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Optimizing the initial cultivation stages of kelp *Ecklonia radiata* for restoration

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Abstract

Restoration of kelp forests typically relies on transplanting sporophylls to new locations and has limited application in regions with low remnant kelp cover. Cultivated kelp requires fewer sporophylls and is a potential alternative and sustainable source of transplants for large-scale restoration projects. Naturally sourced fertile sporophylls, however, are still required as 'seed' stock in cultivation practices, thus optimizing cultivation methods is important to minimize this dependency on wild stocks. To assist in optimizing the early-stage cultivation methods for restoring beds of the laminarian kelp *Ecklonia radiata*, we first tested the effects of sporophyll transport, storage, and dehydration on zoospore release. We then tested for effects of inoculum storage temperature on zoospore abundance, and lastly for media sterilization and inoculum concentration effects on both zoospore settlement and resulting gametophyte densities. Our results show, to maximize zoospore release, sporophylls should be transported dry and inoculated within three hours. Inoculum can be stored at 4°C without affecting zoospore abundance and should be added to sterilized media at lower concentrations to reduce settled zoospore density and improve post-settlement survival of the gametophyte stage. This study provides practical recommendations for optimizing the initial cultivation procedures of *E. radiata*. To develop a full life-cycle cultivation protocol for kelp restoration purposes, future research should focus on optimizing sporophyte production, out-planting and transplanting techniques.

Key words: canopy-forming, dehydration, kelp culture, inoculation, macroalgae, zoospores

Implications for Practice

- Cultivated kelp can be an important source of transplants for restoration projects
- To maximize zoospore release, kelp sporophylls should be transported dry and inoculated within 3 hours
- Kelp inoculum can be stored at 4°C without affecting zoospore abundance and should be added to sterilized media at lower concentrations to improve the post-settlement survival of the gametophyte stage
- This study provides important recommendations for optimizing the production of cultivated kelp to aid restoration efforts

Introduction

Kelps, large brown seaweeds of the order Laminariales, are vital to a diversity of important socio-economic and environmental services, including pollution bioremediation, food and habitat provision, and commercial and recreational fisheries (Bennett et al. 2016; Smale et al. 2013; Vasquez et al. 2014). Despite these important services, there has been a global decline in kelp forest cover, due to anthropogenic stressors such as coastal urbanization, pollution, trophic cascades caused by over-fishing, and climate change (Steneck et al. 2002; Strain et al. 2014; Araújo et al. 2016; Krumhansl et al. 2016). Consequently, restoration is needed to help mitigate these declines (Layton et al. 2020; Morris et al. 2020)

Restoration of kelp forests

Restoration of kelp forests typically relies on transplanting sporophytes from natural populations to new locations (Carney et al. 2005; Campbell et al. 2014; Marzinelli et al. 2016). An alternative technique is to culture these transplants via sexual (e.g., mass culturing of embryonic sporophytes; North 1971) or asexual (e.g. culturing fragments of sporophytes; Westermeier et al. 2013, 2016) propagation (Fig. 1). In restoring degraded systems, finding a sustainable population source of sporophytes may limit the use of transplantation as a restoration tool. Cultivation of kelp for restoration could provide a solution to this problem, however, it requires the development of an efficient cultivation method which can produce enough viable transplants for restoration.

Cultivation of kelp for restoration

When cultivating kelps for restoration, the first step is to collect reproductive sporophytes and create an inoculum of zoospores (North 1971; Mabin et al. 2013). This procedure involves several steps that could affect the concentration of inoculum produced, and hence the densities of zoospores on settlement substrates and the subsequent number of cultivated sporophytes (Kerrison et al. 2017). To develop an inoculum, fertile sporophylls with sori are collected from kelp beds and transported to the cultivation facility where sporophylls are processed. Transported sporophylls are cleaned, and sometimes additional steps (e.g., desiccation, agitation) are undertaken to induce zoospore release from sori before inoculation (Mohring et al. 2013a). The inoculation process involves introducing sori to growing media (i.e., natural or artificial seawater) and allowing the release of zoospores to slowly develop an inoculum, and then using the inoculum to seed settlement substrates (e.g., strings, clam shells, ceramic plates) (Devinny & Leventhal 1979; Westermeier et al. 2014). By optimizing the initial steps in cultivation, more sporophytes could potentially be grown from the same quantity of sori, thus reducing the amount of sori needed from degraded kelp beds to sustain large-scale cultivation for restoration.

To date, the cultivation methods used in previous studies have been varied, and it is unclear whether this is species dependent (Alsuwaiyan et al. 2019). Some cultivation experiments successfully used collected sporophylls to directly inoculate seawater without any procedure to induce zoospore release from sori (North 1971; Devinny & Leventhal 1979; Pang et al. 2006). In these cases, the steps in processing the sori may have induced enough stress for zoospore release, such as during transportation (e.g., change in environment, agitation; North 1971; Devinny & Leventhal 1979; Mohring et al. 2013a), or the cleaning of sporophylls in freshwater (osmotic shock; Gutierrez et al. 2006; Westermeier et al. 2014). In other cases, transported sporophylls

were dehydrated before inoculation, which has been suggested as an essential step in the cultivation process (Gutierrez et al. 2006; Hwang et al. 2009). For instance, Mabin et al. (2013) dehydrated sporophylls for one hour in a cool, dark, and dry environment whereas Hwang et al. (2009; 2013) dehydrated for one hour in a dark and humid environment. Consequently, it is unclear if dehydration enhances zoospore release or whether different methods of dehydration affect zoospore production and how this can impact cultivation success.

Experimental study on Ecklonia radiata

In this study we conducted several experiments aimed at determining the optimal cultivation methods for *Ecklonia radiata,* a kelp species dominating temperate reefs along the southern coastline of Australia (Bennett et al. 2016). *E. radiata* forests are in decline, driven by anthropogenic climate change and increasing populations of herbivorous sea urchins (Azzarello et al. 2014; Bennett et al. 2016; Kriegisch et al. 2016; Wernberg et al. 2016). As highlighted in a recent review (Alsuwaiyan et al. 2019), there has been limited research on the optimal methods for zoospore production in an aquaculture context. To determine the conditions for maximizing the abundance and survival of zoospores and gametophytes in culture, we conducted five experiments that tested the effects of: 1) transport methods of sporophylls; 2) dehydration times of sporophylls; 3) dry transport of sporophylls; 4) storage temperatures of inoculum; and 5) sterilization of media and inoculum concentration on abundances and survival of zoospores and gametophytes in culture.

Materials and methods

Collection, preparation and sampling

Reproductively active *E. radiata* sporophylls were collected from Governor Reef, Port Phillip Bay, Victoria (38°09'12.7" S, 114°43'43" E) on February 27th, March 21st and May 15th 2019 (for experiments one to four) and Williamstown, Port Phillip Bay, Victoria (37°52'08.9" S, 114°53'36.7" E) on June 25th 2019 (for experiment five). On each trip, divers collected 10-20 sporophytes, with sporophylls containing sori that were approximately 2-cm in diameter. These sporophylls were stored in a 50 L cooler and transported to the laboratory at the University of Melbourne (Parkville, Victoria) either dry or wet, depending on the experiment, within three hours of collection.

Sori, indicated by the rougher and darker pigmented area on the blades, were cut out from each sporophyll using a 2-cm-diameter metal cookie-cutter. The sori were sterilized in a solution of 1L filtered seawater (0.2μm) with 1ml commercial iodine (equivalent to 1% iodine w/v) for 30 seconds. To facilitate the even dispersion of zoospores into the inoculum during preparation, the sterilized sori were wiped clean of mucus and epiphytes using a paper towel (*sensu* Mabin et al. 2013).

The method for preparing and sampling the zoospore inoculum was the same for experiments one to four. Five randomly selected sori (pooled from sporophylls collected on the same day) were placed in a replicate 50 ml polypropylene plastic culture jar containing 40 ml filtered (0.2 μm) seawater. Each treatment contained 12 replicate culture jars; each replicate containing 5 sori. The inoculated culture jars were covered with lids and placed in a temperature-controlled water bath (14-16°C) under optimal light levels (15 – 50 μmol/m^2/s) using a GLO Aqua-glo T8 30W fluorescent bulb (Hagen Inc., Montreal, Canada) (Mabin et al. 2013). Two hours following

the introduction of the sori, the inoculum was sampled for zoospores; this length of time was determined by a pilot study (methods adapted from Gutierrez et al. (2006) and Mabin et al. (2013)). For each replicate jar, the solution was agitated by gently shaking the closed jar for ~5 seconds to ensure zoospores were evenly distributed. Three 10 μL subsamples were independently taken from each replicate jar using an adjustable (20uL maximum volume) micropipette. The zoospore density of each subsample was determined using a hemocytometer, and an average density from three subsamples was used to calculate the zoospore density per replicate (except for experiment 5).

Experiment 1: effect of sporophyll transport method

The following four treatments were used to test the effect of transport method on zoospore release: (1) immediate inoculation at collection site (control), (2) sporophylls transported and inoculated immediately at the laboratory; (3) sporophylls transported and refrigerated (4°C) overnight before inoculation the next day; and (4) sporophylls transported and refrigerated (4°C) overnight, dehydrated (one hour, dark, dry, 4°C) and inoculated. For treatments 2-4, all sporophylls were transported wet in coolers containing seawater from the collection site.

Experiment 2: effect of sporophyll dehydration time

To test the effect of dehydrating sporophylls on zoospore release, sporophylls were transported wet and six treatments were used: (1) sporophylls that were not dehydrated (control); and sporophylls refrigerated dry at 4°C for (2) one hour, (3) three hours, (4) six hours, (5) twelve hours, and (6) twenty-four hours in 0.4 x 0.8 m plastic drawstring mesh bags. After each allotted time, sporophylls were removed and the inoculum prepared as described above.

Experiment 3: effect of dry transport

As stress experienced during transport may induce zoospore release, sori could be depleted of zoospores if they are transported wet. To test the effect of dry transport of sporophylls on zoospore release, three treatments were compared: (1) immediate inoculation on site (control), (2) sporophylls transported wet in seawater from the site and inoculated immediately at the laboratory; and (3) sporophylls transported dry from the site within three hours and inoculated immediately at the laboratory. This experiment is an extension of experiment 1 and 2, testing the effect of transport method and dehydration.

