TAXON-SPECIFIC SURVIVAL STRATEGIES TO PROLONGED DARKNESS AND RE-EXPOSURE TO LIGHT IN DIATOMS AND FLAGELLATES: IMPACT ON PHYTOPLANKTON COMMUNITY STRUCTURE IN THE SOUTHERN OCEAN

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ABSTRACT

Phytoplankton populations inhabiting the Southern Ocean have to withstand prolonged periods of darkness (i.e. polar night, sea-ice cover), followed by extended periods of perpetual daylight. The development of adaptations and survival strategies to seasonal changes in solar irradiance were investigated through laboratory experiments. In our study, three Antarctic diatoms (*Thalassiosira antarctica, Fragilaropsis minimum, Chaetoceros brevis*) and three flagellates (*Pyramimonas sp. (Prasinophyceae)*, *Phaeocystis antarctica (Haptophyceae), Polarella glacialis (Dinophyceae)*) were light deprived for 8 weeks. To test the ability to recover upon return of irradiance, a sub-sample was weekly re-exposed to dim light for 5 consecutive days. Chlorophyll *a* (Chl *a*) concentration and photosynthetic characteristics (maximum quantum yield of photosystem II, non-photochemical quenching, etc.) were examined both during the dark treatment and after recovery in irradiance. Both diatoms and flagellates showed a loss of photosynthetic efficiency during darkness. After 5 days of re-exposure to light, diatoms showed better recovery compared to flagellates. The effects of 8 weeks of darkness on Chl-*a* synthesis and PSII functionality were reversible in all three diatom species. Flagellates on the other hand could hardly overcome the photodamage caused by dark incubation and displayed poor shape by the end of the experimental period. The different response to re-exposure in irradiance given by the two groups can be explained by the use of different pigment pools and non-photochemical quenching (NPQ) mechanisms. The ability to stay viable and overcome damage may grant diatoms competitive advantage over flagellates and allow the exploitation of nutrient stocks upon return of irradiance. Eventually, different survival strategies and photosynthetic efficiency determine the spatial distribution in a highly diverse environment such as the Southern Ocean.

INTRODUCTION

Surrounding the Antarctic continent, the Southern Ocean is a rich yet harsh environment. Low temperatures, extreme variations in light regimes, low sun elevations (<40-50°), and strong vertical mixing of the water column (El-Sayed, 1970; Holm-Hansen, 1985) pose severe limitations for marine phytoplankton. Being a high nutrients/low chlorophyll (HNLC) region, light and dissolved-iron concentration are the main controlling factors for phytoplankton growth (Peters and Thomas, 1995; Holm-Hansen, 1977; Gran, 1931; Harvey, 1933). The way iron and light co-limit primary productivity is still uncertain. An opportune strategy to overcome iron and light limitation would be to increase the size of photosystems, thereby increasing light absorption capacity, while also balancing antennae and PSII reaction centres sizes (Falkowski and Raven, 2007; Wientjes *et al*., 2013). Particularly, light availability is controlled by seasonality (i.e. polar night), sea-ice cover, the presence of oceanic

fronts that affect mixing depths (Lutjeharms *et al.,* 1984) and the degree of stratification of the water column. Within the Antarctic circle (i.e. Ross Sea, Weddell Sea, coastal areas) microalgae have to survive a period of near darkness for up to 5 months – as a result of the occurrence of the polar night and sea-ice cover. However, large regions of the Southern Ocean, located outside the Antarctic circle, do not experience polar night. Here, light is mainly limited by sea-ice cover (up to ~40% of the Southern Ocean in winter; Lyon and Mock, 2014). As Sakshaug (1985) suggested, phytoplankton growth can also be limited by the intense and deep mixing of waters. Caught in a water mass, microalgae can be rapidly transferred to dark deep waters and be trapped there for several months or years (Peters and Thomas, 1995). Phytoplanktonic processes of photo-acclimation and adaptation to changing light regimes have received growing attention but are still poorly described (Marra, 1978; Ibelings *et al.*, 1994). On one hand, under low light conditions, algae would adapt by expanding their light-harvesting complexes. The use of stored proteins, lipids and carbohydrates as energy source, combined with a reduced metabolism, has also been observed in polar microalgae as a long-term dark survival strategy (Schaub *et al.*, 2017). At the onset of austral spring (November), phytoplankton blooms are triggered by the return of daylight and/or sea-ice melting. The depth of mixing is also reduced by the formation of a brackish layer (Sakshaug and Slagstad, 1991), increasing light exposure of algae. According to Henley & Ramus (1989) and Kana *et al.* (1997), the algal response to light change entails balancing energy use and energy production, which requires the optimization of their photosystems (PSI and PSII). High-light exposure would trigger photodamage of the photosynthetic apparatus by a surplus of energy produced and not used. Algae attempt to avoid it by reducing the number of photosystems. Additional morpho-physiological adaptations might involve changes to the thylakoid membranes structure, pigment composition and proportion of light-harvesting pigments (i.e. chlorophylls and carotenoids; Lüder *et al*., 2001, 2002; Morgan-Kiss *et al*., 2006), to the photosystems I and II (PSI and PSII) reaction centres and antennae (Reeves *et al.,* 2011), as well as structural changes to the chloroplasts (Baldisserotto *et al.,* 2005*).* The development of blooms during spring and summer suggests the ability of these microorganisms to survive prolonged periods with low levels of Photosynthetically Active Radiation (PAR) and their capability to recover photosynthetic functionality upon return of light.

Different taxonomic groups (i.e. diatoms, flagellates) can develop different photosynthetic apparatus, with diverse pigment composition and cellular structures (Richardson *et al.,* 1983), as well as different strategies to remain viable during prolonged periods of darkness (Peters, 1996; Jochem, 1999). Alternatively, species-specific characteristics – such as nutrients requirement or grazing rates (Van Hilst and Smith, 2002) – may ensue a higher degree of adaptability to dynamic light regimes than taxonomic features (Peters, 1996). For flagellates, other than energy storage and use, mixotrophy (combination of photosynthesis and heterotrophic nutrition) has been suggested as a crucial survival strategy (McKie-Krisberg and Sanders, 2014; Joli *et al*., 2017; Stoecker and Lavrentyev, 2018). In 1999, Jochem identified two different types of adaptations to absence of light: the first type, mainly adopted by diatoms, entails metabolism suppression to endure prolonged darkness, and requires a quick reaction to changing light conditions. The second type, common among flagellates, requires preservation of photosynthetic activity and no metabolic alteration (e.g. reduction of metabolism, shift to mixotrophy, etc.) occurs during darkness. Presumably, Type I algae benefit of a competitive advantage over Type II algae during prolonged dark periods, as the latter would eventually use up and dissipate all energy supplies.

Field observations in the Southern Ocean showed that these blooms are both temporally and spatially different in terms of taxonomic and species composition (Arrigo *et al*., 2010). Diatoms dominate areas of high stratification (stable light conditions), as well as waters of the marginal ice zone (MIZ) and frontal zones (Cailliau *et al*., 1996). Other photosynthetic phytoplankton groups (e.g. Haptophytes) generally dominate less stratified and more deeply mixed waters (DiTullio and Smith, 1996; Arrigo *et al*., 1999, 2000). Each algal group plays a significant role in the biogeochemical and ecological roles in the Southern Ocean. For instance, diatoms play a primary role in the global silica cycle (Caron *et al*., 2000; Goffart *et al*., 2000). Thus, it is highly relevant to understand the taxonomic and/or species-specific photo-physiological characteristics that control the composition of different blooms.

In this study, six different phytoplankton species were investigated to assess photosynthetic performance during darkness and after re-exposure to irradiance for 5 consecutive days. Three diatoms (*Thalassiosira antarctica, Fragilariopsis minimum, Chaetoceros brevis*) and three flagellates (*Pyramimonas sp*. (Prasinophyceae), *Phaeocystis antarctica* (Haptophyceae)*, Polarella glacialis* (Dinophyceae)) were subjected to dark incubation for

a period of 8 weeks. Weekly, samples from each culture were taken and exposed to dim light for a total of 5 days. Subsamples were taken each week from both dark-incubated cultures and recovery cultures. Different growth and photosynthetic parameters were analysed, such as chlorophyll *a* concentration (mg chl a)$^{-1}$, the maximum quantum efficiency of PSII (F_v/F_m), non-photochemical quenching (NPQ), as well as other photosystem-related parameters (σ_{PSII}, τ_{ES}, ρ). Such parameters allowed to assess photosynthetic performance during darkness and the ability to recover upon return of light. Therefore, the main objectives of the study were to: 1) determine for how long the six species were able to stay viable during darkness; 2) assess whether they were able to recover after darkness when re-exposed to irradiance, and how quickly they would react to changing light regime; 3) investigate what role taxon-specific characteristics would play in dark survival and consequent recovery strategy. Based on previous studies on polar phytoplankton, we thereby expected diatoms to be able to survive longer periods of darkness and to more readily react to the return of irradiance compared to flagellates.

MATERIALS AND METHODS

Culture conditions – The three diatoms (*Thalassiosira antarctica, Fragilariopsis minimum, Chaetocerus brevis*), the prasinophyceae *Pyramimonas sp.,* the haptophyceae *Phaeocystis antarctica,* and the dynophyceae *Polarella glacialis*, previously sampled in the Southern Ocean, were cultivated for 4 weeks in F2 enriched seawater, according to Guillard and Ryther (1962). Cultivation was set up in a cold room (~4°C) and under dim light (20 µmol photons m^{-2} s^{-1}) during a 16h light, 8h dark cycle. Cultures were occasionally swirled to avoid the formation of aggregates.

Dark incubation experiment – Each culture was diluted in a 1-liter conical flask, with F2 enriched medium, before dark-incubation. For each species, three replicates were prepared. To recreate dark natural conditions, the flasks were wrapped with aluminum foil and then placed in a box (in a cold room). Weekly subsamples of 15 mL were obtained from each culture. During the short sampling procedure (~1 minute for each culture), irradiance was low, to avoid algae re-adapting to light. Out of the 15 mL, 10 mL were vacuum-filtered, frozen in liquid nitrogen, and stored at -80°C. These subsamples will later be used for POC and pigments analysis. The rest (5 mL) was used for fast repetition rate fluorometry (FRRf, Kolber *et al.,* 1998). This methodology allowed us to assess changes in chlorophyll fluorescence yield and thus photosynthetic parameters of the cultures (see below).

Recovery experiment – Each week, subsamples of 15 mL were taken from each flask and put in small transparent cultivation vessels. These samples were exposed to irradiance (14 µmol photons m^{-2} s^{-1}) for 5 consecutive days. Afterwards, they were subjected to the same analysis as dark-adapted subsamples. Again, 5 mL were used for the analysis of Chl-*a* and photosynthetic parameters, while the rest was used for filters that were snap-frozen in liquid N2 and stored in a -80°C freezer.

FRRf measurements – Measurements obtained through fast repetition rate fluorometry are rapid, sensitive and non-invasive (Falkowski and Kolber, 1995). By estimating the change in quantum yield of chlorophyll fluorescence, this methodology can provide us detailed information of photosynthetic parameters related to PSII (*Krause and Weis, 1991*). In our study, PSII-related parameters from the samples were measured using a FastOcean and Act2 FRRf fluorometer (Chelsea Technology Group, West Moseley, UK). The sample, placed in a cooled sample chamber, is exposed to flashes of saturating actinic light. This series of excitation pulses or 'flashlets' (100 flashlets on a 2 µs pitch for saturation) provoke the closure of the majority of reaction centres. This causes a shift from minimum to maximum fluorescence (*Suggett et al*., *2007*). Two single turnover events (saturation and relaxation) are provided. A mean of multiple turnover acquisitions was obtained and used to estimate the minimum fluorescence-derived Chl-*a* (in µg L^{-1}), the maximum quantum efficiency of PSII (F_v/F_m), the functional absorption cross-section of PSII (σ_{PSII}) and the efficiency of energy transfer from closed to open reaction centres (ρ). Simultaneously, a relaxation phase (40 flashlets at a 60 µs pitch) provided information on the reopening times of closed reaction centres (τ_{ES}) and nonphotochemical quenching (NPQ).

Table 1. Measurements obtained through fast repetition rate fluorometry (FRRf).

Chlorophyll a ($\mu g\ L^{-1}$)	indicator of the physiological state and growth potential during stressful or adverse conditions. When cells are exposed to light, photons are absorbed. Out of a hundred photons absorbed, some are re-emitted as fluorescence by Chl *a (Mobley, 1994).*
F_v/F_m	maximum photochemical efficiency of PSII (when reaction centers are open). F_m is the maximum fluorescence in darkness, F_v is the variable fluorescence in darkness (F_m - F_0) (Fig.1). This parameter is an indicator of photosynthetic performance. Measuring the yield of chlorophyll fluorescence allows to understand changes in photosynthetic efficiency and heat dissipation (*Maxwell and Johnson, 2000*). Lower values are indicators of stress symptoms and poor conditions. Values of F_v/F_m can easily display differences between algal groups (when grown under similar conditions). $$Fv/Fm = (Fm - F0)/(Fm)$$
σ_{PSII} ($nm^{-2}\ PSII^{-1}$)	units: $nm^{-2}\ PSII^{-1}$; the functional absorption cross-section of PSII describes light absorption in PSII (effective target size of the PSII antenna). The larger this cross section, the more easily PSII will be excited. It is controlled by the ratio of light-harvesting pigments of PSII. σ_{PSII} controls the rate of excitation delivery to reaction centers. Exposure to high irradiances reduces the absorption cross-section, thus reducing the rate of delivery of excitation to reaction centers.
τ_{PSII} (μs)	units: μs; minimum turnover time of PSII, represents the reopening time of closed reaction centers.
ρ	also called "connectivity factor" of PSII reaction centers in darkness. It represents the efficiency of energy transfer from closed to open reaction centers.
NPQ/NSV	Normalized Stern-Volmer (NSV) nonphotochemical quenching (NPQ): dimensionless; nonphotochemical quenching represents an important protective process for the photosynthetic apparatus. Excessive energy – caused by light saturation or a quick switch from darkness to light – can cause damage to the photosystem. NPQ allows the algae to dissipate this energy in the form of heat.

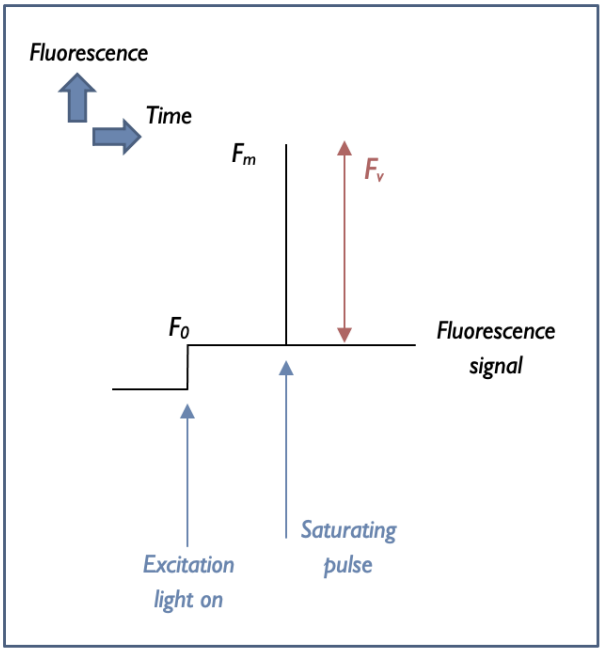

Fig.1. Development of Chl-*a* fluorescence. A light source excites the chlorophyll. The excitation is not high enough to induce electron transport in PSII, and F_0 is at the minimum level of fluorescence. Reaction centers are open. A rapid, saturating pulse of light brings fluorescence to its maximum (F_m). Reaction centers are closed. F_v is the variable fluorescence between the minimum and maximum fluorescence levels. (Based on *Murchie and Lawson, 2013*).

These parameters give us essential information on photosynthetic energy conversion (*Falkowski et al., 1988; Lavergne and Trissl, 1995*), how PSII reacts to environmental stress (*Kolber et al., 1988*), photosynthetic electron transport (*Kolber and Falkowski, 1993*) and the relationship between different photosynthetic apparatus (*Crofts and Yerkes, 1994*).

Statistical analyses – Shapiro-Wilk tests were conducted to check for normality of both dark-incubated and recovery data. All data were normalized to t = 0 of dark-incubated samples. This was done for all three replicates, for each week, both dark-adapted and recovery samples. Change trends (decrease or increase) per week^{-1} were calculated for Chl-*a* concentration and all the other photosynthetic parameters. For both diatoms and flagellates, temporal trends per week^{-1} were calculated from week 0 (before dark incubation) to week 8 by fitting a linear function to the normalized data. For each species, replicates (n=3) were analysed separately. Plots were made from the average of the three replicates. To determine whether time in darkness and species and/or taxonomic groups have an impact on temporal trends, analysis of variance (ANOVA) and TukeyHSD test were performed. Photosynthetic parameters of each species, pooled by taxonomic dominance (diatoms and flagellates), were compared to detect differences or similarities between groups using an ANOVA test. To determine the ability to recovery upon return of irradiance, absolute values for each light regime were pooled at weeks 0, 1, 2 and at weeks 6, 7, 8. Significancy in the difference between the pooled dark-adapted and pooled recovery data was tested by performing an ANOVA and a TukeyHSD test.

RESULTS

Chl-a fluorescence and photosynthetic parameters prior dark-incubation – Absolute values of Chl-*a* concentration during the photo-acclimation process (at 20 µmol photons m^{-2} s^{-1}) prior dark-incubation were around 500 ug m^{-3} for *Pyramimonas sp*. and 300 ug m^{-3} *C. brevis*, and as low as 90 ug m^{-3} for *F.minimum* (Table 5). Accordingly, *Pyramimonas sp*. had the significantly highest maximum quantum yield of PSII (F_v/F_m) absolute value (0,625) at t=0 (Appendix 1). Although, no significant difference was detected between the initial values of F_v/F_m in different species or between the two taxonomic groups. The absorption cross-section (σ_{PSII}) at week 0 was between 5 and 7,5 nm^{-2} $PSII^{-1}$ in *P.antarctica, Polarella and F.minimum*, and around 3,5 nm^{-2} $PSII^{-1}$ in *Pyramimonas sp*. and *T. antarctica* (Appendix 3). Reopening times of closed reaction centres (τ_{PSII}) at t=0 was similar for all species (around 3000 µs). "Connectivity factor" (ρ) absolute values were not significantly different between species and/or taxa, ranging from 310 (*C.brevis, Polarella*) to 490 (*Pyramimonas sp.*) (Appendix 5). Conclusively, Normalized Stern-Volmer non-photochemical quenching before dark-incubation was between 900 and 1130 µmol photons m^{-2} s^{-1} in *T.antarctica*, *C.brevis and P.antarctica*, and between 500 and 800 µmol photons m^{-2} s^{-1} in the other species (Table 5, Appendix 6).

Dark incubation experiments

Chl a fluorescence – FRRf derived Chl-*a* concentration showed similar decline trends in all six species (Table 2; Fig 2). For each replicate, Chl-*a* started declining after the first week in darkness and kept dropping steadily throughout the entire incubation period. After 8 weeks, absolute values were dramatically lower than initial values (t=0) in all species (Table 5, Appendix 1). Weekly decline rates varied from ~ 8.5% $week^{-1}$ (*T.antarctica*, *C. brevis*) to ~16% $week^{-1}$ (*P. antarctica*). No significant difference (ANOVA, $p < 0.05$) was observed either between species or between diatoms and flagellates (Table 3).

Photosynthetic parameters – Maximum quantum efficiency of PSII (F_v/F_m) declined in all species, from both taxonomic groups (Fig. 3). Stronger decline rates were detected in some of the species compared to the others (ANOVA, $p = 0.00042$). For instance, the decline rate of F_v/F_m detected in *Pyramimonas sp*. (-12.6% $week^{-1}$) was significantly higher compared to *C. brevis* (-2.3% $week^{-1}$; $p = 0.00073$) and to *Polarella* (-3.7% $week^{-1}$, $p = 0.00132$; Table 2). No significant difference was observed between diatoms and flagellates (Table 3).

Temporal trends of the functional absorption cross-section (σ_{PSII}) showed that this declined in all species, thereby decreasing the chance of photon absorption (Fig.4). Absolute values were significantly lower after 8 weeks of darkness (compared to t=0) in all species, with the biggest variation detected in *F.minimum* (from 6000 to 1740 nm^{-2} $PSII^{-1}$, -7% $week^{-1}$; Table 5, Table 2, Appendix 3). The variation between decline rates of diatoms and flagellates was not significant (ANOVA, $p = 0.08$).

Reopening times of closed reaction centres (τ_{PSII}) increased over-time (i.e. week 0-8) in all dark-incubated samples (Fig.5), ranging from +7.8% $week^{-1}$ in *Polarella* to +18% $week^{-1}$ in *F.minimum and Pyramimonas sp.* (Table 2), with no significant difference either between diatoms and flagellates or between all the single species.

The "connectivity factor" ρ of PSII reaction centres declined in all species during dark incubation, except for *C. brevis* (+4%, Table 2), showing a downturn in the efficiency of energy transfer from closed to open reaction centres (Fig. 6). While there was no significant difference between diatoms and flagellates (Table 3), the variance in change rates among all species was significant (ANOVA, $p = 2.69^{-5}$; Table 2).

Stern-Volmer Normalized Stern-Volmer non-photochemical quenching (NPQ_{NSV}) increased in all diatoms during dark-treatment (Fig. 7, Table 5). *T.antarctica* and *F.minimum* showed strong increase rates (+78% and +28%, respectively), in contrast with a slight decrease in *C.brevis* (-5%; Table 2), compared to initial values at t = 0. In *Pyramimonas sp*., NSV-NPQ increased for the first 6 weeks of darkness, until it finally dropped at week 7 (+4% $week^{-1}$). In *P.antarctica*, the NSV-NPQ absolute values increased during the first 7 weeks of dark-incubation, and

only dropped in the last week of treatment (+8% week^{-1}; Appendix 6). In *Polarella*, NSV-NPQ increased (18% week $^{-1}$) throughout the entire length of the treatment (Fig. 7).

Table 2. Table showing percentage of change per week (normalized data) derived from linear fits of measurements for each species during 8 weeks of dark incubation (D) and for recovery experiments (R, after dark-incubation and subsequent exposure to the irradiance of 14 µmol photons m^{-2} s^{-1} for 5 consecutive days). R squared values (in brackets) show the fitness of the linear model.

	Thalassiosira antarctica		***Fragilariopsis minimum***		***Chaetoceros brevis***		***Pyramimonas sp.***		***Phaeocystis antarctica***		***Polarella glacialis***	
	D	**R**	**D**	**R**	**D**	**R**	**D**	**R**	**D**	**R**	**D**	**R**
[Chl a]	-8,3 (0,9)	-23,8 (0,9)	-12,66 (0,9)	-21,54 (0,8)	-8,5 (0,8)	-23,21 (0,8)	-10,5 (0,9)	-12,5 (0,9)	-16,33 (0,8)	-50,01 (0,9)	-6,43 (0,7)	-16,2 (0,9)
Fv/Fm	-10,8 (0,9)	-2,22 (0,6)	-6,44 (0,7)	-0,44 (0,1)	-2,25 (0,4)	-0,47 (0,03)	-12,55 (0,8)	-14,20 (0,8)	-8,94 (0,9)	-10,35 (0,7)	-3,7 (0,9)	-2,77 (0,9)
σ_{PSII}	-6,4 (0,9)	-6,6 (0,8)	-7,02 (0,9)	-6,16 (0,9)	-3,9 (0,7)	-5,51 (0,8)	-7,4 (0,9)	-2,8 (0,8)	-4,8 (0,5)	-5,6 (0,7)	-3,30 (0,5)	-4,6 (0,6)
τ_{PSII}	15,06 (0,9)	3,12 (0,4)	18,34 (0,4)	3,21 (0,5)	9,80 (0,3)	-2,87 (0,3)	18,7 (0,8)	1,1 (0,3)	11,6 (0,08)	4,8 (0,4)	7,8 (0,7)	0,05 (0)
ρ	-9,94 (0,9)	0,98 (0,2)	-0,09 (0,1)	0,97 (0,07)	4,1 (0,2)	3,6 (0,5)	-6,97 (0,8)	-4,03 (0,6)	-3,6 (0,2)	-8,13 (0,7)	-4,7 (0,6)	0,7 (0,04)
NPQ_{NSV}	78,5 (0,9)	3,7 (0,5)	28,37 (0,5)	1,65 (0,2)	-5,22 (0,05)	-1,5 (0,02)	4,34 (0,01)	1,8 (0,02)	7,85 (0,01)	34,9 (0,4)	17,03 (0,8)	10,3 (0,9)

