomass or nutrients from the metacommunity. Hydraulic displacement, on the other hand, did involve removing biomass and nutrients from the metacommunity, and replacing the displaced volume with fresh, cell-free media. So, culture vessels described below as isolated patches only experienced hydraulic displacements (flushing events), and culture vessels that were a part of metacommunities experienced flushing events and dispersal events. Flushing events were continued throughout the second stage of research in both the isolated cultures and the connected local assemblages to ensure that changes in species composition and productivity that might occur during the second stage of research were attributed to dispersal and not altered flushing events of the local patches.

Our response variables for these experiments included accumulated biomasses at steady-state. When at steady state, productivity (biomass per time) is the biomass multiplied by the flushing rate, where the flushing rate is equal to the rate of hydraulic displacement (volume per time) divided by the volume of the experimental unit, so final units of flushing are ‘per time’. Because all of our experimental units experienced the same hydraulic flushing over the period of the experiment, trends observed in accumulated biomass were analogous to trends in productivity. Consequently, in the present study we analyze accumulated biomass at steady-state as a surrogate for productivity.

Self-organization - formation of isolated local assemblages

Surface water from the Aegean Sea was collected into 10L Nalgene carboys and transferred to the laboratory shortly after. Upon arrival, a portion of the water was filtered through 47mm Whatman GF/F glass fiber filters, and used for the preparation of f/2 media (Guillard and Ryther, 1962). Water to be used as inoculum for cultures was pre-filtered through a 100μm mesh-size plankton net to avoid bias from mesozooplankton (Katechakis et al., 2002). Experiments were initiated approximately 10h after water collection.

Cultures were kept in a climate-controlled chamber where temperature was held constant at 20°C and photoperiod at a 12-h light:dark cycle. Cool white fluorescent bulbs were used as the light source and irradiance was 200µmol/m^2/s. This value was in the range of typical light saturated photosynthesis rates of phytoplankton (Kirk, 1994). Cultures were kept well mixed with constant and gentle stirring using magnetic stirrers.

The experiment was conducted using semi-continuous cultures in 1L vessels. Three culturing conditions were created where fresh media was pulsed into the cultures at different frequencies (once every day, once every 3 days, and once every 6 days). The volume displacement and magnitude of nutrient loading over time was the same for all culturing conditions. This was achieved by removing a culture volume of 100ml every day, 300ml every 3rd day, and 600ml every 6thday from the respective culture conditions, then replacing the volume removed with fresh f/2 media. These different pulsing frequencies were employed to reflect the different environmental conditions driving local community structure. Each culture condition was replicated thrice, thus nine 1L vessels were used in the first stage of the research.

Cultures were allowed to self-organize and reach steady-state under these pulsed frequency conditions, i.e. the species composition and relative abundances were constant in each of the nine culturing vessels for a period not less than 15 days. At the end of the self-organization period for stage one of the research, which lasted 3 months, one culture vessel from each of the three flushing treatments was randomly selected for use in the second stage of the research.

Dispersal – formation of metacommunities

For the initiation of the second stage of the research, aliquots from the three vessels selected from the first stage of the research were distributed into additional 1L vessels. This occurred during a day of a scheduled pulsed flushing event, so that the disturbance to the isolated cultures would be minimized. Because our goal was to replicate each metacommunity thrice, where each metacommunity was comprised of three vessels (three patches), twenty-seven 1L vessels were used (Fig.1). Patches of the metacommunities designed to receive pulsed flushing of 1-, 3- and 6-day frequency were initiated with assemblages that reached steady state under those same conditions, i.e., 1-, 3- and 6-day frequency. For initiation of the 27 vessels, 100 ml of culture from the first stage of the research was transferred, and the volume raised to 1L using fresh f/2 media. The three vessels from the first experiment that were selected to initiate the second stage of research were maintained with continued hydraulic displacements, but no dispersal, and used as controls for the second stage of the research. To ensure replication for the isolated assemblages, additional 6 vessels (2 per treatment) were formed as above to be used together with the three vessels from the first experiment as controls (Fig.1). All the above vessels were allowed to reach the known steady-state before initiating dispersal.

Using the twenty-seven vessels with transferred culture, three metacommunities were contrived (each replicated thrice, see Fig.1) where dispersal rate was controlled among the three local assemblages. Rates of dispersal were of low ($0.02d^{-1}$), intermediate ($0.1d^{-1}$) and high ($0.2d^{-1}$) magnitude. Because the 1L vessels were well mixed, dispersal was directly proportional to the volume of water exchanged between vessels. For example, at the $0.2\ d^{-1}$ dispersal rate, 200 ml were removed from one vessel, split into 100 ml aliquots with each aliquot being added to the other vessels. This procedure was performed simultaneously between the three local assemblages of each metacommunity. Thus, at the $0.2\ d^{-1}$ dispersal rate each local assemblage lost 200 ml from its assemblage and gained 100 ml from each of the other two local assemblages.

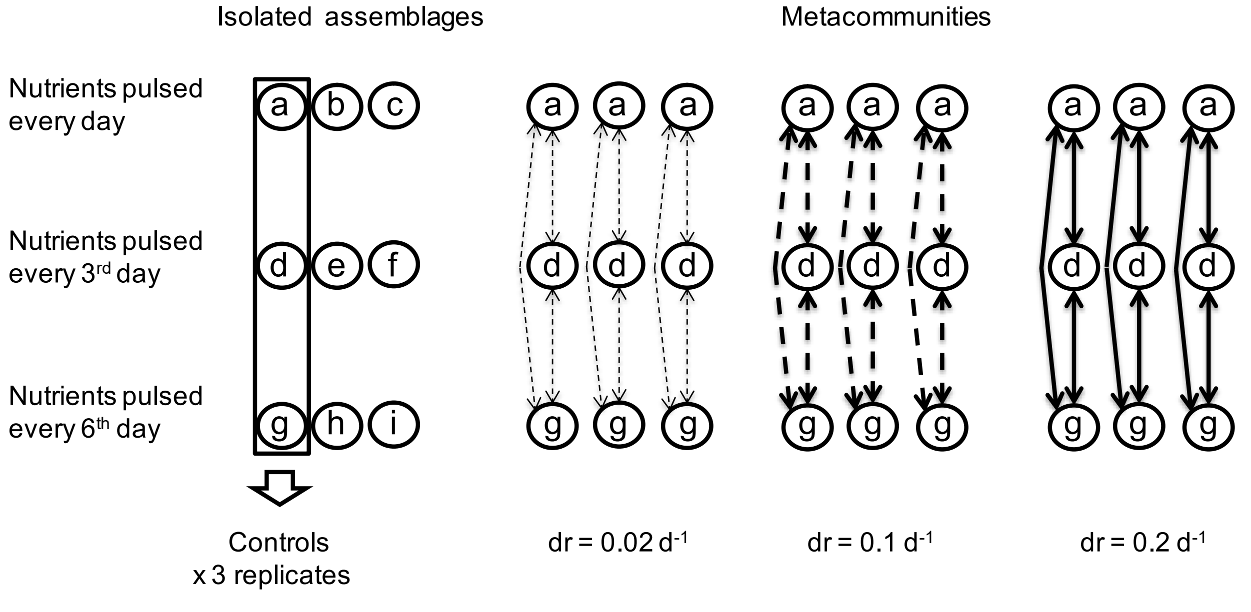

Figure1. Experimental design of metacommunities with differing connectivity. Metacommunities were formed by connecting previously isolated patches where phytoplankton assemblages were allowed to self-organize at three levels of inflow pulsing frequency. The metacommunities differed in their connectivity based on the dispersal rate.

The dispersal rates selected were based on theoretical and experimental studies showing that variation in productivity and species richness usually occurred within this dispersal range (Mouquet and Loreau, 2003; Loreau et al., 2003; Filotas et al., 2010; Howeth and Leibold, 2010). We could not use species traits to define dispersal rates, since our experimental design represents a natural system where exchanges between water masses occur over horizontal distances. For instance, while phytoplankton have varied life-history traits that enable locomotion for some taxa and adjustment of their buoyancy for others, the benefits of locomotion and buoyancy regulation are most realized when considering water-column optimization, that is, species positioning themselves to best take advantage of irradiance and nutrient vertical profiles. Locomotion and buoyancy regulation provide little benefit to phytoplankton when considering horizontal migration, which is associated with water circulation and exchange between adjacent water masses.