Experiment 4: effect of inoculum storage temperature

To test the effect of storing inoculum at different media temperatures on zoospore concentration, three treatments were used: (1) sori inoculated at 15°C and sampled immediately without storage (control); (2) sori inoculated at 4°C and refrigerated (4°C) overnight; and (3) sori inoculated at 15°C and placed in a water bath (15°C) overnight.

Experiment 5: effect of media sterilization and inoculum concentration

A two-factor crossed design was used to test the effects of media sterilization and inoculum concentration on initial zoospore density and gametophyte density after four weeks of culture. The following treatments were used: (1) sterilized, high concentration; (2) sterilized, low concentration; (3) sterilized no inoculum (control); (4) non-sterilized, high concentration; (5) non-sterilized, low concentration; (6) non-sterilized, no inoculum (control). Sporophylls were

collected and transported wet in a 50L cooler filled with seawater to the laboratory. Sporophylls were prepared immediately for inoculation without dehydration.

The high concentration inoculum (4-6 x10^4 zoospores/ml) was prepared by placing the sori (n = 70) into 2L sterilized glass beakers which contained 1L of autoclaved 0.2um-filtered seawater (sterilized treatment) or 1L of 0.2µm filtered seawater (non-sterilized treatment) as inoculum media. To create the low concentration inoculum (50% of the high concentration inoculum), one third of the volume (330ml) of each high concentration inoculum was diluted 1:1 with media of the same type. The inoculum was transferred into four replicate culture beakers, which contained six sterilized glass coverslips (Deckgläser Cover Glasses, 22x22mm). The two control cultures (i.e., without inoculum added): 1) autoclaved 0.2µm-filtered seawater, and 2) 0.2µm-filtered seawater, similarly contained six sterilized glass coverslips (Deckgläser Cover Glasses, 22x22mm). All replicate treatment and control beakers were uncovered and maintained under the same temperature and lighting conditions as in experiments 1-4. The media was changed weekly until the end of the culture period.

One glass coverslip was sampled from each replicate beaker two days after the inoculum was introduced to check if settlement had occurred. Consequently, we removed four coverslips per replicate after three days to count the zoospores and weekly thereafter for 4 weeks (to day 31) to count the gametophytes (Table 1). The above sampling steps were repeated for control cultures without inoculum.

Statistical analyses

For experiments one to four, the effects of sporophyll transport method (fixed; 4 levels including control), the effects of sporophyll dehydration time (fixed; 6 levels: zero, one, three, six, twelve, twenty-four hours), the effects of dry transport (fixed; 3 levels including control), and the effects of inoculum storage and temperature (fixed; 3 levels: control immediate inoculation without storage, 4°C overnight storage, and 16°C overnight storage) on the mean zoospore concentration in inoculum were tested using one-way ANOVAs. As treatment 6 (twenty-four hours) from experiment two contained no zoospores, these data were omitted from the analysis.

In experiment five, the effects of media sterilization (fixed; 2 levels: sterilized, and non-sterilized), inoculum concentrations (fixed; 2 levels: high, low), and replicate beakers nested within treatments and sterilization (random; 4 levels: replicate 1, 2, 3, and 4) on mean zoospore (day 3) and gametophyte (day 31) densities were analyzed with generalized linear mixed-effects models with a Gaussian distribution. The controls (sterilized zero, and non-sterilized zero) were omitted from the analyses, as no zoospores or gametophytes were observed in either treatment.

For all analyses, Boxcox plots were used to identify appropriate transformations to meet model assumptions (log transformation of zoospore concentrations for experiments 1-4). Post-hoc pairwise tests were used to determine the differences among treatment levels, and we present the p-values for these comparisons in the results section below. All statistical analyses were done in R version 1.0.153 (RStudio Team 2016). Full ANOVA and GLMM model results, including the post-hoc pairwise comparisons, can be found in the Supporting Information.

Results

Experiment 1: effect of sporophyll transport method

How sporophylls were transported affected the abundances of released zoospores, with immediate inoculation on site (control) resulting in the highest zoospore concentrations (T1: 37,777 cells/ml) (Fig. 2; Table S1). The post-hoc tests showed that the control (T1), resulted in inoculum with, on average, a 1.90x higher zoospore concentration (cells/ml) than when sporophylls were collected, transported wet, and inoculated in the laboratory on the same day without dehydration (T2; $p = 0.044$), and a 3.36x higher zoospore concentration than when sporophylls were collected, transported wet, and inoculated the following day without dehydration (T3; $p < 0.001$). Inoculating sori on the following day after collection (T3) resulted in a 1.91x lower zoospore concentration than those inoculated on the same day after collection (T2; $p = 0.014$) and a 1.72x lower concentration than those inoculated on the following day but with one hour of dehydration (T4; $p = 0.026$). There was no significant difference between the abundances of zoospores from sporophylls collected, transported wet, and inoculated on the same day without dehydration (T2), and sporophylls which were collected, transported wet, and inoculated the following day with dehydration (T4; $p = 0.996$).