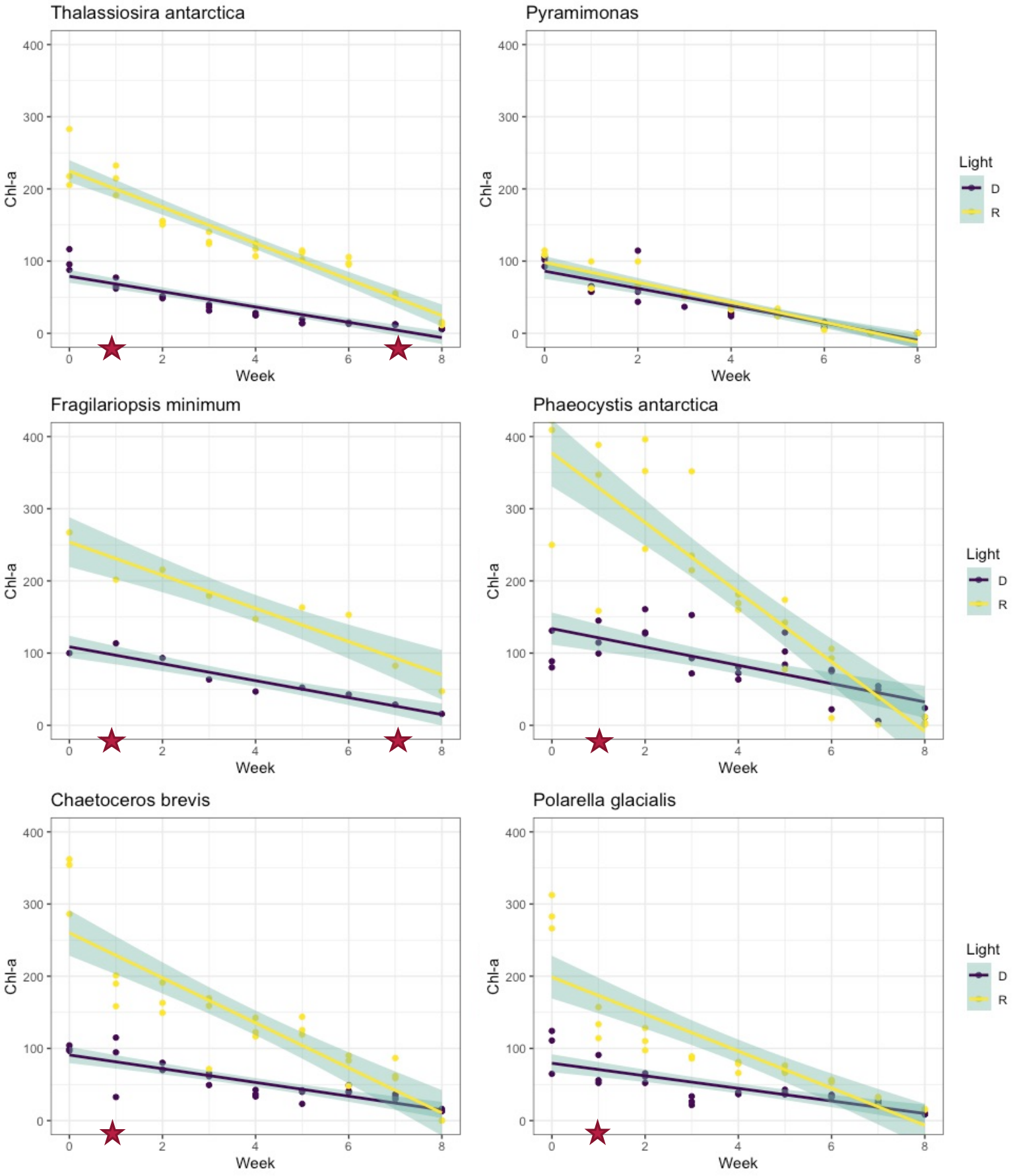

Fig 2. Linear trends of FRRf derived Chl a concentration during 8 weeks of dark incubation (dark lines) and subsequent recovery experiments after 5 days under 14 µmol photons m^{-2} s^{-1} irradiance (yellow lines) of all species (normalized data). Diatoms (*Thalassiosira antarctica, Fragilariopsis minimum, Chaetoceros brevis*) are shown on the left, flagellates (*Pyramimonas sp., Phaeocystis antarctica, Polarella glacialis*) are on the right. The grey line around the linear trend represents the confidence interval. Symbols (stars) mark the points (weeks 0-2, weeks 6-8) where the difference (absolute values) between dark-adapted and recovery samples is significant (Table 4).

Table 3. ANOVA test-output. Significancy of difference between change rates in diatoms and flagellates for each parameter and for both light regimes. Signif. codes of p-value: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 ' ', 1.

	Light regime	Diatoms vs Flagellates
[Chl-a]	**Dark-adapted:**	Not significant
	Recovery:	Not significant
Fv/Fm	**Dark-adapted:**	Not significant
	Recovery:	Significant (***)
σ_{PSII}	**Dark-adapted:**	Not significant
	Recovery:	Not significant
τ_{PSII}	**Dark-adapted:**	Not significant
	Recovery:	Not significant
ρ	**Dark-adapted:**	Not significant
	Recovery:	Significant (***)
NPQ_{NSV}	**Dark-adapted:**	Not significant
	Recovery:	Not significant

Table 4. ANOVA and TukeyHSD test-output. Significancy of difference between dark-incubated and recovery samples. Values (normalized at t=0) were pooled at weeks 0-2 and weeks 6-8 of the experiment (for *Pyramimonas sp*, weeks 4-6 were tested due to missing data after week 6). P-values are between brackets. S indicates significance, NS indicates no significance.

	Thalassiosira antarctica		**Fragilariopsis minimum**		**Chaetoceros brevis**		**Pyramimonas sp.**		**Phaeocystis antarctica**		**Polarella glacialis**	
	0-2	**6-8**	**0-2**	**6-8**	**0-2**	**6-8**	**0-2**	**4-6**	**0-2**	**6-8**	**0-2**	**6-8**
Chl-a	S 127.7 (1.73^{-5})	S 44.2 (0.01)	S 125.8 (8.25^{-5})	S 65.2 (0.01)	S 143.5 (0.0008)	NS	NS	NS	S 210.2 (9.57^{-9})	NS	S 102.7 (0.01)	NS
Fv/Fm	S 16.3 (3.55^{-6})	S 68.6 (3.13^{-10})	NS	S 42.5 (6.84^{-12})	S 22.9 (0.009)	S 36.39 (4.61^{-8})	NS	NS	S 21.21 (0.004)	S 33.9 (0.007)	NS	NS
σ_{PSII}	NS	NS	NS	NS	NS	NS	S -13.5 (0.01)	NS	NS	NS	NS	NS
τ_{PSII}	S -21.4 (0.0009)	S -84.9 (7.09^{-7})	S -55.9 (0.008)	S -220.7 (3^{-10})	S -71.7 (0.03)	S -149.7 (6.34^{-9})	S -13.2 (0.02)	S -64.9 (0.0008)	NS	S -183.1 (0.005)	S -24.8 (0.009)	S -63.6 (1.3^{-5})
ρ	S 20.38 (2.31^{-6})	S 77.9 (2.31^{-6})	S 34.9 (0.0007)	S 44.7 (2.79^{-5})	S 52.4 (0.0005)	S 56.3 (2.29^{-5})	NS	S 20.64 (0.001)	S 37 (0.009)	NS	NS	S 18.1 (0.02)
NPQ_{NSV}	S -38.4 (4.48^{-5})	S -389.7 (8.45^{-6})	NS	S -177.2 (5.9^{-6})	S -19.1 (0.001)	S -71.5 (1.75^{-8})	NS	NS	NS	NS	NS	NS

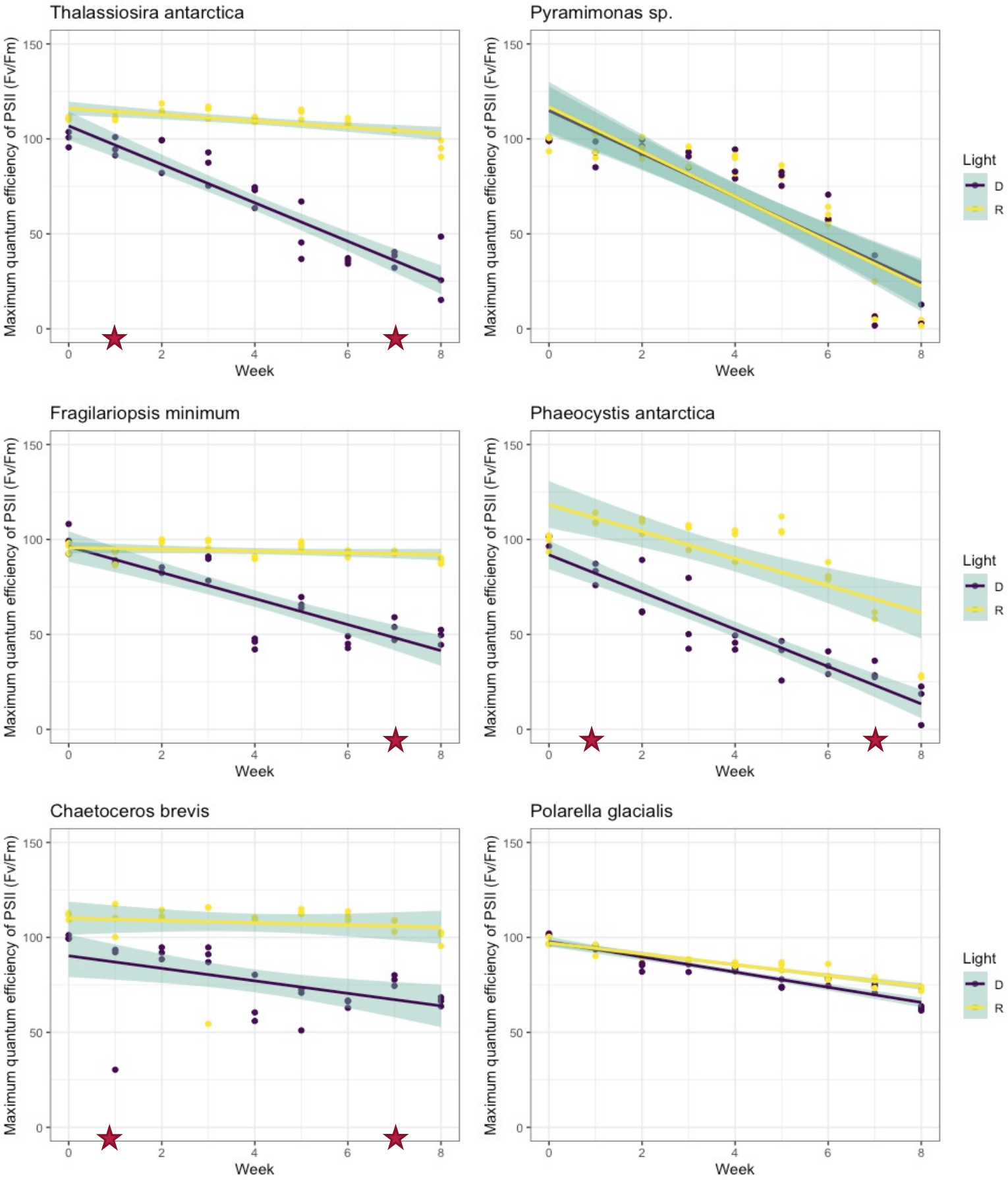

Fig 3. Linear trends of FRRf derived maximum quantum efficiency of PSII (F_v/F_m) during 8 weeks of dark incubation (dark lines) and subsequent recovery experiments under a 5 days-irradiance of 14 µmol photons m^{-2} s^{-1} (yellow lines) of all species (normalized data). Diatoms (*Thalassiosira antarctica, Fragilariopsis minimum, Chaetoceros brevis*) are shown on the left, flagellates (*Pyramimonas sp., Phaeocystis antarctica, Polarella glacialis*) are on the right. The grey line around the linear trend represents the confidence interval. Symbols (stars) mark the points (weeks 0-2, weeks 6-8) where the difference (absolute values) between dark-adapted and recovery samples is significant (Table 4).

Recovery experiments

Chl-*a* fluorescence – Chl-*a* recovery rates (i.e. how long (weeks) species are able to stay viable/recover after dark-incubation) were different for diatoms and flagellates. Diatoms Chl-*a* absolute values were significantly higher after 5 days of irradiance compared to dark-adapted samples during the first weeks (0, 1, 2) of experiment (ANOVA, $p < 0.001$; Fig 2, Table 4, Appendix 1). For the last weeks (6, 7, 8), recovery values were still significantly higher than directly after dark-incubation for *T.antarctica* and *F.minimum* (ANOVA, $p < 0.01$), but not for *C.brevis* (Table 4). *T.antarctica* Chl-*a* went from 94 µg m^{-3} to 193 µg m^{-3} (average of 3 replicates) after 1 week of dark-incubation (and subsequent 5 days of recovery), and from 8 to 18 µg m^{-3} after 8 weeks of darkness and consecutive irradiance (Fig. 2, Table 6). *F.minimum* showed an even stronger increase after 8 weeks of darkness and 5 days of irradiance, from 15 µg m^{-3} when dark incubated to 44 µg m^{-3} after 5 days (Table 6). *C.brevis* recovery was significant until week 7 (Fig. 2), and Chl-*a* absolute values dropped from 1060 µg m^{-3} at t=0 to 0.5 µg m^{-3} at t=8 (Table 6). Declines rates per $week^{-1}$ after recovery were higher in all three diatoms compared to the decline rates through time of dark-adapted samples (Table 2). For flagellates, absolute recovery values for the weeks 0-2 were only significantly different from dark values in *P.antarctica* and *Polarella* (Fig.2). For weeks 6-8, the difference between recovery and dark-adapted values was never significant (Table 4). *Pyramimonas sp.* did not recover the ability to synthetize Chl-*a* after dark-incubation, and recovery values were lower than dark-adapted values for all weeks. *P.antarctica* resumed higher Chl-*a* values after recovery from darkness for the first 4 weeks of experiments. Afterwards, Chl-*a* values decreased during recovery and for weeks 6-8 the difference with dark-adapted values was not significant (Table 4). *Polarella* maintained its ability to recover only until week 6 of the treatment (Fig.2). As in diatoms, Chl-*a* change rates were lower when samples were exposed to irradiance compared to during dark incubation (Table 2).

Photosynthetic parameters – In all diatoms, Chl-*a* and PSII photosynthetic parameters significantly increased after 5 days in irradiance (compared to dark-adapted values). Among flagellates, *P.antarctica* was the only species displaying recovery after 5 days in irradiance, whereas PSII-photosynthetic parameters of *Pyramimonas sp.* and *Polarella* did not significantly recover after 5 days under dim-light (Table 2).

In diatoms, maximum quantum yield of PSII (F_v/F_m) increased significantly after 5 days of recovery compared to directly after darkness (ANOVA, $p < 0.01$; Table 4). The difference with dark-adapted samples was only significant in *T.antarctica* and *C.brevis* for weeks 0-2, and in all three diatoms for weeks 6-8 (Fig.3, Table 4). Between flagellates, *P.antarctica* was the only species to show a significant difference (recovery) after darkness, both at the beginning (weeks 0-2) and at the end (weeks 6-8) of the experiment (ANOVA, $p = 0.0253$; Fig.3, Table 4). *Pyramimonas sp.* had slightly higher F_v/F_m after recovery until week 6. *Polarella* F_v/F_m after 5 days recovery had similar or equal values to directly after darkness (Fig.2). Absolute values were generally higher at t=8 (and compared to t=0) in diatoms than in flagellates (Table 6, Appendix 2). Accordingly, decline rates after recovery were higher in flagellates than in diatoms throughout the 8 weeks of experiments (Table 2).

The functional absorption cross section of PSII (σ_{PSII}) did not show any significant difference after 5 days of recovery in either diatoms or flagellates. Variation between the two taxonomic groups was not significant either (Table 3, Fig. 4). The decline rates of recovery samples were similar to the ones of dark-adapted samples (Table 2, Fig.4).

Reopening times of closed reaction centers of PSII (τ_{PSII}) significantly decreased after recovery compared to directly after darkness in both diatoms and flagellates throughout the whole experiment (Fig.5, Table 4). In *T.antarctica*, the decrease after recovery compared to the dark-adapted sample was almost 50%. *F.minimum* and *C.brevis* τ_{PSII} decrease was > 50% after recovery (Table 6, Fig.5). In *Pyramimonas sp.*, τ_{PSII} was significantly lower after recovery compared to dark-incubated samples both at weeks 0-2 and weeks 4-6 (data is only available up to 6 weeks of dark incubation). *Polarella* showed a similar decline as *Pyramimonas sp.* (Table 4). Whereas *P.antarctica* did not show a significant difference between dark-adapted and recovery samples during the first

weeks of experiment (0-2) (Fig.5). Furthermore, increase rates per week^{-1} were higher in dark-adapted samples than in samples exposed to irradiance (Table 2, Fig.5).

In all three diatoms, the efficiency of energy transfer between PSII reaction centers (ρ) was significantly different after recovery than directly after darkness (Table 4). In contrast to dark-adapted samples, ρ of recovery samples slightly increased all through the 8 weeks of experiments (Table 2, Fig.6). In flagellates, a significant difference with dark-adapted samples after recovery was only detected in *P.antarctica* (ANOVA, p = 0.0127; Table 4). All three flagellates showed declining rates through the 8 weeks of recovery (Table 2, Fig.6).

In diatoms, NSV-non photochemical quenching after recovery increased from t=0 to t=8 (Table 6). The difference between recovery and dark-adapted values was stronger at weeks 6-8 then at weeks 0-2 in all three diatoms (Table 4). In fact, the increase rate after recovery was significantly lower than the one of dark-adapted samples (Table 2, Fig.7). Furthermore, recovery samples had lower absolute values compared to the ones obtained directly after darkness (Table 3, Appendix 6). NPQ of prasinophyceae *(Pyramimonas sp.)* and dinophyceae *(Polarella)* did not significantly change after 5 days of recovery compared to darkness, neither at the beginning nor at the end of the experiments (Table 4). *Pyramimonas sp*. and *Polarella* increased their non-photochemical quenching of respectively +2% and +10% (Table 2, Fig.7) from the start to the end of the experiment (Table 6). The haptophyceae *P.antarctica* NPQ reversed after 5 days of irradiance, and absolute values after irradiance were lower than dark-adapted values by weeks 6-8 (Table 5, 6; Fig 7, Appendix 6).

Table 5. Absolute values of Chl-*a* and photosynthetic parameters in dark-adapted samples, at t=0 (before dark incubation) and t=8 (after 8 weeks of darkness). Values represent the average of the three replicates. Standard deviations are shown between brackets.

	Thalassiosira antarctica		***Fragilariopsis minumum***		***Chaetoceros brevis***		***Pyramimonas sp.***		***Phaeocystis antarctica***		***Polarella glacialis***	
	t=0	**t=8**	**t=0**	**t=8**	**t=0**	**t=8**	**t=0**	**t=8**	**t=0**	**t=8**	**t=0**	**t=8**
Chl-a	137,5 (16,68)	8,176 (0,548)	92,48 (93,39)	14,65 (13,65)	316,5 (12,14)	45,03 (5,51)	502,5 (33,93)	1,87 (0,83)	188,9 (51,54)	23,85 (20,35)	107,5 (33,5)	10,223 (1,11)
Fv/Fm	0,519 (0,021)	0,154 (0,088)	0,543 (0,042)	0,265 (0,021)	0,464 (0,004)	0,307 (0,011)	0,625 (0,006)	0,043 (0,031)	0,442 (0,013)	0,064 (0,047)	0,57 (0,02)	0,355 (0,006)
σ_{PSII}	3,48 (0,158)	1,619 (1,773)	6,038 (0,240)	1,740 (0,220)	4,828 (0,191)	1,619 (0,057)	3,631 (0,26)	1,973 (0,106)	7,442 (0,615)	3,471 (0,13)	5,13 (0,36)	2,602 (0,05)
τ_{PSII}	2870,3 (288,39)	6283,6 (615,6)	2806,3 (487,8)	9400,6 (309,13)	3173,3 (680,2)	8325,3 (692,2)	2960 (111,6)	6000 (249,2)	2844,6 (643,43)	5033,5 (36,06)	3263 (29,4)	6085,3 (243,9)
ρ	375,6 (10,26)	94,33 (70,585)	344 (15,58)	251,3 (57,3)	311 (25)	252,3 (13,57)	489,6 (12,58)	257,3 (39,3)	348 (79,8)	118 (0)	310,6 (4,16)	207,6 (40,4)

NSV-NPQ	903,6 (122,32)	6206,3 (4505,05)	830,3 (127,1)	2082,6 (221,4)	1036,6 (115,1)	1764 (47,46)	542 (39,88)	16,6 (9,29)	1126 (171,5)	5829 (22,8)	738,6 (79,9)	1911,6 (64,2)

Table 6. Absolute values of Chl-*a* and photosynthetic parameters in recovery samples, at t=0 (exposure at 14 µmol photons m^{-2} s^{-1} before dark incubation) and t=8 (recovery subsequent to 8 weeks of darkness). Values represent the average of the three replicates. Standard deviations are shown between brackets.

	Thalassiosira antarctica		***Fragilariopsis minimum***		***Chaetoceros brevis***		***Pyramimonas sp.***		***Phaeocystis antarctica***		***Polarella glacialis***	
	t=0	**t=8**	**t=0**	**t=8**	**t=0**	**t=8**	**t=0**	**t=8**	**t=0**	**t=8**	**t=0**	**t=8**
Chl-a	323,6 (57,33)	17,97 (3,27)	247,04 (274,8)	43,68 (43,32)	1057,6 (131,9)	0,464 (0,018)	556,5 (17,1)	1,40 (0,151)	680,7 (181,14)	10,02 (10,9)	308,8 (25,14)	16,7 (0,50)
Fv/Fm	0,574 (0,004)	0,492 (0,022)	0,520 (0,014)	0,483 (0,009)	0,516 (0,008)	0,464 (0,018)	0,611 (0,02)	0,018 (0,009)	0,435 (0,019)	0,124 (0,002)	0,555 (0,012)	0,413 (0,007)
σ_{PSII}	3,141 (0,123)	1,2473 (0,024)	5,85 (0,17)	2,003 (0,075)	4,477 (0,075)	1,677 (0,081)	3,240 (0,04)	2,194 (0,081)	7,985 (0,45)	2,531 (0,60)	5,27 (0,26)	2,17 (0,07)
τ_{PSII}	2593 (128,13)	3083,6 (237,9)	3041 (225,5)	3292,6 (201,1)	3203 (320)	3297 (250)	2606 (249,7)	3041 (251,5)	2277,3 (360,7)	3731 (565,7)	2819,3 (551,7)	3314 (103,5)
ρ	441 (11,53)	457 (15,39)	398,3 (29,1)	422,3 (9,29)	369 (8,88)	430 (23,9)	452,6 (3,21)	370,6 (28,36)	383,3 (25,5)	142 (87,7)	339,6 (18,4)	286,3 (72,5)
NSV-NPQ	644,3 (644,3)	994 (82,02)	869 (34,04)	1001 (80,7)	846 (43,7)	1034,6 (121)	567,6 (50,76)	1.292 (190,2)	1214 (221,3)	3112,5 (3699,8)	673,6 (96,7)	1411 (98,23)

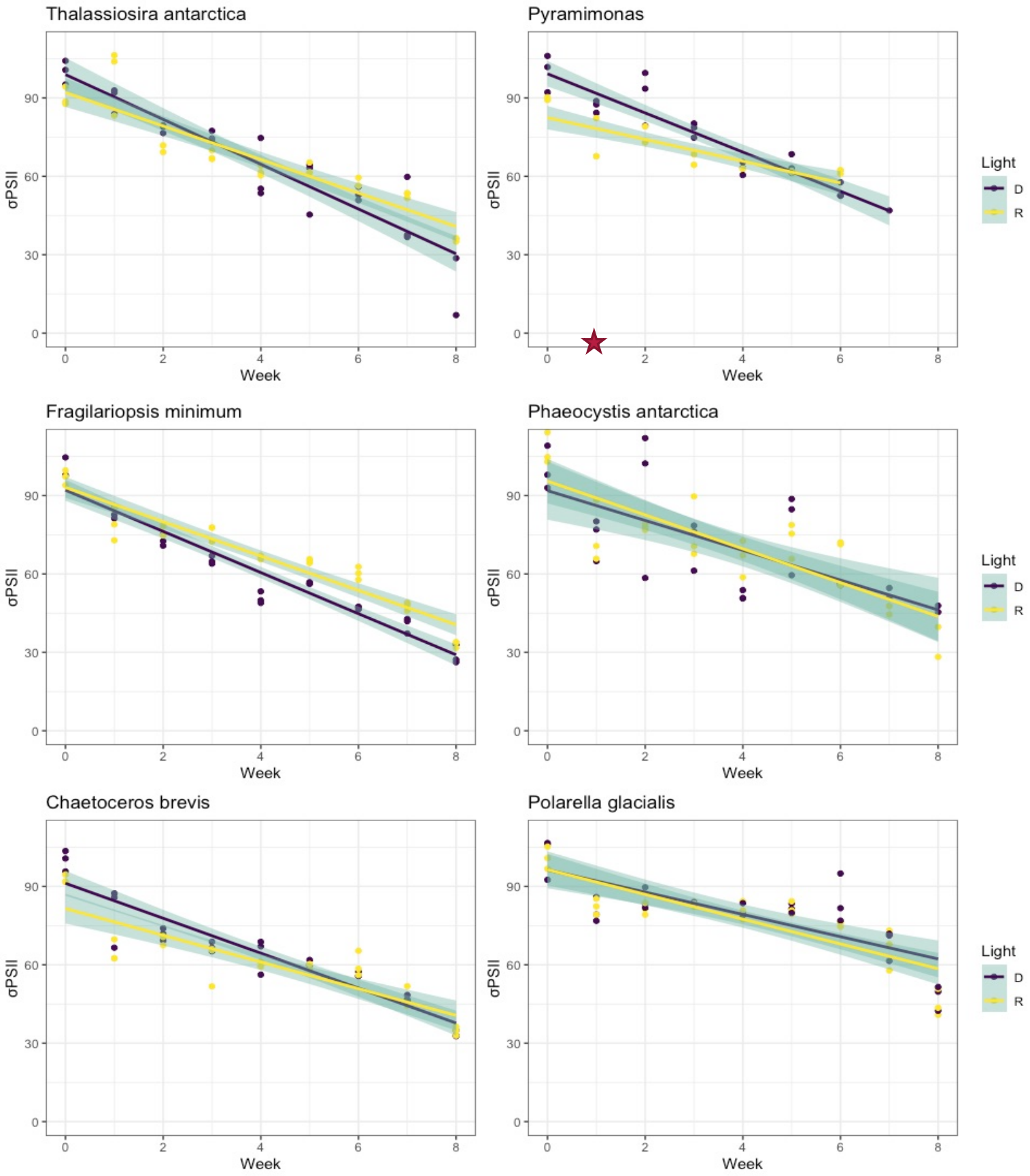

Fig 4. Linear trends of FRRf-derived functional absorption cross section (σ_{PSII}) during 8 weeks of dark incubation (dark lines) and subsequent recovery experiments under a 5 days-irradiance of 14 µmol photons m^{-2} s^{-1} (yellow lines) of all species (normalized data). Diatoms (*Thalassiosira antarctica, Fragilariopsis minimum, Chaetoceros brevis*) are shown on the left, flagellates (*Pyramimonas sp., Phaeocystis antarctica, Polarella glacialis*) are on the right. The grey line around the linear trend represents the confidence interval. Symbols (stars) mark the points (weeks 0-2, weeks 6-8) where the difference (absolute values) between dark-adapted and recovery samples is significant (Table 4).

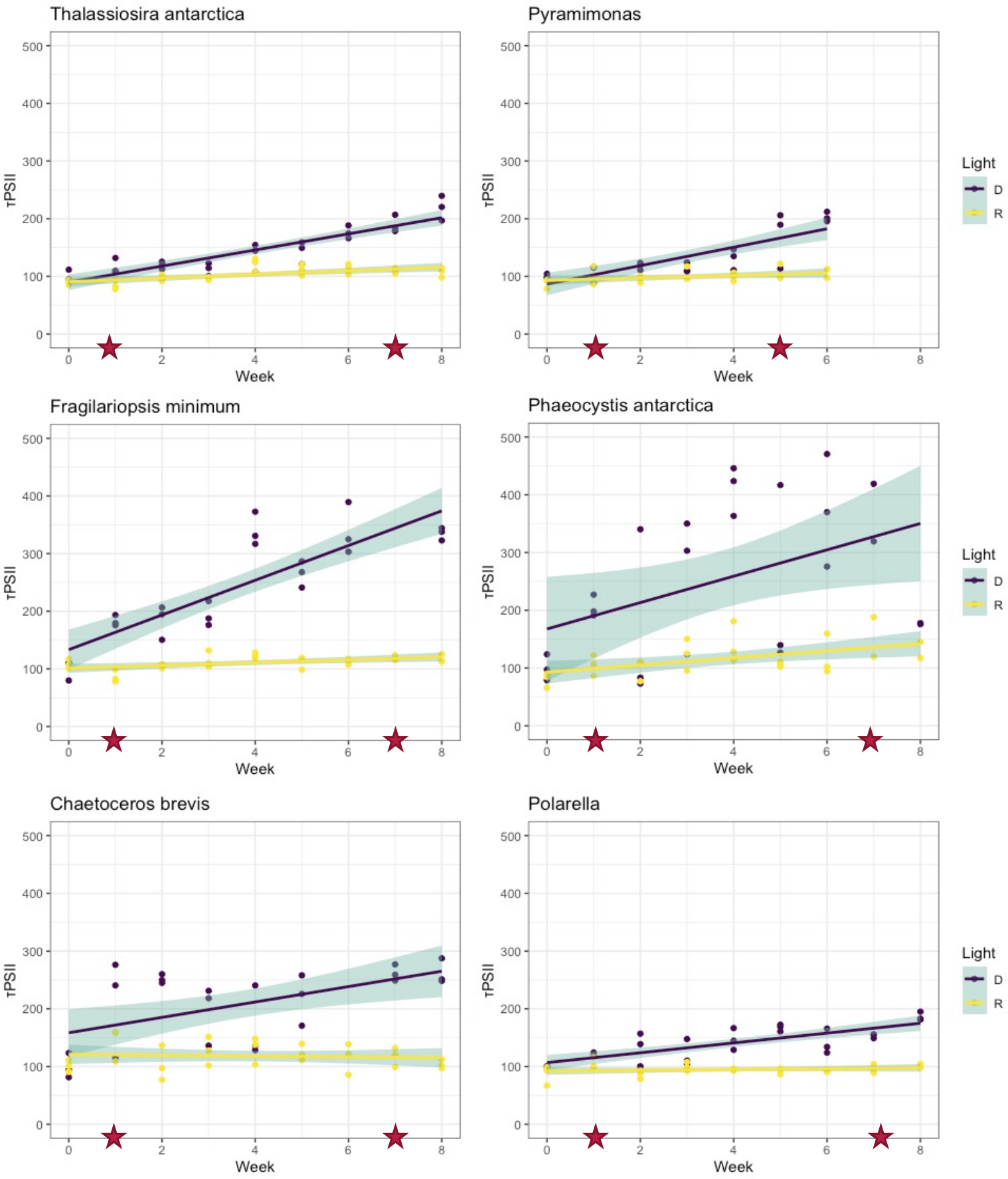

Fig 5. Linear trends of FRRf-derived reopening times of PSII reaction centres (τ_{PSII}) values during 8 weeks of dark incubation (dark lines) and subsequent recovery experiments under a 5 days-irradiance of 14 µmol photons m^{-2} s^{-1} (yellow lines) of all species (normalized data). Diatoms (*Thalassiosira antarctica, Fragilariopsis minimum, Chaetoceros brevis*) are shown on the left, flagellates (*Pyramimonas sp., Phaeocystis antarctica, Polarella glacialis*) are on the right. The grey line around the linear trend represents the confidence interval. Symbols (stars) mark the points (weeks 0-2, weeks 6-8) where the difference (absolute values) between dark-adapted and recovery samples is significant (Table 4).

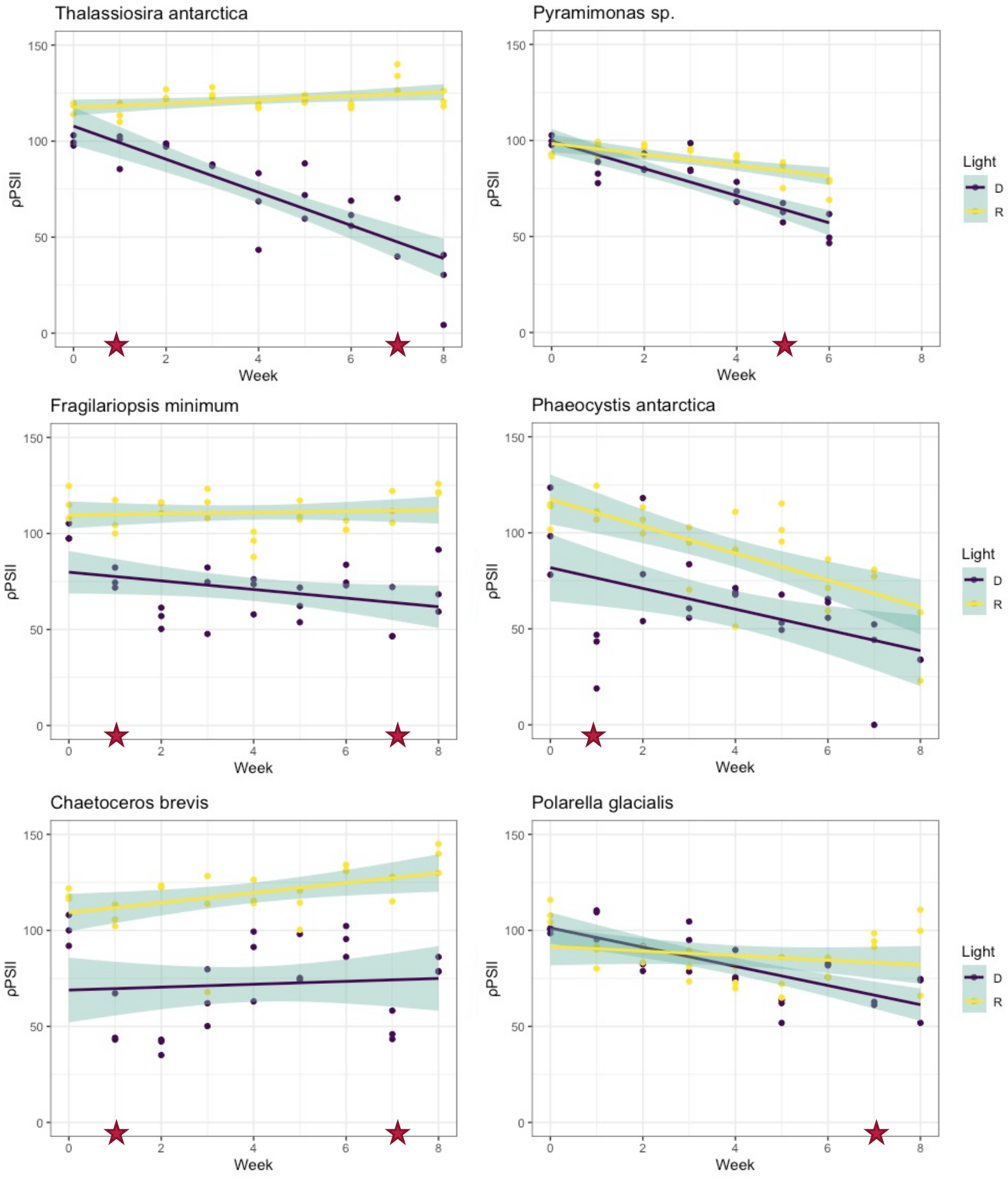

Fig 6. Linear trends of FRRf-derived "connectivity factor" (ρ) values during 8 weeks of dark incubation (dark lines) and subsequent recovery experiments under a 5 days-irradiance of 14 µmol photons m^{-2} s^{-1} (yellow lines) of all species (normalized data). Diatoms (*Thalassiosira antarctica, Fragilariopsis minimum, Chaetoceros brevis*) are shown on the left, flagellates (*Pyramimonas sp., Phaeocystis antarctica, Polarella glacialis*) are on the right. The grey line around the linear trend represents the confidence interval. Symbols (stars) mark the points (weeks 0-2, weeks 6-8) where the difference (absolute values) between dark-adapted and recovery samples is significant (Table 4).

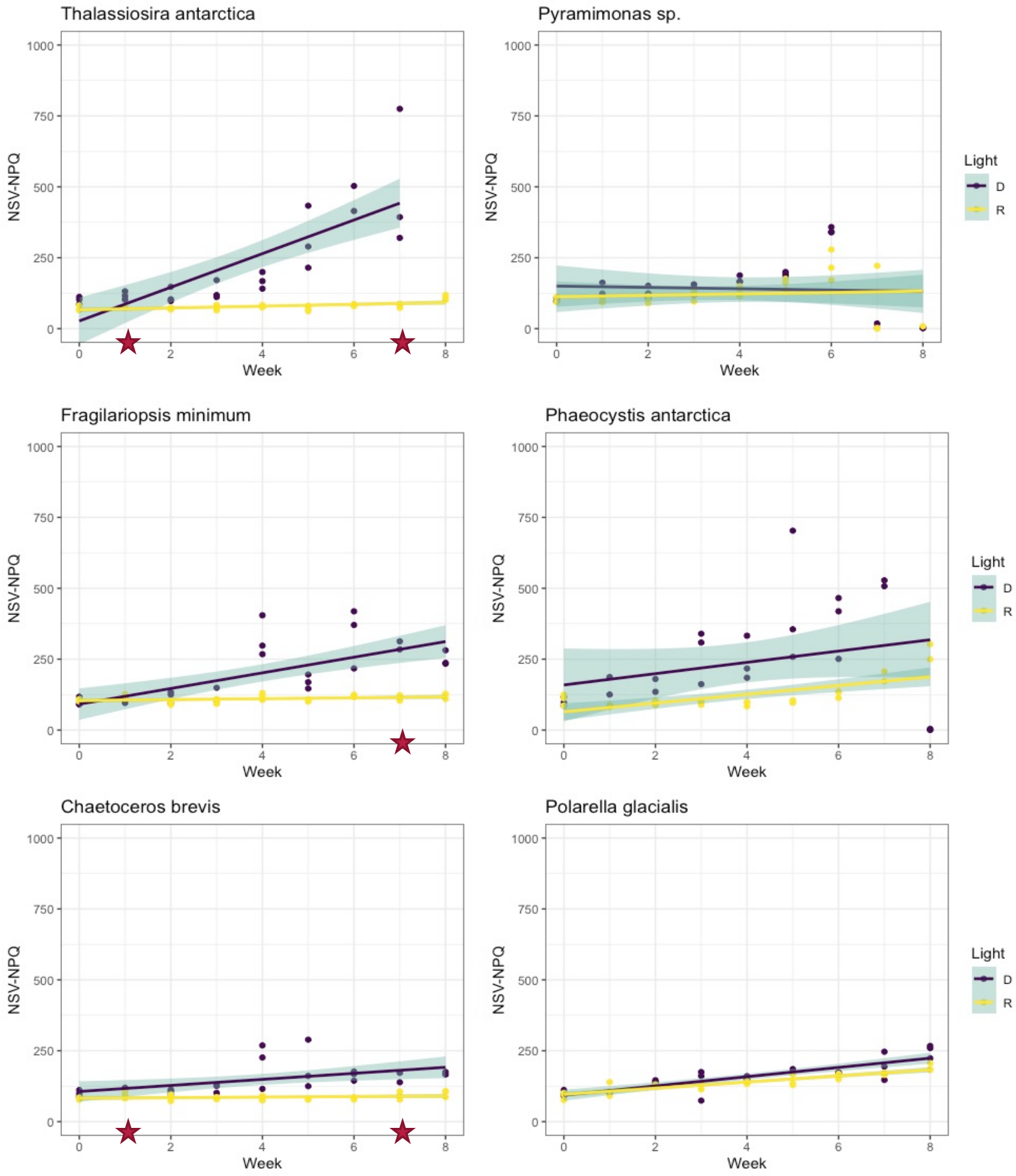

Fig 7. Linear trends of FRRf-derived NSV-non photochemical quenching during 8 weeks of dark incubation (dark lines) and subsequent recovery experiments under a 5 days-irradiance of 14 µmol photons m^{-2} s^{-1} (yellow lines) of all species (normalized data). Diatoms (*Thalassiosira antarctica, Fragilariopsis minimum, Chaetoceros brevis*) are shown on the left, flagellates (*Pyramimonas sp., Phaeocystis antarctica, Polarella glacialis*) are on the right. The grey line around the linear trend represents the confidence interval. Symbols (stars) mark the points (weeks 0-2, weeks 6-8) where the difference (absolute values) between dark-adapted and recovery samples is significant (Table 4).

