Metabolic responses of photosynthetic sea slugs to a changing environment

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Sacoglossan sea slugs have complex interactions with their environment. They are well known for their ability to sequester stolen chloroplasts and utilize them for photoautotrophic CO_2 fixation, yet the dependence on this is not clear in most species. *Elysia stylifera* is an Indo-Pacific tropical sacoglossan that selectively consumes *Halimeda* macroalgae and retains its chloroplasts for two weeks. This association is prone to change as their habitats are subjected to increased ocean warmth and acidification, altering algal distribution and decreasing photosynthetic efficiency. This study aimed to investigate the metabolic response of *E. stylifera* to projected environmental changes. *Elysia stylifera* were subjected to ambient (28°C) or warm (30°C) conditions and five days of food deprivation. On the first and fifth day of starvation, the respiration rates of individuals were measured in the dark to quantify slug response, as well as in the light to characterize their response when given the ability to photosynthesize. Dark treatments showed that slugs deprived of food undergo metabolic suppression in current conditions, but may not undergo suppression in projected warm conditions. The difference between oxygen consumption in dark and light treated slugs demonstrated photosynthesis occurred, but that it was reduced under all stressors. This study reveals that forecasted ocean warming may not be favorable for short-term photosynthetic sea slugs because of its impact on both the sea slugs and their ingested kleptoplasts. It also presents new uncertainties about the benefits of kleptoplasty and how it may transform with climate change.

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Abstract

Sacoglossan sea slugs have complex interactions with their environment. They are well known for their ability to sequester stolen chloroplasts and utilize them for photoautotrophic CO_2 fixation, yet the dependence on this is not clear in most species. *Elysia stylifera* is an Indo-Pacific tropical sacoglossan that selectively consumes *Halimeda* macroalgae and retains its chloroplasts for two weeks. This association is prone to change as their habitats are subjected to increased ocean warmth and acidification, altering algal distribution and decreasing photosynthetic efficiency. This study aimed to investigate the metabolic response of *E. stylifera* to projected environmental changes. *Elysia stylifera* were subjected to ambient (28°C) or warm (30°C) conditions and five days of food deprivation. On the first and fifth day of starvation, the respiration rates of individuals were measured in the dark to quantify slug response, as well as in the light to characterize their response when given the ability to photosynthesize. Dark treatments showed that slugs deprived of food undergo metabolic suppression in current conditions, but may not undergo suppression in projected warm conditions. The difference between oxygen consumption in dark and light treated slugs demonstrated photosynthesis occurred, but that it was reduced under all stressors. This study reveals that forecasted ocean warming may not be favorable for short-term photosynthetic sea slugs because of its impact on both the sea slugs and their ingested kleptoplasts. It also presents new uncertainties about the benefits of kleptoplasty and how it may transform with climate change.

Introduction

Species interactions influence organisms' responses to environmental change. In cases of symbiosis, where different species are closely associated with each other, the dynamics are more complex and prone to alterations (Chapin et al., 2000). For instance, the forecasted levels of water temperature in the euphotic zone in oceans have been found to disrupt the behavior, ecology, and physiological processes of marine invertebrates (Tomanek, 2001; Bindoff et al., 2007; Doney et al., 2009). With increasing ocean temperatures, the physiological processes of marine invertebrates will be altered (Doney, 2010; Pörtner & Farrell, 2008).

Environmental stressors are predicted to cause suboptimal physiological performance and reduce the success of certain marine species (Doney et al., 2012). The responses may be more life-threatening when species are closely associated with each other and have evolved physical or behavioral traits that require the function of the other species (Lawrence et al., 2012). For example, the symbiotic interactions between coral and zooxanthellae, in which photosynthetic dinoflagellate algae provide coral with nutrients and receive compounds for photosynthesis, respond severely to ocean acidification and global warming (Muscatine, 1967; Muller-Parker & D'Elia, 1997; Bellwood et al., 2004). Stressed and overheated corals expel the zooxanthellae and as a result lose their primary carbon source (Hughes et al., 2003). This sensitive system portrays how tropical marine interactions may be disturbed with shifting environmental conditions (Hay et al., 2004; Bellwood et al., 2004).

Several other taxa also evolved mechanisms to obtain photosynthetic products through associations with photosynthetic bacteria, algae, or plants. One adaptation is kleptoplasty, where organisms have the ability to retain only the functional plastids from their photosynthetic prey

and utilize them for photoautotrophy (Kawaguti, Yamamoto & Kamishima, 1965, Clark, Jensen & Stirts, 1990). This is a specialized form of predation and tightly links the predator to its particular prey (Rumpho et al., 2011; Johnson, 2011). Kleptoplasty has evolved several times and is seen across different lineages, including dinoflagellates, ciliates, foraminiferans, and metazoans, yet its role in physiology and behavior and overall benefit is not entirely understood (Johnson, 2011).

Sacoglossan sea slugs are specialized marine gastropods (Mollusca, Plakobranchoidea) with kleptoplasty (Kawaguti, Yamamoto & Kamishima, 1965). Most species are herbivorous grazers that consume contents from siphonaceous or coenocytic algae and store the kleptoplasts intracellularly in the digestive gland (Taylor, 1968; Jensen, 1996). These kleptoplasts fix carbon dioxide and produce oxygen and reduced products (carbohydrates, lipids, and even proteins), providing the individual with an additional source of energy (Greene, 1970; Trench, Trench & Muscatine, 1972). The role of this energetic contribution and overall importance is not very clear. Scientists suggest that kleptoplasty provides strategies to allow the slugs to live on seasonal diets, in patchy distributions, on low effort diets, or to radiate out from their ancestral diet (Williams & Walker, 1999). It also may aid in crypsis because the ingested chloroplasts are displayed in the epithelium and blend in with the prey (algae) that also serves as their habitat (Greene & Muscatine, 1972; Händeler et al., 2009). Although the kleptoplasts provide an additional source of energy, in most cases, the slugs are unable to completely maintain the kleptoplasts, and therefore they must be replaced regularly for optimal functionality (Clark, Jensen & Stirts, 1990; Händeler et al., 2009). This regular replacement of the kleptoplasts brings up the question of kleptoplasty function and contribution in sacoglossan sea slugs.

Projected environmental changes likely have implications for slugs with kleptoplasty because it may impact habitat, algal food source, behavior and physiology (Middlebrooks, Pierce & Bell, 2011). *Elysia stylifera* is such a kleptoplastic sea slug (Jensen, 1997; Phuong, 2010). It is prominent in French Polynesia and selectively consumes the green macroalgae *Halimeda* (Jensen, 1993; Phuong, 2010). *Elysia stylifera* is thought to have short-term kleptoplast retention, though this is not confirmed (Jensen, 1993; Phuong, 2010). This species inhabits coral reefs in the Indo-Pacific and will be exposed to potential stressors, including increased ocean temperatures and decreased food availability (Phuong, 2010; Bellwood et al., 2004; Sinutok et al., 2012; IPCC, 2013). The response of *E. stylifera* to these conditions may be acclimatized by kleptoplasty and can be measured in different ways. One metric that is indicative of the overall performance of an organism is the respiration rate (Miller et al., 2014). Generally, during stressful conditions, oxygen is more rapidly depleted (Miller et al., 2014). