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Limited evolutionary responses to harvesting regime in the intensive production of algae

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Abstract Plastic changes in the growth and productivity of algae in response to environment and stocking density are well established. In contrast, the capacity for such changes to persist once environmental differences cease, potentially signalling an evolutionary response, have rarely been tested for algae in intensive production systems. We tested whether continuous differences in harvesting regime (a high stocking density/low-yield regime versus low stocking density/high-yield regime) generated changes in biomass productivity and other growth metrics within several strains of the clonal macroalga *Oedogonium* (Chlorophyta, Oedogoniales) and whether such changes persisted once differential harvesting yields ceased. We found considerable plasticity in growth rate and biomass productivity over a 12-week period of active selection (i.e. repeated high-yield and low-yield harvesting of clonal lineages within strains) and that strains responded differently to this selection pressure over time. While small, but significant, differences in growth rates of clonal lineages exposed to high-yield vs low-yield harvesting regimes were maintained after prolonged culture under a common selection regime (i.e. medium-yield harvesting), differences in biomass productivity were not. There was no evidence for positive or negative effects of maintaining multiple strains in polyculture on growth and biomass productivity. Overall, we detected limited potential for evolutionary responses to harvesting regime in the main commercial trait of interest—biomass productivity. This outcome is important for commercial cultivation in intensive production systems, since it identifies a low risk that harvesting practices will impact negatively on biomass productivity in the longer term.

Keywords Aquaculture · *Oedogonium* · Chlorophyceae · Growth · Selection · Polyculture · Monoculture

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Introduction

The production of algae in intensive, land-based culture systems is a relatively new approach with significant commercial potential and interest. Algae have diverse applications as targets for a broad range of biofuels (Mata et al. 2010; Elliott et al. 2015) and as a source of new bioproducts (Gosch et al. 2012; Jiménez-Escrig et al. 2012). More recently, the potential to provide a water treatment service and generate bioproducts are being considered as complementary revenue-raising activities for companies (Woertz et al. 2009; Christenson and Sims 2011; Hafting et al. 2012; Neveux et al. 2014a; Cole et al. 2015). Irrespective of the type of product or service, or the type of algae (micro or macro) considered, optimising the biomass productivity of the system is critical to maximise the value per unit of capital investment (Mata et al. 2010; Park and Craggs 2011; Mata et al., 2016). What is typically proposed for the intensive production of algae in land-based systems is the clonal propagation of a single species (i.e. via cell division for microalgae and fragmentation followed by cell division for macroalgae). These land-based systems are therefore characterised by the continuous, high-density culture

of clonally reproducing algae, with regular partial harvest of the standing stock (Capo et al. 1999; Moheimani and Borowitzka 2006; Rodolfi et al. 2009; Mata et al., 2016). Harvesting regimes are by default high-yield (i.e. a large proportion of the standing stock biomass is removed at each harvest), as species are deliberately targeted for their rapid growth rates. Algal growth and biomass productivity are phenotypically plastic, and their responses to culture conditions as an immediate result of plasticity are well described (Rodolfi et al. 2009; Cole et al. 2014; Samocha et al. 2015). Less is known, however, about the capacity for continual high-yield harvesting to generate stable changes in algal production that could potentially reflect an evolutionary response to this selection pressure.

High-yield harvesting regimes have been shown to cause the rapid and often unwanted evolution of life history traits, including growth rates, biomass productivity and body size, in harvested populations (Hendry et al. 2011). These populations are usually sexually reproducing vertebrates (game animals, ungulates and fish; Conover and Munch 2002; Coltman et al. 2003; Garel et al. 2007; Proaktor et al. 2007; Enberg et al. 2009) or plants (Law and Salick 2005; Mooney and McGraw 2009) with relatively long generation times, for whom evolutionary change relies largely on standing genetic variation (Hendry et al. 2011). Yet for clonal organisms, generation times are often poorly defined and mutations, or other sources of novel variation arising in somatic growth, may contribute to evolutionary change when standing variation is low or depleted (Gill et al. 1995; Fagerström et al. 1998; Orive 2001). Such potential is widely acknowledged for cultivated plants (Larkin and Scowcroft 1981; Meins 1983) and macroalgae (Meneses and Santelices 1999; Poore and Fagerström 2000). Evidence from these groups suggests that cultivation can favour clonal reproduction and enhance mutation accumulation (Pujol et al. 2005; Guillemin et al. 2008), and that mutations can accumulate within surprisingly short periods of time (i.e. 30 days; Meneses and Santelices 1999). From a production perspective, novel variation arising within algal strains may become problematic if it causes strain performance to gradually deteriorate. Alternatively, the selection imposed by harvesting could improve biomass productivity over time—for example, by favouring clonal lineages with high biomass yields under the specific harvesting regime. However, the existing evidence for positive evolutionary responses to harvesting is equivocal, and there are no empirical tests of this concept for algae in intensive culture systems. Thus, there is a clear need to quantify the risks and potential benefits that evolutionary responses to harvesting may present, in order to develop management strategies for large-scale algal production.

An alternative approach to optimising large-scale algal production is to include diversity in cultures through intentional polyculture or mixed assemblages, with the goal of mitigating any negative impacts of high-yield harvesting on algal life history traits. A range of studies on natural systems, including marine and freshwater algae (Bruno et al. 2006; Stachowicz et al. 2008a, 2008b; Cardinale 2011), macrophytes (Engelhardt and Ritchie 2001) and terrestrial grasses (Tilman et al. 1996, 2001; Hector et al. 1999), have shown that mixtures of species, genotypes or functional groups can be more productive than monocultures. Yet, other studies have found weak or non-existent effects of diversity on productivity, or that species composition mattered more than diversity itself (Hector et al. 2002; Hooper and Dukes 2004; Stachowicz et al. 2007; Salo et al. 2009; Lawton et al. 2013). In agricultural systems, however, the belief that monocultures and intensively managed single species are more productive than are diversified systems has been a challenge to moving these systems toward greater diversity (Lin 2011). While few large-scale experiments have explored diversity effects on productivity in such systems, research has shown that high diversity within agricultural plots can increase production levels relative to less diverse systems (Tilman et al. 2006), and that polycultures can outyield monocultures in biomass (Picasso et al. 2008). Elsewhere, however, temporal diversity in terms of crop rotation resulted in lower yield relative to monocropped systems, but the yield was of higher quality (Snapp et al. 2010). These contrasting results suggest that diversity effects on productivity are context-dependent and should therefore be evaluated for algae in intensive, land-based culture systems to determine whether increasing the diversity of cultures can offset potential declines in growth and biomass productivity.

The aim of this study was to evaluate the potential for evolutionary responses to high-yield harvesting in the production of a freshwater macroalgal genus, *Oedogonium*, maintained in intensive culture systems. We also sought to understand whether responses to harvesting were contingent on the initial algal strain. We employed principles from laboratory natural selection (Garland and Rose, 2009) to test these aims, exposing replicate cultures from each of three *Oedogonium* strains to 12 weeks of active selection imposed by high-yield and low-yield harvesting regimes. We did so for each strain isolated in monoculture and for all strains combined in polyculture. At the end of this active-selection period, all cultures were maintained for a further 4 weeks under a common selection regime (i.e. medium-yield harvesting) that relaxed differences in selection within strains. We then compared growth and biomass productivity across harvesting regimes after active selection, and again after relaxed selection, to determine whether responses to harvesting were due to the immediate effects of phenotypic plasticity (and therefore dissipated once selection was

relaxed) or could potentially be attributed to evolutionary change (and therefore persisted beyond this point).

Methods

Study system

Oedogonium is a cosmopolitan genus of freshwater green macroalgae. Rapid growth and high productivity make it a robust, competitively dominant genus that has consequently been identified as a key target for the treatment of freshwater waste streams (Roberts et al. 2013; Cole et al. 2014) and as feedstock biomass for bioenergy applications (Lawton et al. 2013; Neveux et al. 2014b). *Oedogonium* has filaments that are one cell thick, and all cells can divide to initiate new growth. It has no predetermined lifespan or generation time in the usual sense. In our cultures of up to 4 years (Lawton et al. 2014), reproduction is exclusively clonal (female reproductive structures have occasionally been observed but male structures have not), with filaments multiplying via fragmentation and subsequent growth of new fragments. Like other macroalgae with similar biology, this combination of rapid, filamentous growth and prolonged clonality allows genetic variation to accumulate during cell division (e.g. via somatic mutation, mitotic recombination and plaoidy changes) and be passed onto all subsequent cells within a lineage (Gill et al. 1995; Meneses and Santelices 1999; Poore and Fagerström 2000). Cultures of filaments that are clonal lineages of a single strain may therefore become genetically variable over time (Meneses and Santelices 1999; Monro and Poore 2004), allowing selection among clonal lineages to potentially cause evolutionary change within strains by purging deleterious mutations and spreading beneficial ones (Orive 2001; Monro and Poore 2009).

Sample collection and isolation of strains

This experiment used three strains of *Oedogonium*—Tsv1, Tsv2 and Tsv11 (Genbank accession numbers KC701473, KU195821 and KU170196, respectively)—that were originally isolated from samples of natural water bodies and wetland areas around Townsville, Queensland, Australia (Lawton et al. 2014, Supporting Information, Appendix 1). Strains were genetically distinct according to sequence data (Supporting information, Fig. S1). Tsv2 was identified as *O. intermedium* using taxonomic keys (Entwisle et al. 2007). It was not possible to identify Tsv1 or Tsv11 to species level as diagnostic characteristics (i.e. reproductive structures) were not visible in either strain and their ITS sequences did not match those of any *Oedogonium* species in Genbank (Lawton et al. 2014, Supporting Information, Appendix 1). Following isolation, strains were maintained in nutrient-enriched (0.1 g L^{-1} of MAF growth medium: 13.4% N, 1.4% P; Manutech Pty Ltd) autoclaved freshwater in a temperature and light controlled laboratory (12:12 light/dark cycle, 50 μmol photons m^{-2} s^{-1}, 23 °C) at James Cook University (JCU) for at least 2 years prior to the experiment. Stock cultures of each strain were established in 1000-L plastic tanks maintained in a greenhouse with ambient natural light at the Marine and Aquaculture Research Facility Unit, James Cook University. Cultures were provided with aeration by a continuous stream of air entering the cultures through multiple inlets around the base of the tanks. Stock cultures were maintained for 3 weeks under these conditions prior to the start of the experiment.