DISCUSSION

The microalgal species analysed in our experiment have to witness harsh seasonal fluctuations in light conditions when found in their natural environment, where prolonged darkness can be followed by prolonged irradiance (due to e.g. polar night, sea-ice melt, stratification). For this, polar microalgae went through evolutionary adaptations that differentiate them from temperate and tropical microalgae (Hopes and Mock, 2015*).* Polar microalgae need to maintain their photosynthetic capacity during the long dark season and subsequently prevent photodamage during protracted periods of irradiance (Osmond, 1981; Hopes and Mock, 2015). The capability of a species to survive and successfully photo-acclimate determines its competitiveness (Moisan and Olaizola, 1998).

In our study, all six types of phytoplankton species revealed species-specific photosynthetic responses to light-deprivation for a prolonged period of time. In conditions of light deprivation, taxon-specific characteristics did not seem to determine changes in photosynthetic potential, and all groups showed similar responses to darkness. In 1996, Peters already uncovered species-specific traits as the main adaptability factor to changing environmental conditions, compared to taxonomic traits. However, diatoms and haptophyceae (*Phaeocystis antarctica*) showed faster recovery after darkness and upon re-exposure. In this case, taxonomic characteristics may have granted the two groups more flexibility and favoured recovery upon re-exposure to irradiance, compared to the other two flagellate groups. A faster and better recovery would allow these species to outcompete flagellates and exploit nutrient stocks in the early austral spring. Taxon/species-specific responses to environmental variations heavily control the spatial distribution of algae in the Southern Ocean (Scharek *et al.,* 1994), and consequently regional biomass and primary production rates.

Adaptations to darkness – A similar decline trend in Chl-*a* concentration could be recognised in all six species. Chl-*a* started declining after the first week of dark incubation and kept dropping steadily throughout the entire length of the experiment in both diatoms and flagellates. By the end of the dark incubation period (week 8), Chl-*a* concentration was close to zero in all species. Such results highlighted how light can be a major limiting factor for algal growth. These observations of Chl-*a* concentration contrast with the research findings of van de Poll *et al.* (2019) on polar diatoms (*Thalassiosira antartica, Thalassiosira nordenskioeldii*) and flagellates (*Rhodomonas sp., Micromonas sp*.) from the Arctic Ocean. Assertedly, diatoms were more resistant to darkness compared to flagellates, thus superior at surviving and competing after long dark incubation. This difference might be due to the different length of polar night/dark season experienced by Arctic and Antarctic species. Due to geographical conformation, Arctic microalgae are exposed to longer polar nights than Antarctic species, which in turn often experience short or no polar night (although light can still be limited by sea-ice cover). Furthermore, being the Southern Ocean mostly disconnected from other oceans (i.e. strong currents around the Antarctic circle), Antarctic species evolved separately from other species for millions of years, hereby evolving peculiar adaptations.

Parallel to Chl-*a* content, the decline in F_v/F_m symbolizes a considerable deterioration of the cellular photosynthetic apparatus due to prolonged absence of light. Gradual loss of PSII efficiency was likewise confirmed by other FRRf-derived photosynthetic parameters. For instance, the reduction of the functional absorption cross section (σ_{PSII}), experienced at similar rates by all species, revealed a major drop in the efficiency of light utilization by the photosynthetic apparatus. Accordingly, turnover times of the electron transfer chain (τ_{PSII}) were 2 or 3-fold longer after 8 weeks of darkness compared to pre-treatment times. The increase in turnover times induced a decrease in photosynthetic rates and energy conversion efficiency (Han *et al.,* 2000). The only significant difference between groups was detected for the non-photochemical quenching of chlorophyll fluorescence (NPQ). During dark-incubation, diatoms NPQ was low at the beginning of the dark period and was induced throughout the dark-incubation. Whereas *Pyramimonas sp*. and *P.antarctica* NPQ decreased during the 8 weeks of dark treatment. The induction of NPQ during darkness in diatoms has already been reported by previous studies (Falkowski *et al.,* 1986*;* Demers *et al.,* 1991*;* Ting and Owens, 1993*;* Geel *et al.,* 1997*;* Jakob *et al.,* 1999*,* Mouget and Tremblin, 2002*).* Diatoms rely on the heat-dissipation through the interconversion between the pigments diadinoxanthin (Ddx) and diatoxanthin (Dtx) (xanthophyll cycle) as a non-photochemical quenching mechanism (Welschmeyer and Hoepffner, 1986; Demers *et al.,* 1991; Brunet *et al.,* 1993) in the PSII antenna system. This cycle responds faster to high light compared to the VAZ (violaxanthin, antheraxanthin and

zeaxanthin) cycle, which is common among green flagellates (Demrnig-Adams, 1990; Arsalane *et al.,* 1994*).* Haptophytes such as *Phaeocystis antarctica* display the Ddx/Dtx cycle as well. Fernández-Marín *et al.* (2011) observed a correlation between the de-epoxidation of xanthophylls with a decrease in F_v/F_m in the downregulation of photosynthetic activity during darkness. In our study, we also observed a linear correlation between NPQ and F_v/F_m values (r^2=0.82), where higher non-photochemical quenching linearly relates to a lower quantum efficiency of the PSII. An increase in the Ddx pool size, which can be induced by low temperatures (Anning *et al.,* 2001), allows the accumulation of high levels of Dtx, which usually accumulates under high irradiances. Dtx seems to be able to induce NPQ even in absence of a proton gradient (Goss *et al.,* 2006b). At the same time, the xanthophyll pool size is determined by initial conditions of acclimation (Demers *et al.,* 1991; Willemoës and Monas, 1991; Moisan *et al.,* 1998). Furthermore, the exposure to high irradiances under low temperature conditions has been proven damaging, as low temperatures can slow down the photoprotective response (Serôdio *et al.,* 2005). The enhancement of NPQ in darkness, and consequent lower F_v/F_m, is regarded as side-effect of the increase of the Ddx pool. Such ability to induce NPQ in darkness is deemed to be an adaptive advantage for diatoms and crucial to avoid the degradation of xanthophyll cycle pigments during prolonged darkness (Hoefnagel *et al.,* 1998; Jakob *et al.,* 1999). The xanthophyll cycle and acclimation can be a major contributing factor for a species' ability to photo-acclimate and be competitive in varying light conditions (Meyer *et al.,* 2000).

Re-exposure to irradiance – The exposure of polar microalgae to strong irradiances triggers photoinhibition, resulting from the damage of PSII reaction centers (Ibelings *et al.,* 1991; Kok, 1956*).* This down-regulation of the photosynthetic activity is required to avoid the production of excessive energy, thus radical formation and damage to the photosynthetic apparatus (van Leeuwe *et al.,* 2005*).* At lower temperatures, the biochemical process of reparation after photo-damage is even slower (Young and Schmidt, 2020). Furthermore, adaptation to lower irradiances might leave algae more vulnerable to strong irradiances and a drastic shift from low to high-light conditions can make acclimation even more challenging (Behrenfeld *et al.,* 1998; Arrigo *et al.,* 2010). The photoprotection potential is a key functional feature of algal heterogeneity (Jakob *et al.,* 1999).

All three diatoms exhibited substantially higher Chl-*a* values after 5 days of irradiance compared to directly after dark-incubation, both at the beginning (weeks 0-2) and at the end (weeks 6-8) of the experiment. This revealed their ability to preserve the PSII functionality and stay viable – despite the poor conditions displayed during darkness – in order to resume photosynthetic activity upon return of light. Flagellates on the other hand did not exhibit any significant recovery of Chl-a production after irradiance by the end of the experimental period (weeks 6-8; for *Pyramimonas* sp., weeks 4-6), revealing a deficiency in the ability to resume Chl-a synthesis after darkness.

Accordingly, diatoms F_v/F_m increased significantly after 5 days of irradiance. Among flagellates, *Phaeocystis antarctica* was the only species displaying F_v/F_m recovery after re-exposure, while *Pyramimonas* sp. and *Polarella* showed little or no recovery. Seemingly, the Dd/Dt xanthophylls cycle found in diatoms and *P.antarctica* played a role in the recovery of PSII functionality upon light re-exposure. In diatoms, non-photochemical quenching was induced during darkness, but absolute values after recovery from darkness did not significantly increase or decrease compared to initial values at t=0 (Appendix 2). Consequently, diatoms and haptophyceae-NPQ was reversed during recovery from darkness. On the other hand, *Pyramimonas* sp. and *Polarella* displayed higher NPQ absolute values after 5 days of recovery compared to diatoms. In green flagellates, such as *Pyramimonas* sp., the NPQ mechanism is connected to the violaxanthin cycle, which might explain the stronger NPQ enhancement after illumination compared to the other groups. According to Goss *et al.* (2006a), the conversion of Zx to Vx slightly increases during high light illumination, whereas the Dtx epoxidation cycle is inhibited by the light-induced proton gradient (Goss *et al.,* 2006b). As previously mentioned, the NPQ mechanism is connected to the quantum efficiency of PSII (F_v/F_m), and the reversal of NPQ supported the recovery of photosynthetic efficiency of PSII in diatoms and *P.antarctica*. Despite displaying reversal of the NPQ mechanism and recovery of F_v/F_m, *P.antarctica* was not able to resume Chl-a synthesis as fast as diatoms by the end of the experiment (week 6-8), highlighting diversity between the two taxonomic groups' responses.

Our observations after recovery are in line with those of van de Poll *et al.* (2019) on Arctic microalgae. The previously mentioned Arctic diatoms *T.antarctica* and *T. nordenskioeldii* were able to recover from 8 weeks of darkness, in contrast to the flagellates *Rhodomonas* sp. and *Micromonas* sp.. Prolonged darkness thus seemed to be detrimental for the photosynthetic apparatus of (some) flagellates and unfavourable for growth upon restoration of light conditions. Nonetheless, both diatoms and flagellates showed inter-group variability, as each species showed unique recovery or degradation rates. Two possible explanations were hypothesized for the lack of recovery after darkness. Algae might be permanently damaged by light deprivation and not able to recover full PSII functionality after long periods of darkness (i.e. 8 weeks). Alternatively, once adapted to little or no irradiance, they are not able to react to the photodamage caused by higher irradiances (photoinhibition). Furthermore, the VAZ cycle-NPQ is not as quickly reversible as the xanthophyll cycle-NPQ, which might suggest that 5 days of irradiance are not enough for them to resume photosynthesis. The latter hypothesis would be in line with previous discoveries from Ibelings *et al.* (1994) and Dijkman (2001). In such case, growth reduction would be explained by a down-regulation of the efficiency of PSII and consequent cut in energy production, required to avoid photodamage. It has been hypothesized that, in conditions of changing light regimes, algae might adapt by maintaining their light-harvesting capacity to a minimum during darkness – but sufficiently high as to keep the cell viable (van Leeuwe *et al.,* 2005). Concurrently, upon return of light, generation of excess energy causes photodamage. This can be prevented by increasing PSII turnover rates (Kana *et al.,* 2002). Eventually, the enhancement of maintenance costs may lead the algae to a reduction of the energy invested in growth.

CONCLUSIONS

Despite species-specific variability in the degradation rates of PSII functionality, both diatoms and flagellates exhibited poor photosynthetic efficiency during the 8 weeks of incubation. Nevertheless, diatoms adapted readily to the return of light and showed better photosynthetic efficiency after recovery from 6-8 weeks of darkness, (compared to directly after darkness) than flagellate groups. Flagellates were hardly able to overcome the damage and get back in shape by the end of the experimental period (except for *Phaeocystis antarctica*). Certainly, the ability to withstand long darkness and recover from it strongly determines the competitive advantage of a species/group over the others and affects its spatial distribution. In the Southern Ocean, characterized by great geographic and hydrographic variability, the degree of water mixing and light availability at surface change dramatically according to both season and geographical location (Arrigo *et al.,* 2010). Individual traits are thereby crucial in determing the ability of a species to thrive in a specific area and to exploit light and nutrients stocks. The ability to recover faster might grant diatoms the chance to exploit nutrients supply at the onset of the austral spring. Concurrently, they might have a competitive advantage in regions that lack solar irradiance for longer periods of time. In Southern Ocean regions where the dark season lasts the longest (i.e. closer to the Antarctic Circle), we would thus expect phytoplankton blooms to be dominated by diatoms rather than flagellates. However, it is important to point out that our observations were based on static light-conditions, whereas ambient light-conditions are commonly dynamic. Our findings can thus be limited by the (possibly) different developments and strategies adopted in a laboratory setup, compared to the natural environment.

Ecological implications – In the near future, polar microalgae will have to adapt to climate change and its various implication. In the Southern Ocean, these might imply thinning of sea-ice, which allows increasing light penetration (Lyon and Mock, 2014). Algae trapped in sea-ice would not directly experience temperature changes, although the thinning of sea ice would imply temporal and spatial reductions of their habitat (Young and Schmidt, 2020). However, increasing temperatures would cause a stronger stratification of the water column. Increased stratification can impact the algal community structure in the different ways. On one hand, the onset of shallow mixed layer, characterised by higher irradiance, might favour diatoms dominance. Whereas, on the other hand, strong stratification means loss of intensity of the upwelling, which can in turn negatively impact large diatoms in favour of smaller phytoplankton species such as flagellates (Marinov *et al*., 2010). Furthermore, according to Young and Schmidt (2020), increased temperatures could enhance metabolic rates and disrupt cellular

homeostasis, resulting in substantial loss of energy stores due to the thermal sensitiveness of algal biochemical processes. This would lead to a decrease in photosynthetic efficiency. The intensity and duration of these events will change in different locations and thus affect the spatial distribution of these algae. Consequently, dark-survival adaptations and light-dependent photosynthetic processes of both diatoms and flagellates are expected to change within the next decades.