Each dispersal experiment lasted 30 days, enough time for local assemblages to reach a new steady-state. To follow species dynamics in the metacommunities and to monitor steady-states in the isolated assemblages, time series sampling was conducted. Every 3 days, 5ml of culture were removed from vessels and fixed with Lugol's iodine solution for microscopic analysis. For the patches receiving 1- and 3-day pulsed flushing events, this coincided with a scheduled hydraulic displacement. For the patches receiving 6d pulsing frequency, the 5ml of culture removed on days between the scheduled 6d pulsed flushing event were not replaced with fresh media until the next scheduled pulse. In the isolated assemblages culture samples for analysis were removed every 6 days, since those assemblages were at steady-state and their populations remained invariable with time.

Cell counting was performed using inverted light microscopy (Motic AE31, 400X) (Utermöhl, 1958). Cell biovolumes, a common measure of algal biomass, were estimated by measuring cellular dimensions corresponding to representative geometrical shapes (Hillebrand et al., 1999). In addition, intracellular carbon was approximated using taxon-specific equations that converted cellular volume to carbon mass (Menden-Deuer and Lessard, 2000). Phosphate and nitrate concentrations were measured according to Parsons et al. (1984) in the control cultures and in each local assemblage at the end of the dispersal experiments (after 30 days).

The Berger-Parker dominance index (BP) was calculated along with species richness as a measure of assemblage structure. The unimodal relationship between dispersal and species richness or productivity at local scale was statistically evaluated according to Cusens et al. (2012). Analysis of variance (one-way ANOVA) was used to reveal differences in accumulated biomass and nutrient concentrations among the different dispersal treatments, between isolated cultures and between isolated and connected assemblages. Since nutrient concentrations and species biovolumes between dispersal levels, were not significantly different, they were aggregated (*n*=9) in this analysis. Statistical analysis was performed in R (package v.2.15.1; R Development Core Team 2012).

RESULTS

The self-organized phytoplankton assemblages used in the initial condition of the metacommunity experiments differed in species composition, assemblage structure, biomass, and nutrient concentrations (Fig.2d,e). The selected culture receiving daily pulses while in isolation had a relatively even assemblage (BP=0.53) that consisted of three diatoms, *Navicula* sp., *Cyclotella* sp. and an unidentified species (Fig.4a). On the other hand, the selected cultures receiving pulses every 3rd and 6th day while in isolation were more speciose and characterized by high dominance (BP>0.93) of *Nitzschia* sp. and *Nannochloris* sp. respectively (Fig.4b,c, Fig.2a-c). The three selected assemblages from stage one of the research were highly differentiated from the original assemblage from the Aegean Sea with respect to species richness and the identity of dominant species.