Laboratory natural selection experiment

We used a laboratory natural selection experiment (Garland and Rose, 2009) to explore the potential for clonal lineages of *Oedogonium* to diverge in response to differential harvesting (the agent of selection). To do so, we established 20 replicate monocultures of each strain ($M_{1–3}$), as well as 20 replicate polycultures (P) containing equal quantities of fresh weight (FW) biomass per strain. Note that polycultures contained an additional strain (Tsv4, Genbank accession number KU170195 and identical to Tsv2 genetically) whose monocultures were excluded from analyses due to the loss of all replicates in one harvest regime treatment. We applied different harvesting regime treatments within each strain, and within the polyculture, by randomly assigning ten replicate cultures of each M_n and P to a high-yield harvest treatment and the other ten replicates to a low-yield harvest treatment. The treatments were created by initially stocking high-yield cultures at a density of 0.5 g FW L^{-1} and low-yield cultures at a density of 2 g FW L^{-1}, then returning all stocking densities to their initial values at each harvest. In effect, this meant that ~70% of the biomass was removed from high-yield cultures and ~20% of the biomass was removed from low-yield cultures at each harvest (Supporting information, Fig. S3). Stocking densities were based on average growth rates in our production system and initial growth-curve data (Supporting Information, Appendix 2 and Fig. S2), ensuring that cultures in both treatments had final densities well below the level at which growth was limited (see the "Growth curves" section and Supporting Information, Appendix 2 and Fig. S2). Each replicate culture was harvested every 7 days. The harvested biomass was briefly spun in a centrifuge to remove excess water, and then weighed to determine FW. Each culture was then restocked using the same biomass but with excess biomass removed to reset stocking density to the relevant treatment level (0.5 or 2 g FW L^{-1}). Biomass from each culture was kept separate throughout the experiment, maintaining a pure isolate of each strain × treatment combination.

This entire process was repeated every week for a total of 12 weeks, representing 12 cycles of active selection (i.e. repeated application of high-yield and low-yield harvest treatments within strains). The process was then repeated for a further 4 weeks (representing 4 cycles) of relaxed selection. During this period, all replicates were cultured under a common, intermediate selection regime that removed differences in selection within strains, to evaluate whether any effects of differential harvesting persisted once this selection pressure eased (Garland and Rose 2009). Here, stocking densities were reset to 1 g FW L^{-1} following harvesting, equivalent to a medium-yield harvesting treatment with ~50% of the biomass removed at each harvest. In total, therefore, the selection experiment ran for 16 weeks, which can potentially harbour significant levels of broad-sense heritability in life history traits such as growth and productivity (Lawton et al. 2015).

Culture conditions

Cultures were grown in 20-L plastic buckets in a greenhouse with ambient natural light at the Marine and Aquaculture Research Facility Unit, James Cook University. The culture water was enriched (0.1 g L^{-1}) with MAF growth medium (13.4% N, 1.4% P; Manutech Pty Ltd). Buckets were placed in a water bath with continuous flow to minimise large temperature fluctuations. Average water temperature throughout the experiment was 25.6 °C (±0.7 S.D.) and cultures received an average total photosynthetically active radiation of 150 mol photons m^{-2} $week^{-1}$ (±33 S.D.) (Supporting information, Fig. S4). Cultures were provided with aeration by a continuous stream of air entering the cultures through multiple inlets around the base of the buckets. This served to move the biomass throughout the tank in a "tumble" culture. The stocking density treatments essentially created two different starting points for light availability, as biomass in the high-stocking density (low yield) treatment was effectively in a low-light environment compared to that of the low stocking density (for additional information on the interaction between stocking density, light availability and photosynthesis in tumble culture see Magnusson et al. 2015).