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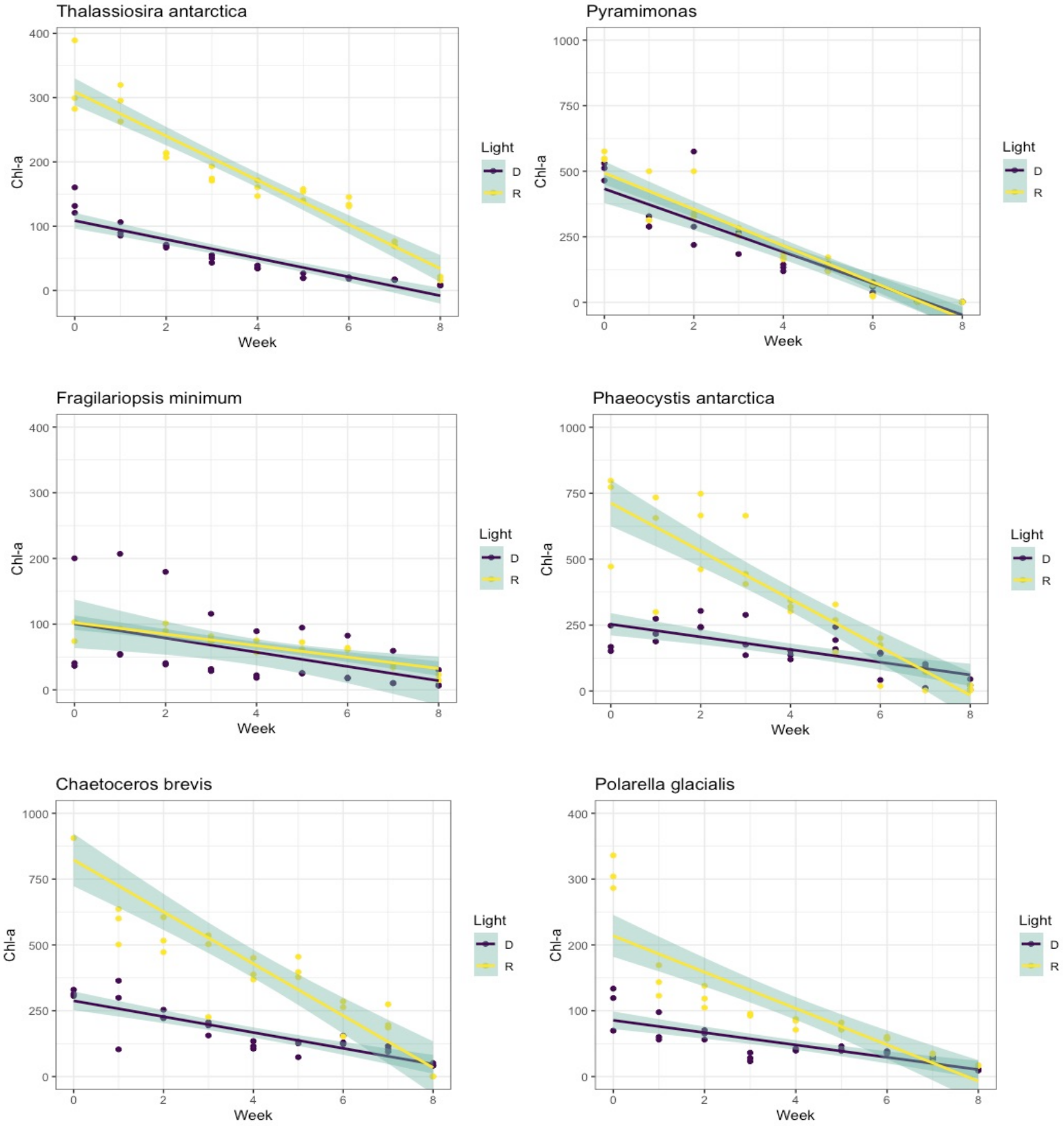
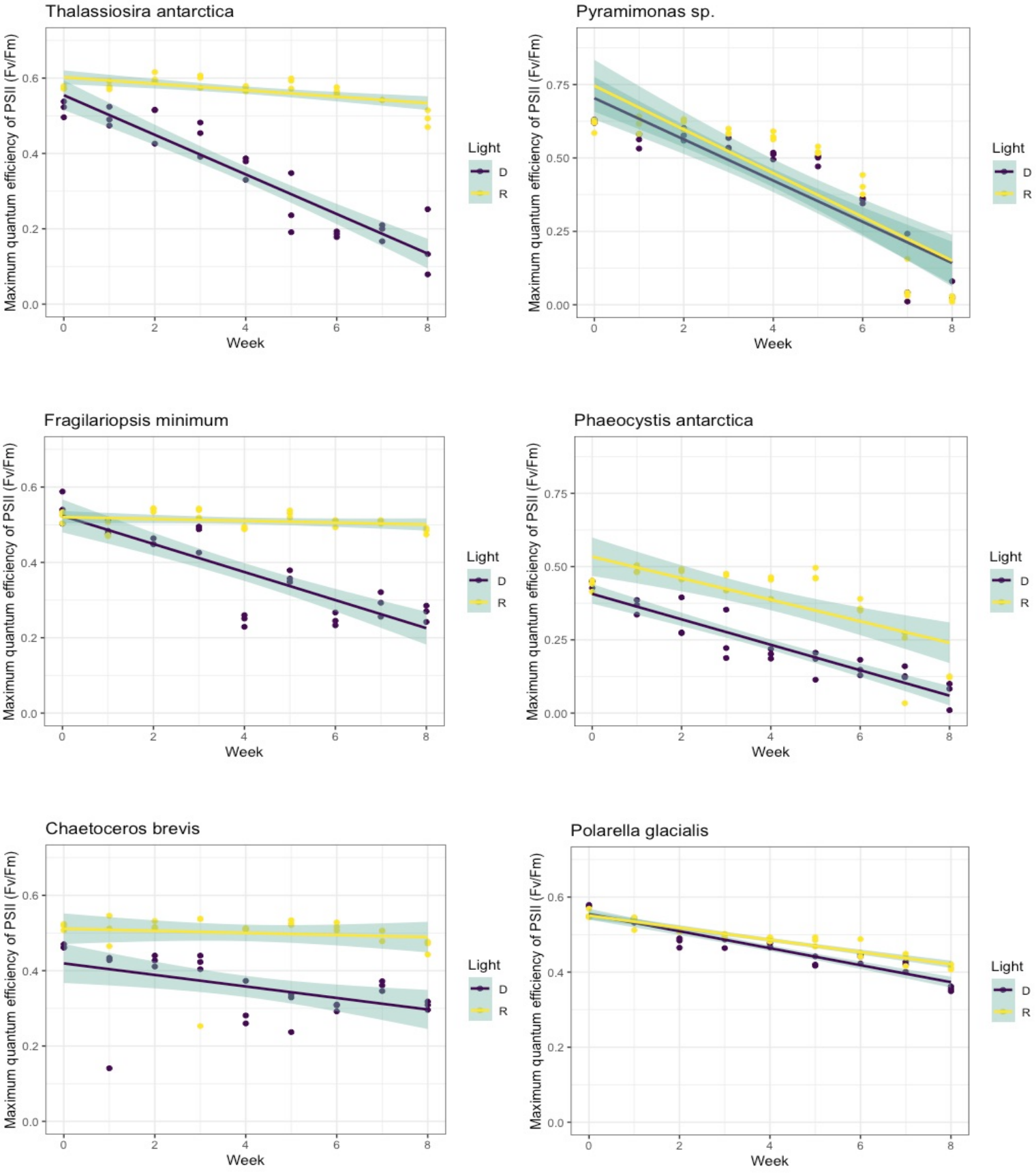
APPENDIX I



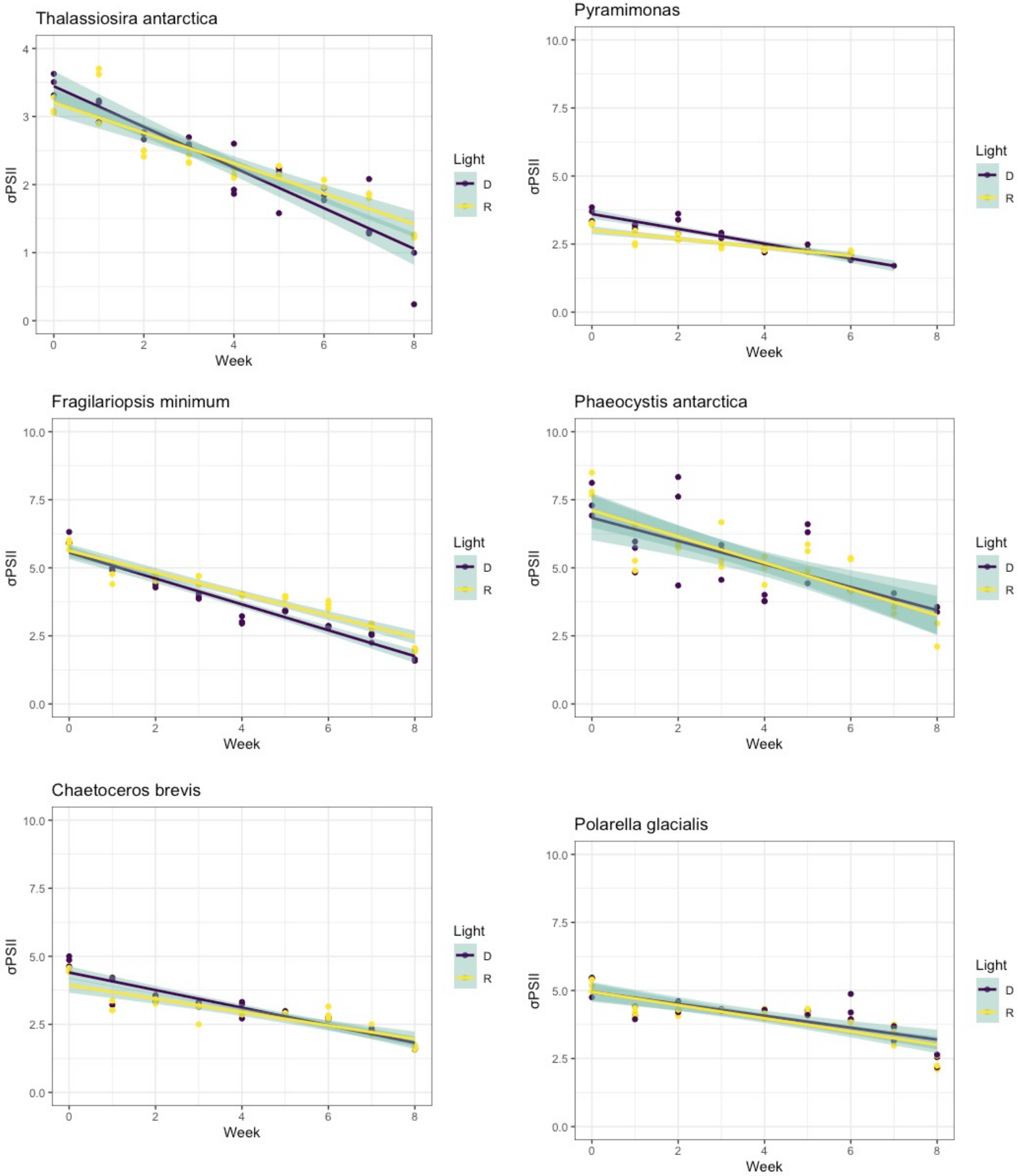
Appendix I - Linear trends (absolute values) of FRRf-derived Chl-*a* concentration during 8 weeks of dark incubation (dark lines) and subsequent recovery experiments under a 5 days-irradiance of 14 µmol photons m^{-2} s^{-1} (yellow lines) of all species. Diatoms (*Thalassiosira antarctica, Fragilariopsis minimum, Chaetoceros brevis*) are shown on the left, flagellates (*Pyramimonas sp., Phaeocystis antarctica, Polarella glacialis*) are on the right. The grey line around the linear trend represents the confidence interval.