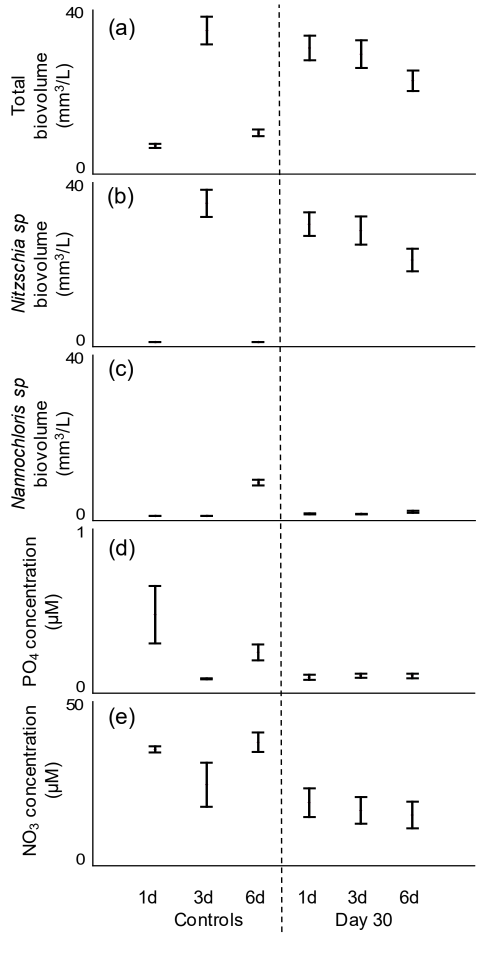

Figure2. Total biovolume (mm^3/L) (a) and the biovolume of the two most dominant species, namely *Nitzschia* sp. (b) and *Nannochloris*sp. (c), along with the change in nutrient concentrations in the isolated patches (left-Controls, n=3) and at the end of dispersal experiments (right-Day 30, n=9). 1d: every day nutrient pulsing frequency, 3d: every 3 days nutrient pulsing frequency, 6d: every 6 days nutrient pulsing frequency.

In the metacommunity with low dispersal, local species richness (i.e. species richness inside each culture vessel) was higher at the end of the dispersal experiment compared to the culture vessels maintained in isolation. In addition, regional richness (the combined richness of the three culture vessels connected through dispersal, i.e., the metacommunity) remained high (Fig.3a). In the metacommunities with intermediate and high dispersal between culture vessels, richness decreased at both local and regional scales. This resulted in a unimodal relationship between local richness and dispersal (Fig.3a), according to the significant quadratic term of the ordinary least squares regression analysis (p-value<0.01) and the locally weighted sums of squares (LOWESS) plot (not presented). Productivity was higher at the end of the experiment at all dispersal levels compared to isolated cultures but no differences were observed between levels of dispersal (Fig.3b). The quadratic term was not significant (p-value>0.05), revealing a positive linear relationship at both local and metacommunity level.

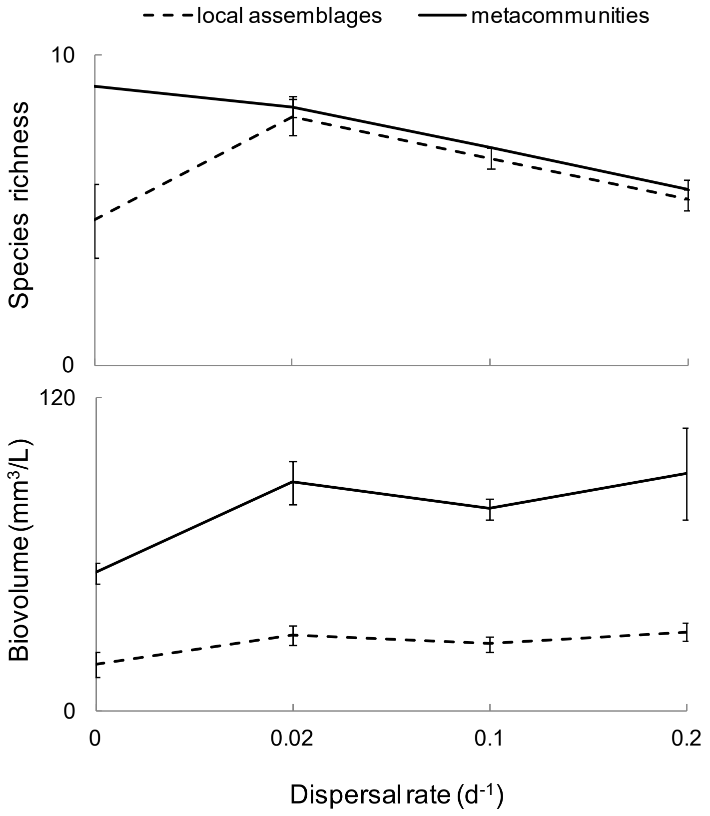

Figure3. Species richness (a) and productivity presented as biovolume (mm^3/L) (b) as a function of dispersal rate. Error bars in local assemblages present standard errors of the means of the three local assemblages connected through dispersal. Error bars in the metacommunities are standard errors of the three replicate metacommunities.