Experiment 2: effect of sporophyll dehydration time

In general, dehydration resulted in fewer zoospores released relative to controls (without dehydration) (Fig. 3; Table S2). Although a dehydration time of three hours induced the highest zoospore concentrations (mean: 8.9×10^4 cells/ml), this was not significantly higher than controls ($p = 0.863$). Similarly, there were no detectable differences in the abundances of zoospores between sporophylls that were dehydrated for one (T2) or 6 (T4) hours relative to controls (T1) ($p = 0.131$ and $p = 0.206$, respectively). In contrast, the zoospore concentration in

the inoculum from twelve hours of dehydration (T5) was 25.81x lower relative to control (T1) ($p < 0.001$). Twenty-four hours of dehydration (T6) resulted in zero zoospores being released by sori.

Experiment 3: effect of dry transport

Dry transport of sporophylls resulted in higher zoospore concentrations (Fig. 4; $p < 0.001$; Table S3). Transporting sporophylls dry (T3) resulted in zoospore abundances 6.29x higher than controls (T1; $p = 0.001$), and 11.96x higher than wet transport (T2; $p < 0.001$).

Experiment 4: effect of inoculum storage temperature

Inoculum storage temperature had no detectable effect on zoospore abundance (Fig. 5; $p = 0.135$; Table S4). While there was a high degree of variation among replicates within treatments, inoculation and overnight storage of inoculum at ambient sea temperature (T3) had 1.76x higher zoospore abundances than the control inoculation at ambient sea temperature without overnight storage (T1) and 3.29x higher abundances than cold overnight storage (T2).

Experiment 5: effect of media sterilization and inoculum concentration

There was a significant interactive effect of media sterilization and inoculum concentration on both zoospore density at Day 3 and gametophyte density at Day 31 ($p = 0.010$ and $p = 0.022$, respectively; Table S5). After 3 days, in sterilized media treatments, the zoospore counts in the 'High' concentration treatments were 2.99x higher than the 'Low' concentration treatment (Fig. 6a; $p = 0.047$). In contrast, in non-sterilized media treatment, there were no detectable differences in zoospore densities between 'High' and 'Low' concentration treatments (Fig. 6a; p

= 0.998). After 4 weeks, there were 19x more gametophytes in the 'Low' concentration, sterilized media treatment than the 'Low' concentration, non-sterilized media treatment (Fig. 6b; p = 0.012). No gametophytes were observed in either of the 'High' concentration treatment groups (Fig. 6b).

Discussion

This is the first study to test the effects of different cultivation methods on the release of *E. radiata* zoospores. We demonstrated that the method of transport can influence sori productivity and zoospore density in the inoculum (North 1971; Devinny & Leventhal 1979; Mohring et al. 2013a). In this study the highest counts of zoospores from sori were observed when sporophylls were inoculated immediately on site. The lower counts of zoospores resulting from transport or delayed inoculation of sporophylls were likely a result of premature release of zoospores into the storage seawater. Hence our results strongly suggest that sporophytes should be sampled directly after collection to maximize zoospore production. Similarly, Gutierrez et al. (2006) and Hwang et al. (2013) suggest that sori should be inoculated as soon as possible, though these studies did not elaborate why it is important to do so.

To avoid the loss of zoospores during transport and increase transport efficiency, dry transport of sporophylls has been suggested as a potential method to maximize the production of zoospores (Kim et al. 2017; Bak et al. 2018). While we found dry transport of sporophylls to be the best method for transporting large quantities of sporophylls and maximizing the production of zoospores from sori, its utility depends on the capacity to collect and transport sporophylls quickly and effectively back to the laboratory to prevent declines in zoospore production (e.g. a

few hours after collection; Devinny & Leventhal 1979). If wet transport is required due to transport times longer than a few hours, cultivation will be limited by the practicality and costs of transporting large volumes of seawater when scaled up (Kim et al. 2017; Bak et al. 2018). Hence transport time is an important factor when deciding on the appropriate method for transporting sporophylls.

Optimizing the processing of stored sporophytes is critical for maximizing zoospore production. We found that the optimal period of dehydration of *E. radiata* sporophytes was 3 hours (~89,000 cells/ml). Our findings are largely consistent with other studies that suggest sori dehydrated for less than 6 hours still produce enough zoospores to culture macroalgae (e.g., > 40,000 cells/ml; Gutierrez et al. 2006). These studies, however, did not quantify zoospore output in the inoculum (Hwang et al. 2009, 2013; Mabin et al. 2013). Consequently, more research is needed to determine if a dehydration time of 3 hours is optimal for other brown seaweeds.

Temperature can be a key driver in zoospore release for *E. radiata* (Fredersdorf et al. 2009; Mohring et al. 2013a). We found that storing inoculum at warmer temperature (15°C) resulted in three times the abundance of zoospores released than when the inoculum was stored at a colder temperature (4°C). Fertile kelps release viable zoospores under a wide range of temperatures (2-23°C) (Fredersdorf et al. 2009; Mohring et al. 2013b). However, field studies have also demonstrated that warmer temperatures of 21-23°C resulted in higher releases of zoospores from *E. radiata* sporophytes and increased growth and development of gametophytes (Mohring et al. 2013a, b). Thus, it may be more advantageous to inoculate sori in warmer rather than colder media temperatures.