APPENDIX 2



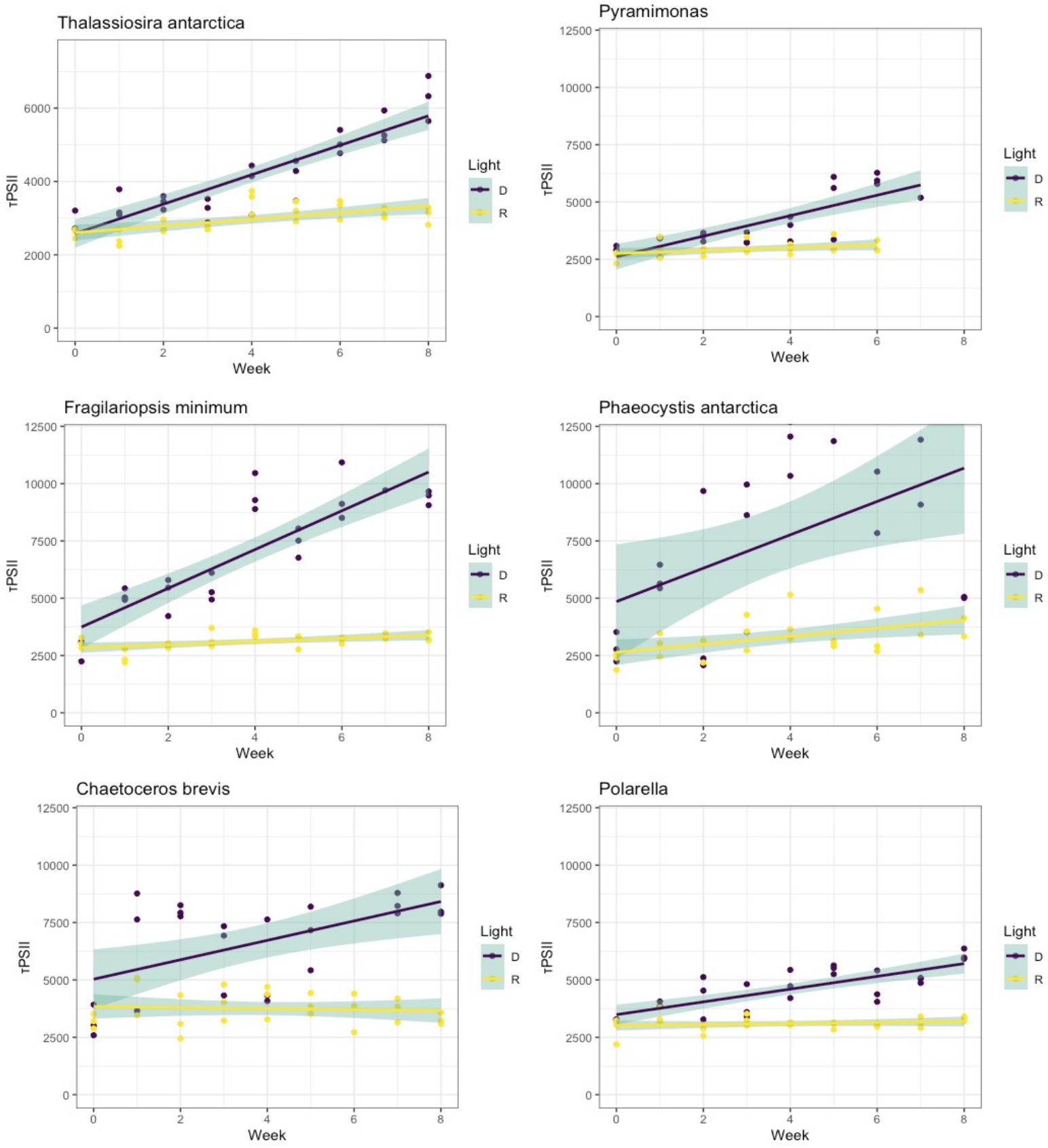
Appendix 2 - Linear trends (absolute values) of FRRf-derived maximum quantum efficiency of PSII (F_v/F_m) during 8 weeks of dark incubation (dark lines) and subsequent recovery experiments under a 5 days-irradiance of 14 µmol photons m^{-2} s^{-1} (yellow lines) of all species. Diatoms (*Thalassiosira antarctica, Fragilariopsis minimum, Chaetoceros brevis*) are shown on the left, flagellates (*Pyramimonas sp., Phaeocystis antarctica, Polarella glacialis*) are on the right. The grey line around the linear trend represents the confidence interval.

APPENDIX 3



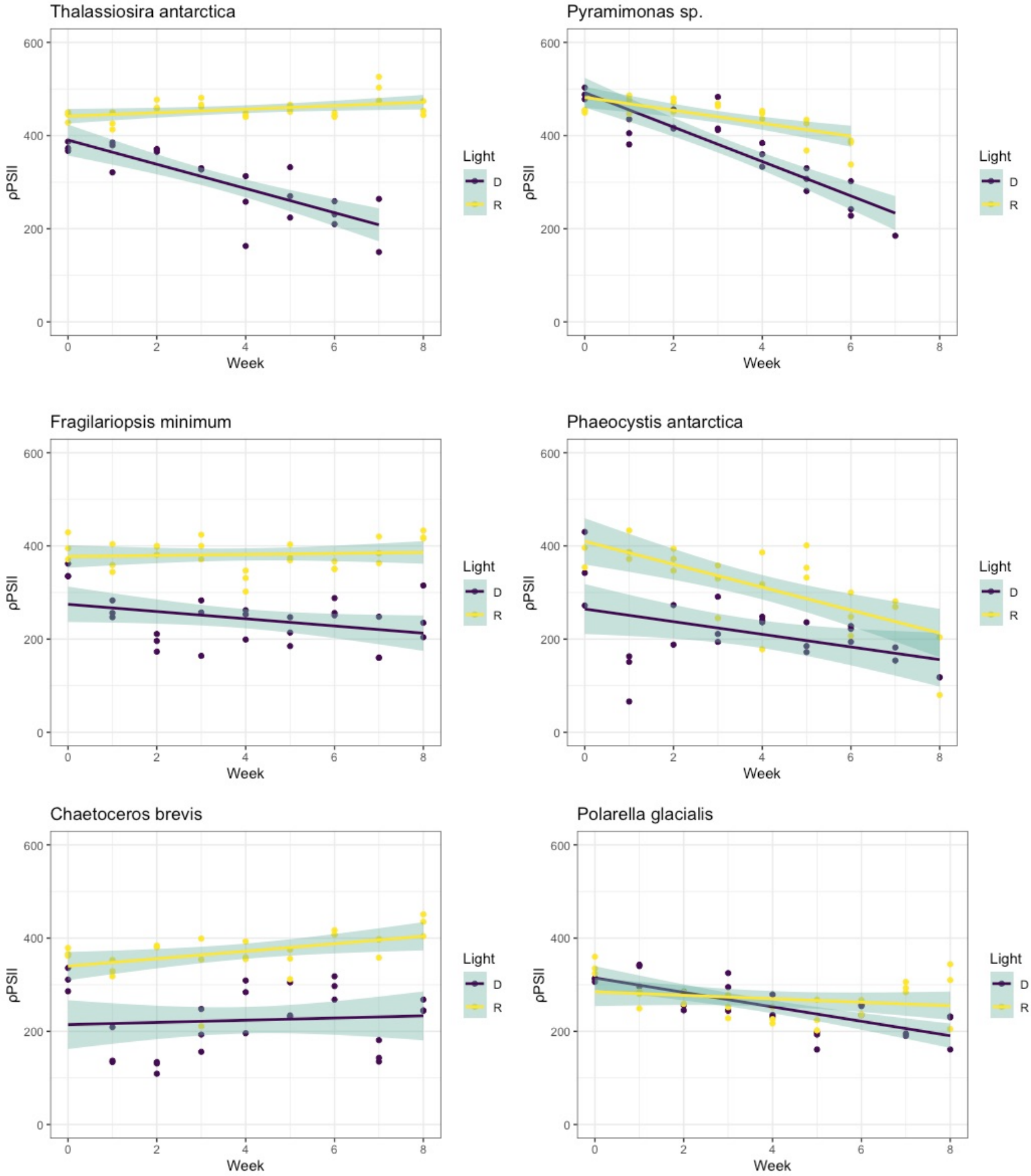
Appendix 3 - Linear trends (absolute values) of FRRf-derived absorption cross section (σ_{PSII}) during 8 weeks of dark incubation (dark lines) and subsequent recovery experiments under a 5 days-irradiance of 14 µmol photons m^{-2} s^{-1} (yellow lines) of all species. Diatoms (*Thalassiosira antarctica, Fragilariopsis minimum, Chaetoceros brevis*) are shown on the left, flagellates (*Pyramimonas sp., Phaeocystis antarctica, Polarella glacialis*) are on the right. The grey line around the linear trend represents the confidence interval.

APPENDIX 4



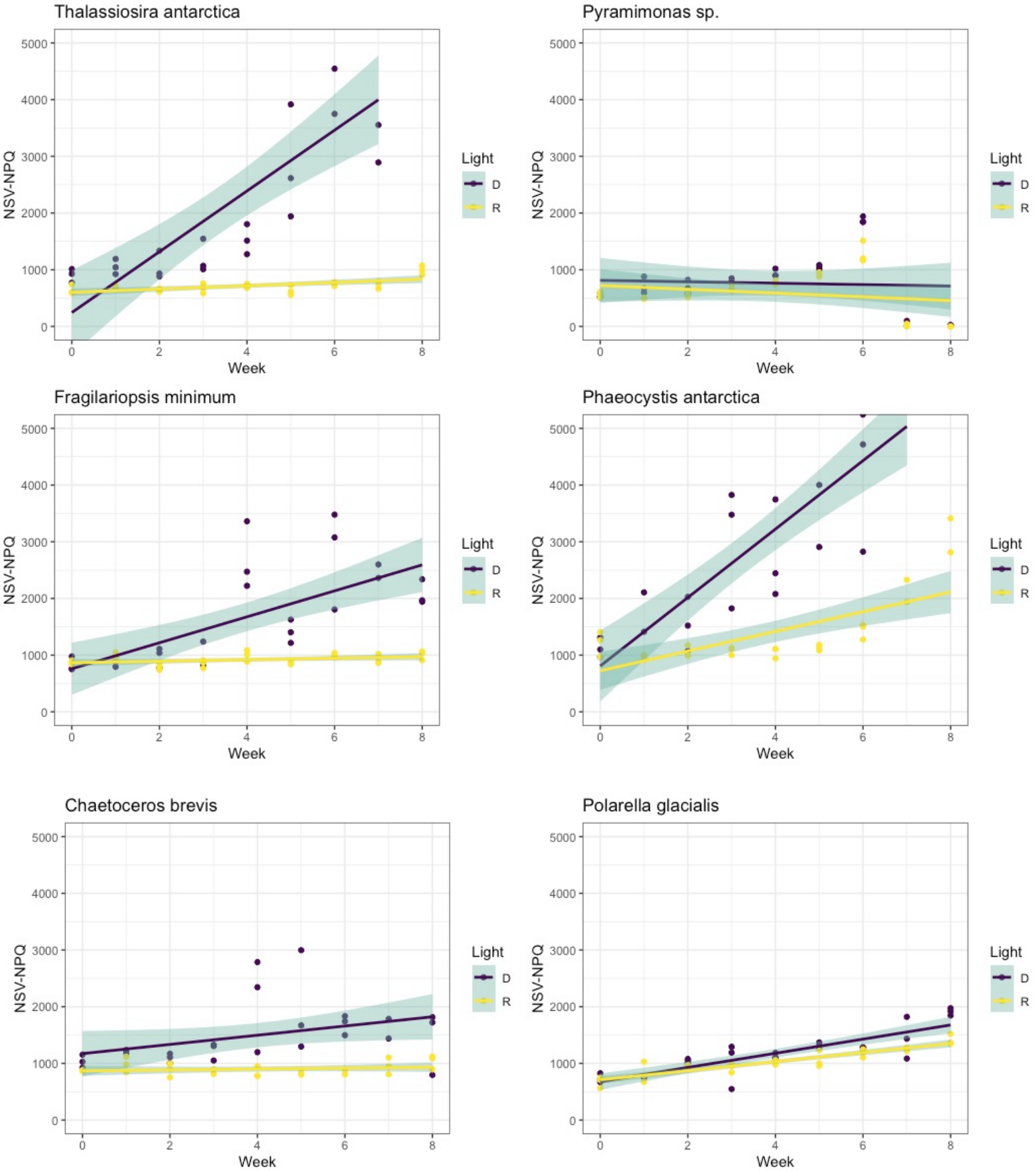
Appendix 4 - Linear trends (absolute values) of FRRf-derived turnover times (τ_{PSII}) during 8 weeks of dark incubation (dark lines) and subsequent recovery experiments under a 5 days-irradiance of 14 µmol photons m^{-2} s^{-1} (yellow lines) of all species. Diatoms (*Thalassiosira antarctica, Fragilariopsis minimum, Chaetoceros brevis*) are shown on the left, flagellates (*Pyramimonas sp., Phaeocystis antarctica, Polarella glacialis*) are on the right. The grey line around the linear trend represents the confidence interval.

APPENDIX 5



Appendix 5 - Linear trends (absolute values) of FRRf-derived "connectivity factor" (ρ) during 8 weeks of dark incubation (dark lines) and subsequent recovery experiments under a 5 days-irradiance of 14 µmol photons m^{-2} s^{-1} (yellow lines) of all species. Diatoms (*Thalassiosira antarctica, Fragilariopsis minimum, Chaetoceros brevis*) are shown on the left, flagellates (*Pyramimonas sp., Phaeocystis antarctica, Polarella glacialis*) are on the right. The grey line around the linear trend represents the confidence interval.

APPENDIX 6



Appendix 6 - Linear trends (absolute values) of FRRf-derived non-photochemical quenching (NPQ_{nsv}) during 8 weeks of dark incubation (dark lines) and subsequent recovery experiments under a 5 days-irradiance of 14 µmol photons m^{-2} s^{-1} (yellow lines) of all species. Diatoms (*Thalassiosira antarctica, Fragilariopsis minimum, Chaetoceros brevis*) are shown on the left, flagellates (*Pyramimonas sp., Phaeocystis antarctica, Polarella glacialis*) are on the right. The grey line around the linear trend represents the confidence interval.