Measured phenotypes

Growth rate and productivity Specific growth rates (SGR) and dry weight (DW) productivity were calculated weekly for each replicate culture. SGR was calculated using the equation SGR (% day^{-1}) = $\mathrm{Ln}(B_f/B_i)/T*100$, where B_f and B_i were the final and initial algal biomasses (g) and T was the number of days in culture. DW productivity (g DW m^{-2} day^{-1}) was calculated using the eq. $P = [(B_f - B_i)/FW/DW]/A/T$, where B_f and B_i were the final and initial algal biomasses (g), $FW{:}DW$ was the fresh weight to dry weight ratio, A was the area (m^2) of culture tanks and T was the number of days in culture. FW/DW was obtained by taking the excess biomass that was not used to restock each culture and weighing it before (FW) and after (DW) drying in an oven at 65 °C for at least 48 h. Ash-free dry weight (AFDW) productivity was calculated for each replicate culture at week 12 (end of active selection) and week 16 (end of relaxed selection). AFDW productivity (g AFDW m^{-2} day^{-1}) was calculated using the eq. $P = \{[(B_f - B_i)/FW/DW]*(1 - ash)\}/A/T$, where B_f and B_i were the final and initial algal biomasses (g), FW/DW was the fresh weight to dry weight ratio, ash was the proportional ash content of the dried biomass, A was the area (m^2) of culture tanks and T was the number of days in culture. The ash content of three randomly chosen replicate cultures from each strain × treatment combination was quantified at week 12 (end of active selection) and week 16 (end of relaxed selection) by combusting a 100–300-mg subsample of dried biomass at 550 °C in a muffle furnace until constant weight was reached.

Growth curves Initial 4-week growth curves were constructed for each strain immediately before the active selection period (see Supporting Information, Appendix 2 and Fig. S2) to inform our choice of stocking densities and ensure that they were unlikely to limit growth. We constructed another set of growth curves at the end of active selection (i.e. immediately after week 12) to evaluate whether differential harvesting had caused growth trajectories to diverge among clonal lineages of each strain. Here, biomass harvested at the end of active selection was used to stock three replicate cultures of each strain × treatment combination (for each one, we chose the three fastest-growing replicates at week 12) at an initial density of 0.2 g FW L^{-1}. Cultures were then maintained in 5-L plastic buckets under identical conditions to those described above (see the "Culture conditions" section). Each culture was harvested weekly. The entire biomass per culture was briefly spun in a centrifuge to remove excess water, weighed and then restocked into the same culture, resulting in a continual increase in the stocking density and total biomass of each culture over time. Biomass was never mixed between cultures. This process was repeated for 4 weeks. Water was exchanged once per week to maintain cultures under the same conditions experienced in the selection experiment.

Statistical analysis

Data were analysed in general linear models, fitted using maximum likelihood in SAS 9.3. Strain and harvesting treatment were modelled as fixed effects. Denominator degrees of freedom were computed using the Kenward-Roger correction to account for missing data due to the death of eight cultures (three polycultures, two monocultures of Tsv1 and Tsv2 and one monoculture of Tsv11 from the high-yield treatment) throughout the experiment (Littell et al. 2006). In the case of growth curves, which were evaluated over 4 weeks at the end

of the active selection period, the model also included week as a repeated measure. Weeks were modelled using a heterogeneous first-order regressive error structure, allowing growth to differ from week to week and covariation between weeks to weaken over time (Littell et al. 2006).

Results

Temporal effects of active selection on growth rate and productivity

Across all strains and the polyculture, weekly SGRs were higher in the high-yield harvest treatment than the low-yield harvest treatment during the active selection phase (weeks 1–12), although the relative differences in SGRs between the treatments varied among strains and weeks (Fig. 1; Supporting information, Table S1). Average SGRs across the 12 weeks for the high-yield treatment ranged from 12.1% day^{-1} (±0.8 S.E.) for Tsv1 to 15.5% day^{-1} (±0.4 S.E.) for the polyculture, while average SGRs in the low-yield treatment ranged from 2.5% day^{-1} (±0.3 S.E.) for Tsv11 to 4.5% day^{-1} (±0.2 S.E.) for Tsv2. Similarly, DW productivity during the active selection phase was higher in the high-yield treatment compared to the low-yield treatment across all strains and the polyculture but also varied among strains and weeks (Fig. 2, Supporting information, Table S1). Across the 12 weeks, average DW productivity for the high-yield treatment ranged from 7.2 g DW m^{-2} day^{-1} (±0.1 S.E.) for Tsv11 to 8.2 g DW m^{-2} day^{-1} (±0.3 S.E.) for the polyculture, while average DW productivity in the low-yield treatment ranged from 3.4 g DW m^{-2} day^{-1} (±0.4 S.E.) for Tsv11 to 6.9 g DW m^{-2} day^{-1} (±0.3 S.E.) for Tsv2. There were clear changes in the DW productivity of some strains under the high-yield vs. low-yield treatments over time. The most pronounced change was in the polyculture, where DW productivity of the low-yield treatment gradually increased over time, while the DW productivity of the high-yield treatment gradually decreased (Fig. 2).