We observed the highest counts of zoospores in the sterilized, high-density inoculum treatment. Although we had hypothesized that the high-density inoculum would result in the highest densities of gametophytes, by the fourth week of culture (Day 31), gametophytes were only observed in the low-concentration inoculum treatments. High settlement density (of at least 1 spore/mm; (Reed 1990; Reed et al. 1991; Kerrison et al. 2017)) can result in strong intraspecific competition for space and nutrients, leading to strong negative density-dependent mortality of gametophytes (Steen 2003; Kerrison et al. 2017). The overall low gametophyte survival in all treatments may have been caused by strong interspecific competition from diatoms observed between weeks one to four. It is possible that improper sterilization of sori and/or the open-culture set-up led to contamination of diatoms from the seawater table in which culture beakers were placed. Future research should consider the addition of germanium dioxide to culture media to reduce the occurrence of diatoms (Shea & Chopin 2007; Kerrison et al. 2016). Eliminating or reducing interspecific competition would allow for better assessment of the relationship between inoculum concentration and zoospore settlement and the resulting impact of intraspecific competition on gametophyte mortality (Steen 2003). Survival of gametophytes in culture could also be improved by enriching culture media to decrease competition for nutrients (Steen 2003; Kerrison et al. 2016).

These results have important implications for studies seeking to restore kelps through cultivation of transplants. Future research should investigate: the optimal settlement substrate for *E. radiata*; efficient out-planting methods for cultured micro-sporophytes; and methods for minimizing the

density-dependent mortality of developing macro-sporophytes, to successfully up-scale kelp cultivation for the purposes of restoration.

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Table 1 Developmental stages of zoospores seeded onto glass slides during four weeks of culture in Experiment 5. Note- coverslip quality was too poor in Week 4 to include an informative image.

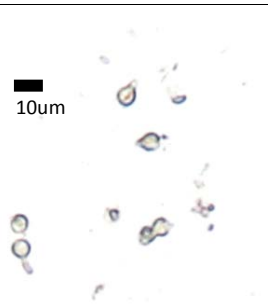
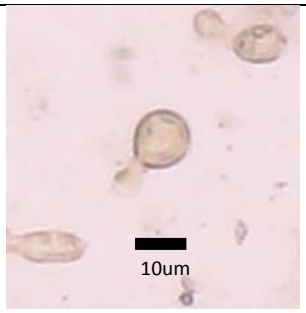
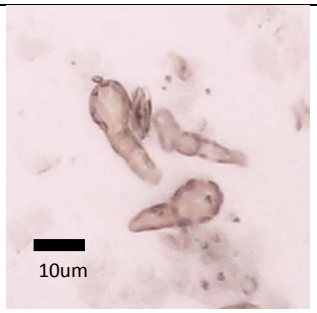
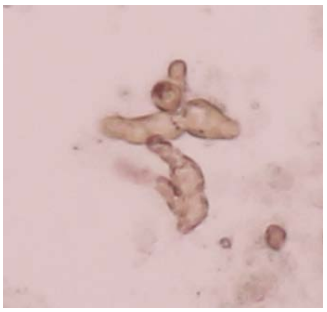
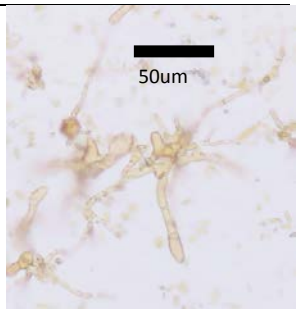
Week 0	Week 1	Week 2	Week 3
		 	
Zoospores at 3 days after inoculation.	Gametophytes first observed at 10 days after inoculation.	Female (above) and Male (below) gametophytes at 17 days after inoculation.	Male gametophyte at 24 days after inoculation.

Figure Captions

Figure 1 Diagram showing the life stages of *E. radiata* (modified from www.adlysia.wordpress.com).

Figure 2 Effect of different transport methods of *E. radiata* sporophylls on the mean zoospore concentrations in the inoculum (cells/ml ± SE). Treatments are: immediate inoculation on site (control; T1), wet transport and same day inoculation (T2), wet transport and next day inoculation without dehydration (T3), wet transport and next day inoculation with one-hour dehydration (T4). Different letters denote significant differences between treatments.

Figure 3 Effect of dehydration time for *E. radiata* sporophylls on mean zoospore concentrations in the inoculum (cells/ml ± SE). Treatments are: 0-hour (control; T1), 1-hour (T2), 3-hours (T3), 6-hours (T4), 12-hours (T5), 24-hours (T6). Different letters denote significant differences between treatments.

Figure 4 Effect of wet vs dry transport methods of *E. radiata* sporophylls on mean zoospore concentrations in the inoculum (cells/ml ± SE). Treatments are: control immediate inoculation on site (T1), wet transport and same day inoculation (T2), and dry transport same day inoculation (T3). Different letters denote significant differences between treatments.