As mentioned above, the diatom *Nitzschia* sp. was best adapted in the isolated culture vessels of intermediate pulsing frequency (i.e. pulses every 3rd day) (Fig.4b). While *Nitzschia* sp. was present in the initial species pool, it went extinct during the process of self-organization in the isolated culture vessels receiving daily pulses (Fig.4a), and it was very rare in the isolated culture vessels receiving pulses every 6th day (Fig.4c). With dispersal, *Nitzschia* sp. remained dominant in the local assemblages receiving pulses at an intermediate frequency. In addition, with dispersal it became dominant in the local assemblages of low- and high-pulsing frequency, environmental conditions that did not favor its growth in the isolated culture vessels. The biomasses of species that were best adapted at low- and high-pulsing frequency when isolated were drastically reduced when connected in a metacommunity (Fig.4d,g,j and Fig.4f,i,l). This is clearly seen in the displacement of *Nannochloris* sp. in the local assemblages receiving pulses every 6th day (Fig.4f,i,l). The takeover of *Nitzschia* sp. occurred at all dispersal levels and happened quicker as dispersal rate increased. When cellular biovolumes were converted to taxon-specific carbon equivalents, results were identical (not shown).

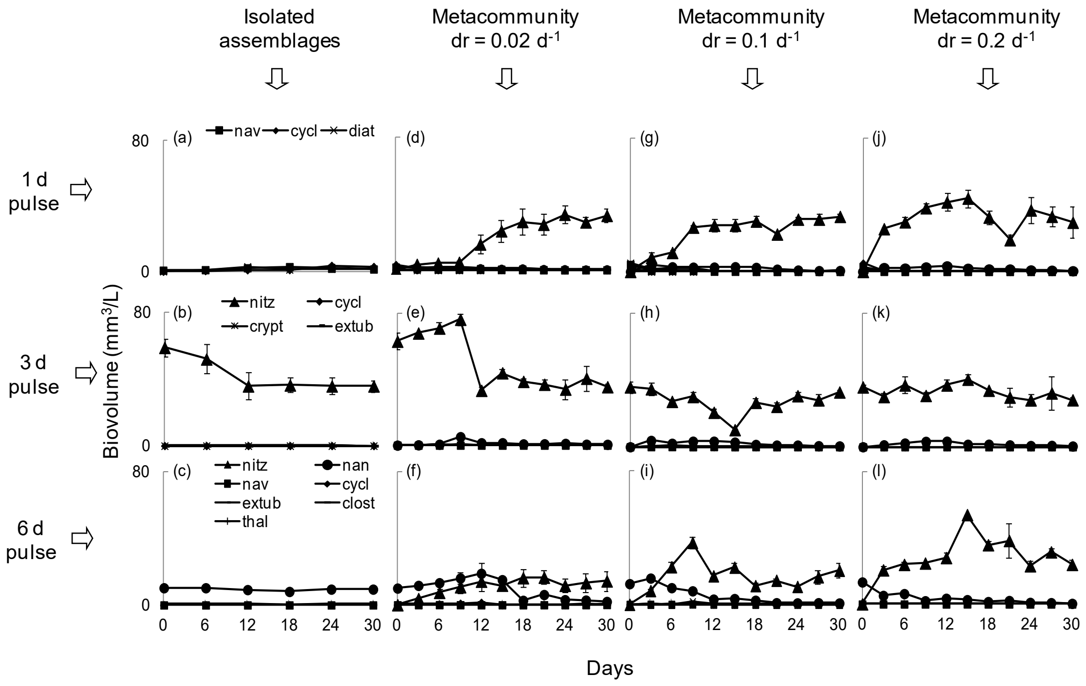

Figure4. Phytoplankton population dynamics in each isolated patch with patches differing in inflow pulsing frequency (a-c), and the three metacommunities with connected patches still having different inflow pulsing frequency (shown vertically in figure panels) but connected using a gradient of dispersal rates (shown horizontally in figure panels).
nitz: *Nitzschia* sp., nan: *Nannochloris* sp*.,* cycl: *Cyclotella* sp*.,* nav: *Navicula* sp.*,* diat: diatom, crypt: cryptophyceae, extub: *Extubocellulus* sp.*,* clost: *Cylindrotheca closterium,* thal: *Thalassiosira* sp. Species with low biovolume range from 0.01 to 1.5 mm^3/L.