Responses to active selection vs relaxed selection

Growth rate and productivity By the end of active selection (week 12), SGR, DW productivity and AFDW productivity all differed significantly between high-yield and low-yield harvest treatments. However, the magnitudes of these differences were inconsistent among strains (Figs. 1, 2, and 3 and Table 1). SGRs were higher in the high-yield treatment compared to the low-yield treatment for all strains and the polyculture (Fig. 1), ranging from 9.6% day^{-1} (±0.9 S.E.) for Tsv1 to 13.6% day^{-1} (±0.5 S.E.) for Tsv11 in the high-yield treatment, and from 1.5% day^{-1} (±0.2 S.E.) for Tsv11 to 3.4% day^{-1} (±0.5 S.E.) for Tsv2 in the low-yield one. DW productivity was also higher in the high-yield treatment compared to the low-yield treatment for Tsv1 (5.4 ± 0.5 S.E. vs 4.0 ± 0.2 S.E. g DW m^{-2} day^{-1}), Tsv11 (6.3 ± 0.2 S.E. vs 2.3 ± 0.3 S.E. g DW m^{-2} day^{-1}) and the polyculture (5.7 ± 1.0 S.E. vs 5.2 ± 0.3 S.E. g DW m^{-2} day^{-1}) but similar across treatments for Tsv2 (4.7 ± 0.7 S.E. vs 4.5 ± 0.7 S.E. g DW m^{-2} day^{-1}) (Fig. 2). Similarly, AFDW productivity was higher in the high-yield treatment compared to the low-yield treatment for Tsv1 (5.0 ± 0.4 S.E. vs 3.8 ± 0.2 S.E. g AFDW m^{-2} day^{-1}), Tsv11

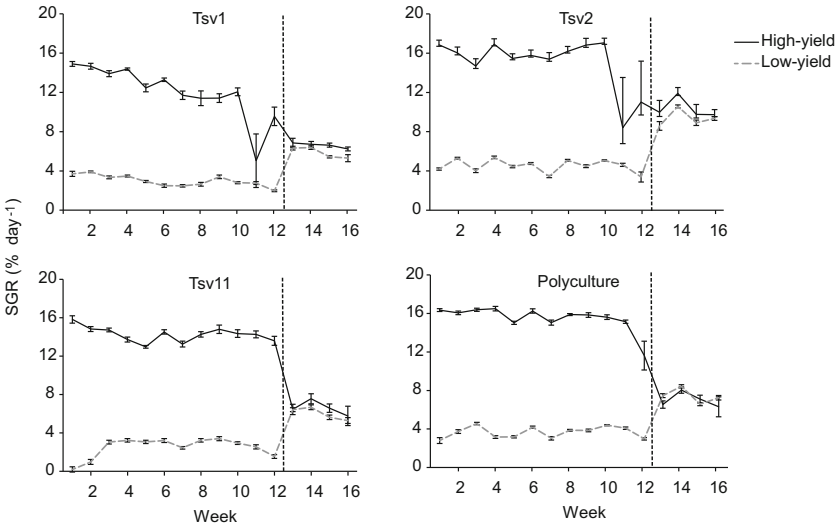

Fig. 1 Specific growth rates (SGR, in % day^{-1}) of *Oedogonium* strains in monoculture (Tsv1, Tsv2, Tsv11) and polyculture (with the addition of Tsv4) maintained under a high-yield harvest treatment (stocking density of 0.5 g FW L^{-1}) and a low-yield harvest treatment (stocking density of 2 g FW L^{-1}). Harvest treatments were applied weekly during 12 weeks of active selection (weeks 1–12), with selection applied within each strain and the polyculture. All cultures were subsequently maintained in an intermediate harvest treatment (stocking density of 1 g FW L^{-1}) for 4 weeks of relaxed selection (weeks 13–16) that removed differences in harvesting within each strain and the polyculture. *Vertical dashed line* indicates where active selection stops and relaxed selection begins. Values shown are means ± SE

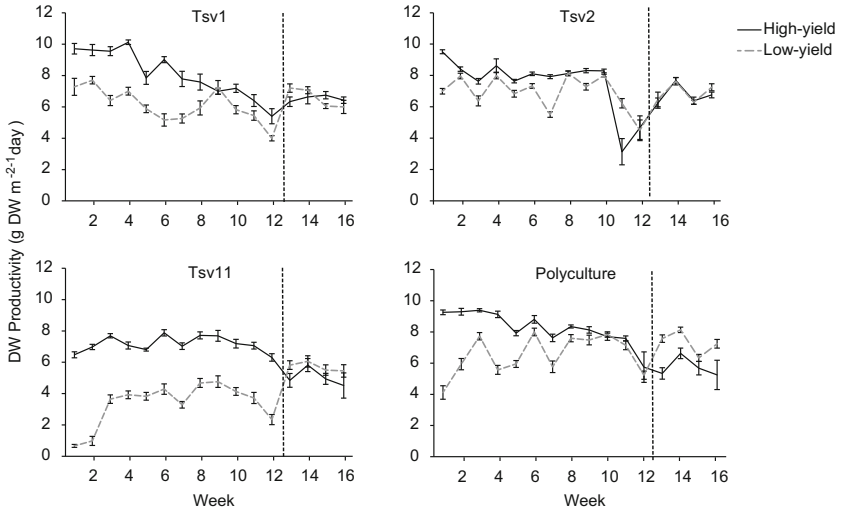

Fig. 2 Dry weight biomass productivity (DW, in g m^{-2} day^{-1}) of *Oedogonium* strains in monoculture (Tsv1, Tsv2, Tsv11) and polyculture (with the addition of Tsv4) maintained under a high-yield harvest treatment (stocking density of 0.5 g FW L^{-1}) and a low-yield harvest treatment (stocking density of 2 g FW L^{-1}). Harvest treatments were applied weekly during 12 weeks of active selection (weeks 1–12), with selection applied within each strain and the polyculture. All cultures were subsequently maintained in an intermediate harvest treatment (stocking density of 1 g FW L^{-1}) for 4 weeks of relaxed selection (weeks 13–16) that removed differences in harvesting within each strain and the polyculture. *Vertical dashed line* indicates where active selection stops and relaxed selection begins. Values shown are means ± SE