Figure 5 Effect of inoculating and storing *E. radiata* inoculum at different temperatures on mean zoospore concentrations in the inoculum (cells/ml ± SE). Treatments are: inoculum inoculated at

15°C on same day without overnight storage (T1), inoculum inoculated with sori and stored overnight at 4°C (T2), and inoculum inoculated with sori and stored overnight at 15°C (T3). Control group inoculated at 15°C on same day without overnight storage (T1). Same letters denote no differences between treatments.

Figure 6 Effect of inoculum concentration and media sterilization on the mean densities (#/mm^2 ± SE) of *E. radiata* (a) settled zoospores at three days after inoculation, and (b) gametophytes at 31 days after inoculation. Treatments are: 'Sterilized-High', 'Sterilized-Low', 'Non-Sterilized-High', and 'Non-Sterilized-Low'. Different letters denote significant differences between treatments.

Figure 1

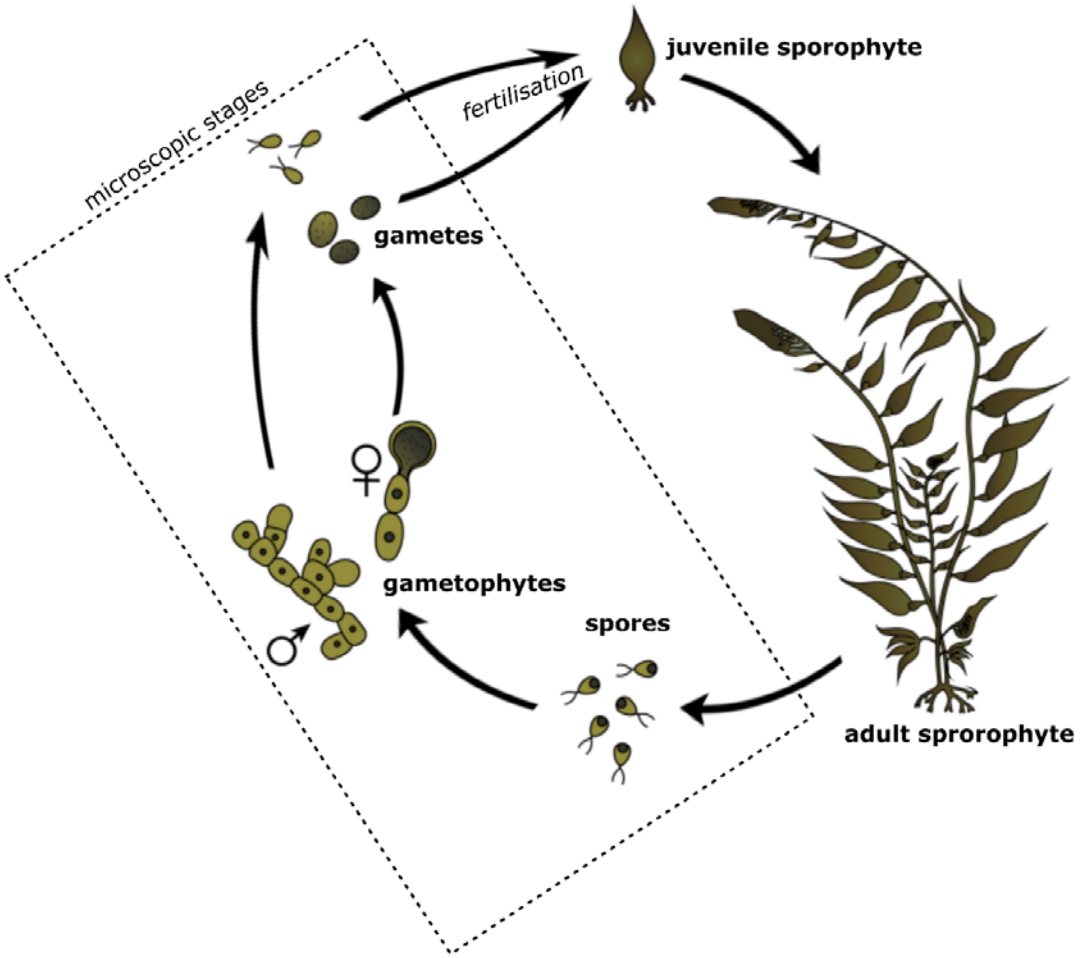


Figure 2

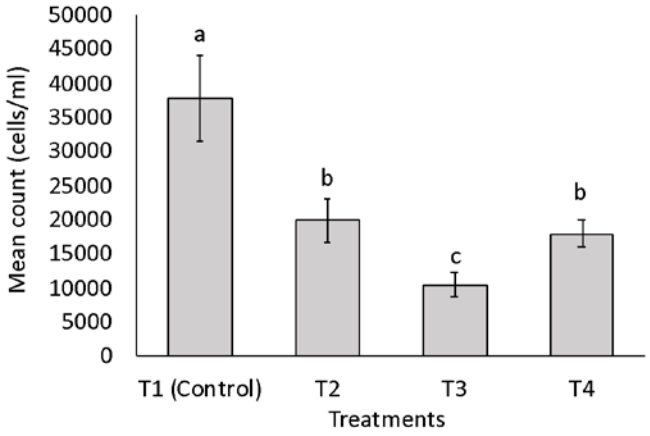


Figure 3

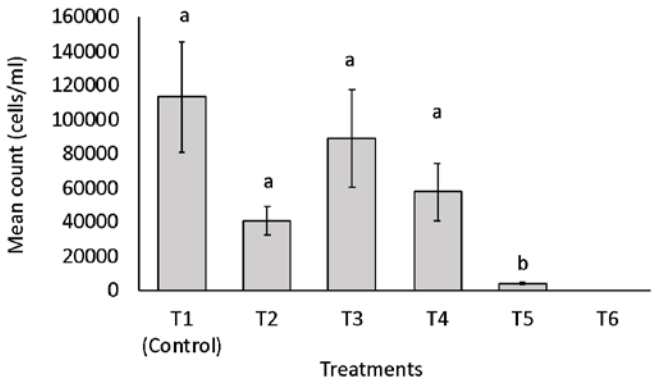


Figure 4

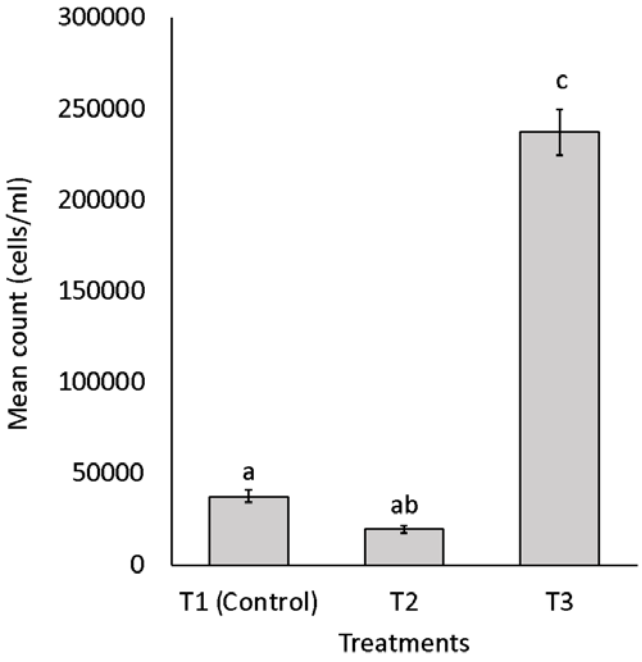


Figure 5

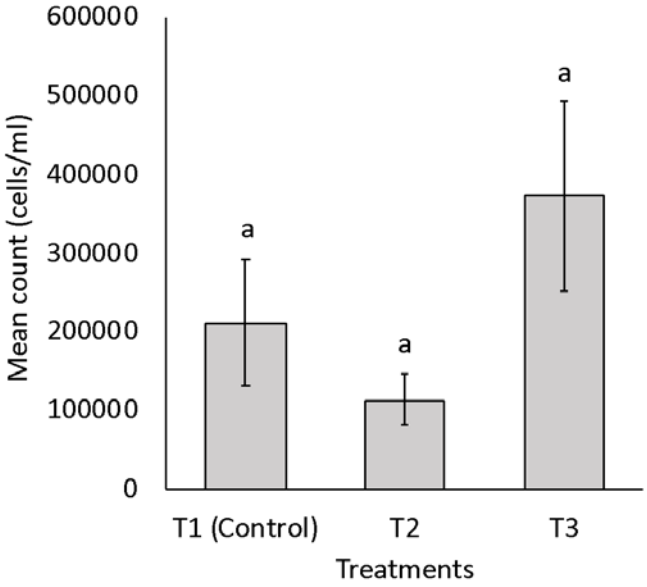
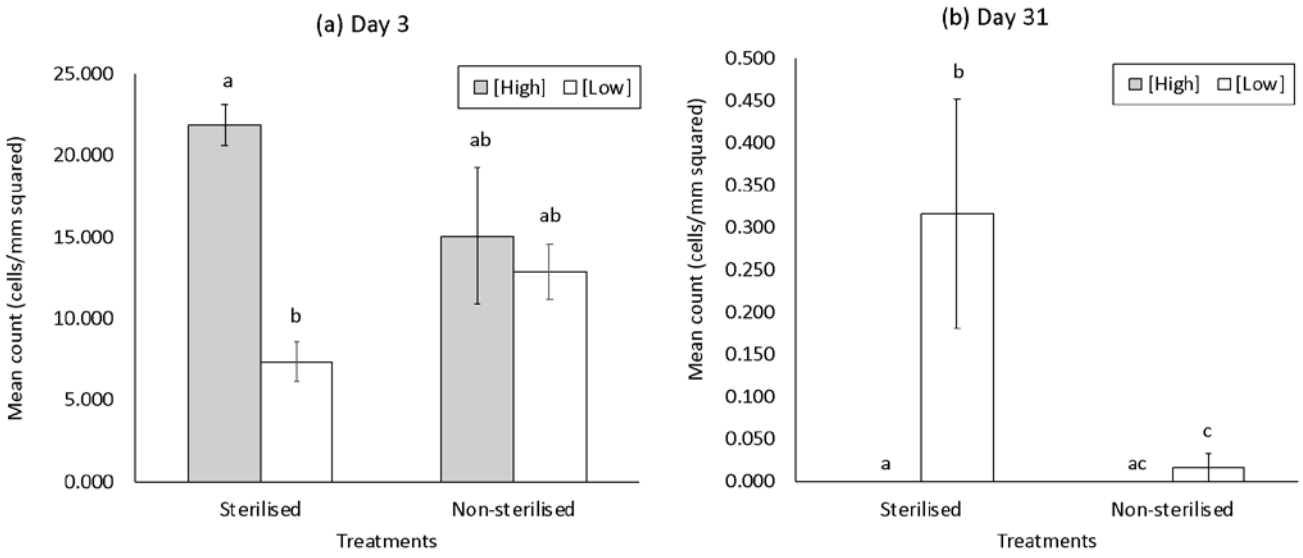