Nutrient concentrations varied among isolated culture vessels according to the nutrient pulsing frequency they received, as well as between the isolated culture vessels and the connected local assemblages. In the isolated culture vessels, phosphate concentration was the lowest at the intermediate pulsing frequency (pulses every 3rd day), where *Nitzschia* sp. dominated the assemblage (ANOVA, p-value <0.01). After 30 days of dispersal, phosphate levels in all patches of the metacommunity dropped to the same level as the isolated culture vessel receiving pulsed flushing of intermediate frequency (ANOVA, p-value >0.05). This decrease in phosphate concentration in the patches of the metacommunities corresponded to the biomass increases of *Nitzschia* sp. in these culture vessels, which suggests that the *Nitzschia* sp. was the most competitive of all species observed for phosphate (Fig.2). Nitrate concentration in the isolated culture vessels was also lower at the intermediate pulsing frequency. However, the difference was not statistically significant (ANOVA, p-value >0.5). With dispersal, all culture vessels presented the same nitrate concentration regardless of pulsing frequency (Fig.2e).

DISCUSSION

The importance of inflow pulsing frequencies in shaping phytoplankton assemblage composition and accumulated biomass was demonstrated in experimental and field studies (Roelke et al., 1997; Roelke et al., 1999; Roelke, 2000; Buyukates and Roelke, 2005; Spatharis et al., 2007; Roelke and Eldridge, 2008). This is in agreement with the present findings in regards to the assemblages in the isolated culture vessels. In those, different inflow pulsing frequencies resulted in self-organized assemblages of varied structure, composition and biomass despite the use of the same initial species pool. This implies that the dominant species in each pulse has its niche optimum at the given environmental condition, allowing us to assume niche complementarity following Loreau et al. (2003). Inflow pulses of intermediate frequency have been shown to present higher species richness compared to lower and higher frequency inflow pulses (Padisak, 1993; Sommer, 1995). In our study the assemblage with the lowest inflow pulsing frequency (once every 6th day) retained the highest number of species (7 species), suggesting that this pulsing frequency cannot be regarded as low but rather as intermediate.

In regards to the metacommunity experiments, our observations of declining metacommunity richness at high dispersal rates are in agreement to theory (Mouquet and Loreau, 2003; Holyoak et al., 2005) and previous experimental studies (Cadotte, 2006). At high connectivity, dispersal homogenizes the environmental conditions in the metacommunity, and competitive exclusion leads some species to extinction (Leibold and Miller, 2004; Holyoak et al., 2005). This unimodal species richness-dispersal relationship indicates that important structural changes in aquatic metacommunities are expected to occur over the range of dispersal rates employed here (0.02-0.2 d^{-1}). On the other hand, productivity in the interconnected assemblages was higher than the isolated ones and remained high as dispersal increased. This is in contrast to theory, which predicts a decrease in productivity at high dispersal rates (Loreau et al., 2003). When considering the two mechanisms driving the productivity-dispersal relationship (i.e. species richness and spatial averaging), spatial averaging by the species best adapted to the intermediate inflow pulse conditions seems to play a more predominant role than the respective decrease in species richness. This suggests that in natural systems, the ecosystem function of productivity in the metacommunity may be maintained with increasing dispersal rates, despite the loss of species in the metacommunity.

In our experiments, the role of spatial averaging might have been more pronounced than species loss because the species that was best adapted to the conditions of intermediate pulsing frequency was also a highly productive species. This high productivity can be attributed to its likely low phosphorus requirements (Tilman et al., 1997), as evidenced by the lower phosphate concentrations observed in the isolated culture vessels where this species dominated. Having a lower phosphorus requirement would have provided this species a competitive advantage over other species that were dominant in the isolated culture vessels with daily and 6-day pulsing frequencies (Tilman, 1982). To further validate this assumption, physiological traits of the species involved need to be measured and the population dynamics modeled.