(5.6 ± 0.2 S.E. vs 2.1 ± 0.3 S.E. g AFDW m^{-2} day^{-1}) and the polyculture (5.3 ± 0.8 S.E. vs 4.9 ± 0.2 S.E. g AFDW m^{-2} day^{-1}) but was similar across treatments for Tsv2 (4.3 ± 0.7 S.E. vs 4.2 ± 0.6 S.E. g AFDW m^{-2} day^{-1}) (Fig. 3).

By the end of relaxed selection (week 16), the significant difference in SGR between harvest treatments persisted and was now consistent among strains (Table 2 and Fig. 1). However, this difference was relatively small, with high-yield harvesting increasing the daily SGR by 0.65% (±0.26 S.E.). In contrast, the effects of harvest treatment on DW productivity or AFDW productivity had dissipated by the end of relaxed selection (Table 2 and Figs. 2 and 3), leaving only among-strain variation in these metrics.

Growth curves Growth curves were significantly altered by harvesting treatment and remained so over the 4-week evaluation period at the end of active selection (Table 3 and Fig. 4). Specifically, growth rates were higher in cultures stocked with biomass grown under the high-yield harvest treatment compared to cultures stocked with biomass grown under the low-yield harvest treatment. Although growth curves differed among strains (demonstrated by the significant strain × week interaction in Table 3), strains did not differ in their responses to harvesting (Table 3).

Discussion

Despite significant potential for continual high-yield harvesting to exert selective pressures on life history traits (Law 2000; Reznick and Ghalambor 2005; Proaktor et al. 2007; Enberg et al. 2009; Hendry et al. 2011), its potential impacts beyond

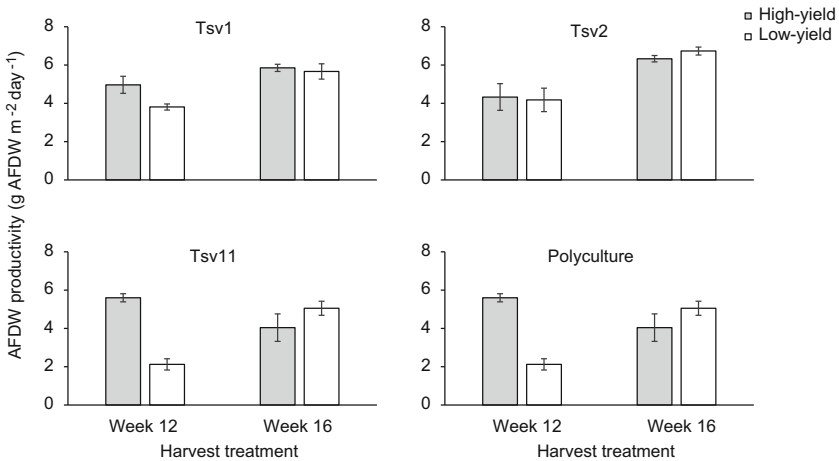

Fig. 3 Ash-free dry weight productivity (AFDW, in g m^{-2} day^{-1}) of *Oedogonium* strains in monoculture (Tsv1, Tsv2, Tsv11) and polyculture (with the addition of Tsv4) at week 12 (end of active selection) and week 16 (end of relaxed selection). Values shown are means ± SE

Table 1 Results of general linear models testing the effects of strain and harvest treatment on the specific growth rates, dry weight (DW) productivity and ash-free dry weight (AFDW) productivity of *Oedogonium* strains in monoculture and polyculture at week 12 (end of active selection)

		Specific growth rate			DW productivity			AFDW productivity		
Effect	Num DF	Den DF	F Value	Pr > F	Den DF	F value	Pr > F	Den DF	F Value	Pr > F
Strain	3	65	15.45	**<0.001**	63	11.43	**<0.001**	63	11.09	**<0.001**
Harvest	1	65	1823.07	**<0.001**	63	33.81	**<0.001**	63	34.17	**<0.001**
Strain × harvest	3	65	9.94	**<0.001**	63	20.97	**<0.001**	63	20.51	**<0.001**