Figure 6



Supporting Information

Table S1 ANOVA and Tukey HSD test results of experiment 1: the effect of different transport methods on zoospore abundances (log-transformed). * indicate significant differences between group means.

Effect	**df**	**F**	**P**
Treatment	3	11.60	<0.001*
Residuals	44		
Contrasts	**Estimate**	**T ratio**	**P**
T1 (Control) – T2	0.258	2.728	0.044*
T1 (Control) – T3	0.556	5.893	<0.001*
T1 (Control) – T4	0.279	2.959	0.025*
T2-T3	0.299	3.165	0.014*
T4-T2	0.022	0.231	0.996
T4 -T3	-0.277	-2.934	0.026*

Table S2 ANOVA and Tukey HSD test results of experiment 2: the effect of different dehydration times on zoospore abundances (log-transformed). The analysis excludes treatment six as all data were zero counts. * indicate significant differences between group means.

Effect	**df**	**F**	**P**
Treatment	4	21.48	<0.001*
Residuals	55		
Contrasts	**Estimate**	**T ratio**	**P**
T2-T1 (Control)	0.376	2.399	0.131
T3-T1 (Control)	0.154	0.981	0.863
T4-T1 (Control)	0.340	2.171	0.206
T5-T1 (Control)	1.314	8.391	<0.001*
T3-T2	-0.222	-1.419	0.619
T4-T2	-0.036	-0.229	0.999
T5-T2	0.938	5.991	<0.001*
T4-T3	0.186	1.190	0.757
T5-T3	1.161	7.410	<0.001*
T5-T4	0.974	6.220	<0.001*

Table S3 ANOVA and Tukey HSD test results of experiment 3: the effect of different dry transport (T5) on zoospore abundances (log-transformed). T1 and T2 raw data are from experiment 1. * indicate significant differences between group means.

Effect	**df**	**F**	**P**
Treatment	2	17.21	<0.001*
Residuals	33		
Contrasts	**Estimate**	**T ratio**	**P**
T2-T1 (Control)	0.593	1.805	0.184
T3-T1 (Control)	-1.291	-3.932	0.001*
T3-T2	-1.884	-5.738	<0.001*

Table S4 ANOVA and Tukey HSD test results of experiment 4: the effect of inoculum storage temperatures on zoospore abundances (log-transformed).

Effect	**df**	**F**	**P**
Treatment	2	2.132	0.135
Residuals	33		
Contrasts	**Estimate**	**T ratio**	**P**
T2-T1 (Control)	0.479	1.066	0.542
T3-T1 (Control)	-0.449	-0.999	0.583
T3-T2	-0.928	-2.064	0.113

Table S5 glmmTMB (Gaussian) and pairwise (emmeans) results for Experiment 5: the effects of media sterilisation and inoculum concentrations on zoospore density. * indicate significant differences between group means.

Day 3	**Estimates**	**SE**	**P**	
(Intercept)	12.85	3.809	0.001*	
Sterilisation	-5.533	5.387	0.304	
Concentration	2.233	5.387	0.678	
Sterilisation:Concentration	12.35	7.619	0.010*	
Contrasts	**Estimate**	**SE**	**T ratio**	**P**
NS Low – NS High	-2.230	5.390	-0.415	0.976
NS Low – S Low	5.530	5.390	1.027	0.735
NS Low – S High	-9.050	-5.390	-1.680	0.347
NS High – S Low	7.770	5.390	1.442	0.481
NS High – S High	-6.820	5.390	-1.265	0.590
S Low – S High	-14.58	5.390	-2.707	0.047*
Day 31	**Estimates**	**SE**	**P**	
(Intercept)	0.017	0.066	0.799	
Sterilisation	0.300	0.093	0.001*	
Concentration	-0.017	0.093	0.857	
Sterilisation:Concentration	-0.300	0.131	0.022*	
Contrasts	**Estimate**	**SE**	**T ratio**	**P**
NS Low – NS High	0.017	0.093	0.180	0.998
NS Low – S Low	-0.300	0.093	-3.237	0.012*
NS Low – S High	0.017	0.093	0.180	0.998
NS High – S Low	-0.317	0.093	-3.417	0.008*

NS High – S High	0.000	0.093	0.000	1.000
S Low – S High	0.317	0.093	3.417	0.008*