During the self-organization process in the isolated culture vessels, the diatom *Nitzschia* sp. was best adapted to pulsed flushing of intermediate frequency, as evidenced by this taxa becoming dominant in those treatments (i.e. the local patches receiving pulses every 3rd day). It went extinct in the culture vessels receiving pulsed flushing of high frequency (daily pulses) and was very rare in the culture vessels receiving pulsed flushing of low frequency (pulses every 6th day). This probably occurred because the species was unfit to compete in isolation at the high- and low-pulsed flushing frequencies. At the onset of dispersal the mass effect mechanism (i.e. the immigration of cells from the intermediate pulsing frequency patches being more important than the species interactions within patches) likely supplemented the growth of *Nitzschia* sp. in patches receiving pulses of high and low frequency (Amarasekare and Nisbet, 2001; Mouquet and Loreau, 2003; Matthiessen and Hillebrand, 2006) augmenting its populations there. This recurrent immigration of *Nitzschia* cells seemed to enable the species to gradually facilitate environmental conditions in the high- and low- pulsing frequency patches of the metacommunity (i.e. lowering the concentration of phosphate in those patches below the growth thresholds of competing species), favoring persistence of future immigrants. This possibly allowed *Nitzschia* to eventually dominate across the metacommunity. Although the dominance of *Nitzschia* was observed at all dispersal rates, the species came to dominance faster at higher connectivity. This observation is in agreement with Limberger and Wickham (2012) who argued that effects of connectivity are time-dependent. The biomass increase of *Nitzschia* sp. occasionally observed along the self-organization stage is a pattern typically observed for small-sized *Nitzschia* spp. due to their heterotrophic behavior (Hellebust and Lewin, 1977). Although this pattern did not affect the final local assemblage composition and biomass, it might influence the time the species needed to dominate, as also shown for the different dispersal rates in the present study.

Our findings are directly applicable to heterogeneous environments where patches are recurrently isolated then connected, such as estuarine lakes, interconnected lagoons, salt ponds and other coastal aquatic environments. In such systems, local assemblages self-organize according to local conditions during periods of isolation, and these assemblages are often distinct from other local assemblages within the metacommunity. Metacommunity dynamics ensue during periods of connection. Generally, species richness has been regarded as the main driver of ecosystem productivity (Matthiessen and Hillebrand, 2006; Venail et al., 2010). However, the present study provides evidence on the importance of spatial averaging in maintaining high productivity at high ecosystem connectivity. In agreement to generalized theory (Loreau et al., 2003), our observations showed that spatial averaging did occur and the species best adapted to the intermediate environmental condition eventually became dominant in the metacommunities. In the assemblages employed here, this species was also highly productive, resulting in highly productive metacommunities. This observation may be generalized beyond the specific species, as the highly temporal and spatially variable conditions in aquatic metacommunities such as estuaries and interconnected lagoons, favor generalist species tolerant of a wide range of environmental conditions (Costanza et al., 1993). Furthermore, experimental studies have demonstrated that generalist species tend to be more productive, due to their better exploitation of heterogeneous environments (Gravel et al., 2011). The present findings may have important implications regarding ecosystem management practices in response to anticipated effects of climate change (i.e. changing fragmentation and connectivity patterns, see Whitfield and Taylor, 2009), as it highlights the pivotal role of dispersal in the functioning of aquatic ecosystems.

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PUBLISHED AND SUBMITTED WORK

Journals

1. E. Smeti, S. Spatharis, D.L. Roelke. Spatial averaging compensates for species loss in maintaining productivity at high ecosystem connectivity, submitted to Journal of Ecology
2. E. Smeti, D.L. Roelke, G. Gremion, S. Spatharis. Competition between two globally important Pseudo-nitzschia species under N- and P- limitation (in preparation)
3. E. Smeti,G.Tsirtsis, , D.L. Roelke, G.Kokkoris, S. Spatharis. Increasing functional diversity with decreasing resource pulsing frequency following fragmentation (in preparation)

Conferences

1. E. Smeti, G. Gremion , S. Spatharis. Combination of cultures and numerical modeling as a tool for predicting phytoplankton assemblage structure, 6th Panhellenic Conference of Ecology, Athens 2012
2. E. Smeti, S. Spatharis, D. Roelke. Spatial averaging sustains increased productivity at high ecosystem connectivity, ESA 98th Annual Meeting, Minneapolis 2013
3. E.Smeti, S. Spatharis, D. Roelke. The role of dispersal as a mechanism controlling productivity in fragmented ecosystems, AIMEN Workshop, Brest 2013