Significant *P* values (<0.05) are shown in bold

immediate, plastic changes in such traits have not been considered for algae in intensive land-based culture systems. We addressed this knowledge gap by experimentally testing whether different continual harvesting regimes (i.e. active selection) generated changes in biomass productivity and other life-history traits within *Oedogonium* strains and whether such changes persisted once they were no longer maintained by differences in harvesting (i.e. under relaxed selection). This latter step was essential to infer that harvesting-induced changes could potentially have an evolutionary basis and are not simply immediate, plastic responses to harvesting. After 12 weeks of active selection, we found significant differences in the SGR and biomass productivity of algae maintained under high-yield vs low-yield harvesting regimes. Significant differences in SGR between harvesting regimes were maintained when all replicate cultures were grown under a common, medium-yield harvesting regime, demonstrating the potential for evolutionary responses to harvesting for the first time for algae. Moreover, growth curves varied significantly between algae maintained under high-yield vs low-yield harvesting regimes. These results show that different harvesting regimes can lead to persistent changes in growth in algae maintained in intensive production systems. However, significant differences in biomass productivity (both DW productivity and AFDW productivity) between harvesting regimes, the key metric for commercial algal production, dissipated once differences in harvesting were relaxed, demonstrating that differential harvesting resulted in plastic rather than evolutionary changes in production traits, with positive implications for the biomass production of algae.

The fact that our high-yield harvesting regime produced positive evolutionary changes in growth, without negatively impacting biomass productivity, is in contrast to the negative evolutionary changes in these traits that have been documented in intensively harvested populations for a range of organisms (Conover and Munch 2002; Coltman et al. 2003; Law and Salick 2005; Edeline et al. 2007; Garel et al. 2007; Mooney and McGraw 2009). However, in each of these studies, larger or older individuals were selectively harvested from the population. In contrast, size- or age-selective harvesting was unlikely to be occurring in our algal cultures as each filament had an equal chance of removal from the culture at each harvest point. Therefore, the differing outcomes between the current study and previous studies may be due to the effects of size- or age-selective harvesting, which may be strong drivers of evolutionary change in growth and population biomass (Ernande et al. 2004). Alternatively, most previous studies have documented evolutionary responses to harvesting that were presumably driven by standing genetic variation among individuals. Except for polycultures, evolutionary responses to harvesting here in *Oedogonium* can only have been driven by somatic mutations arising de novo within strains during cultivation or else by some kind of carry-over environmental effect that can persist even when the selective pressure that created it ceases. Both of these phenomena are potentially important sources of variation in clonally propagated plants and macroalgae, including *Oedogonium* (Schwaegerle et al. 2000; Monro and Poore 2004, 2009; Lawton et al. 2015) but may promote weaker, slower or idiosyncratic evolutionary responses to harvesting relative to standing genetic variation.

Table 2 Results of general linear models testing the effects of strain and harvest treatment on the specific growth rates, dry weight (DW) productivity and ash-free dry weight (AFDW) productivity of *Oedogonium* strains in monoculture and polyculture at week 16 (end of relaxed selection)

		Specific growth rate			DW productivity			AFDW productivity		
Effect	Num DF	Den DF	F Value	Pr > F	Den DF	F Value	Pr > F	Den DF	F Value	Pr > F
strain	3	72	44.59	**<0.001**	72	7.97	**<0.001**	72	7.75	**<0.001**
harvest	1	72	5.68	**0.020**	72	2.37	0.128	72	2.21	0.142
strain*harvest	3	72	1.05	0.378	72	1.50	0.223	72	1.50	0.222

Significant *P* values (<0.05) are shown in bold

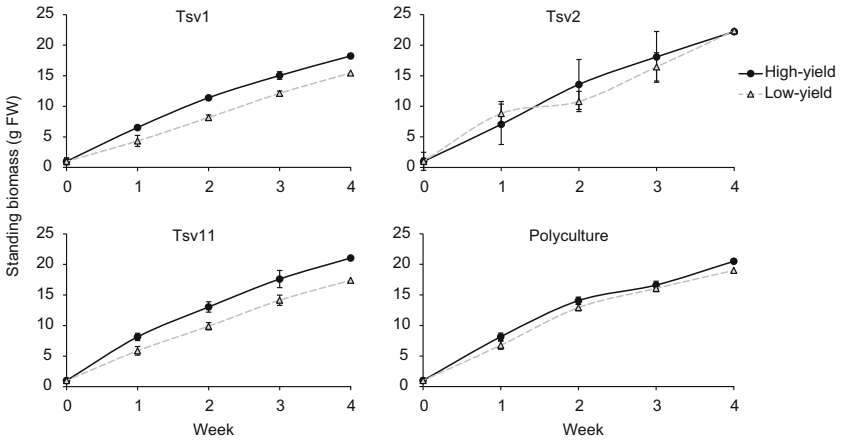

Fig. 4 Growth curves, measured by the standing biomass of fresh weight (FW, in g) of *Oedogonium* strains in monoculture (Tsv1, Tsv2, Tsv11) and polyculture (with the addition of Tsv4), recorded in continuous batch cultures over a 4-week period starting at the end of active selection. Values shown are means ± SE

Regardless of the causes of the differing effect of high-yield harvesting regimes on algae compared to other organisms, our results have several important implications for the commercial production of algae. The key metric for commercial production of algae is areal biomass productivity, rather than growth (Goldman and Ryther 1975; Park and Craggs 2011). This is because most industrial applications and potential end-product uses of algae require large amounts of biomass, and the key parameter for their success therefore is the total amount of algal biomass produced per unit area (Mata et al. 2010; Park and Craggs 2011; Mata et al. 2016). The lack of evolutionary changes in biomass productivity in response to harvesting regime in our experiment implies that intensive algal cultures can be maintained under continuous high-yield harvesting regimes without risk of negative evolutionary changes in biomass productivity. This outcome is particularly important, as maintaining a relatively consistent biomass productivity is imperative for successful commercial algal cultivation (Gellenbeck 2012). Our results also demonstrate that effects on biomass productivity for organisms harvested in natural systems, such as capture fisheries or wild game animals, do not necessary apply to algae cultivated in large-scale industrial systems where production is tightly managed. Finally, the high productivities maintained in our study demonstrate that intensive production of algae via asexual propagation can be maintained for extended periods in monoculture without crashing.

Table 3 Results of a general linear repeated-measures model testing the effects of week, strain and harvest treatment on growth curves (i.e. specific growth rates measured over a 4-week period) of *Oedogonium* strains

Effect	Num DF	Den DF	F Value	Pr > F
Week	3	36	414.82	**<0.001**
Strain	3	24	3.87	**0.022**
Harvest	1	24	7.62	**0.011**
Strain × week	9	46	2.92	**0.008**
Harvest × week	3	36	2.58	0.069
Strain × harvest	3	24	0.52	0.669
Strain × harvest × week	9	46	1.74	0.107

Significant *P* values (<0.05) are shown in bold

The risks of variable biomass productivity in commercial algal cultivation may also be mitigated by including diversity in cultures through intentional polyculture, as mixed species assemblages can have higher productivity than assemblages comprised of a single species (Tilman et al. 1996; Engelhardt and Ritchie 2001; Bruno et al. 2006; Stachowicz et al. 2008b; Cardinale 2011). We did not find that polycultures of the four strains of *Oedogonium* had significantly higher biomass productivity than monocultures of individual strains. Although we found significant strain × treatment interactions for the DW productivity and AFDW productivity of algae maintained under high-yield vs low-yield harvesting regimes, these significant interactions were all driven by differences in the response of strain Tsv2 to the harvesting regimes compared to monocultures of the other strains and the polyculture. Moreover, there were no significant strain × treatment interactions for either variable when all algae were grown under a medium-yield harvesting regime (i.e. relaxed selection). These results demonstrate firstly that increasing the diversity of cultures with multiple strains from a single genus has no phenotypic impact, positive or negative, on the biomass productivity of algae. It is possible that there was not enough niche-variation between strains in the current experiment to lead to significant differences in the biomass productivity of the polyculture compared to monocultures. However, several studies have also found a weak or non-existent effect of increased species or genus diversity on productivity (Hector et al. 2002; Hooper and Dukes 2004; Bruno et al. 2005; Stachowicz et al. 2007, 2008a; Salo et al. 2009; Lawton et al. 2013). Secondly, our results demonstrate that increasing the diversity of cultures does alter the evolutionary response of

algae to harvesting regimes. Commercial cultivation of algae is focused on the production of biomass from pure cultures of a single algal species as this produces a high-quality biomass with a consistent biochemical profile, usually containing specifically targeted natural products (e.g. Borowitzka and Borowitzka 1990; Guerin et al. 2003). Our findings are important therefore from a management perspective as they indicate that any potential costs of maintaining mixed species cultures of algae (e.g. reduced quality of algal biomass due to mixed species composition) as a risk-management strategy will not be offset by increased growth or productivity.

There is a growing recognition that eco-evolutionary dynamics are an important driver of harvested systems more generally. We therefore anticipated stronger, more persistent consequences of intensive harvesting in our system but instead found that most responses were relatively transitory. Given the simplified ecology of intensive production, and resource-rich conditions under which the algae are grown, it could be that ecological effects trump any evolutionary outcomes in our study system. As such, even though we observe some evolutionary change in growth in response to intensive harvesting, the net effect on yield (biomass productivity) is negligible because the resource availability dominates the dynamics of weekly yield. In contrast, evolutionary shifts in the life history in response to harvesting in the wild has more pervasive (and often negative) effects on subsequent yield and sustainability (Walsh et al. 2006).

Although we found only limited potential for the effects of high-yield harvesting on life history characteristics of *Oedogonium* to persist once harvesting ceased, it is possible that effects may have persisted in other traits, including biochemical composition. Seasonal and environmental variation in the biochemical composition of algae is well documented in both wild and cultivated biomass (de Castro and Garcia, 2005; Adams et al. 2011; Gosch et al. 2012). Cultivation conditions, such as nutrient flux and stocking density, can also affect biochemical composition (Pereira et al. 2006; Xin et al. 2010; Cole et al. 2014). Second to areal biomass productivity, the concentration of targeted natural products in the biomass is an imperative feature for successful commercial production of algae and the long-term effects of high-yield harvesting regimes on the biochemical composition of algae should be considered next. In the meantime, the growth and biomass productivity for products and water treatment services are reliable propositions for expanding the algae industry.

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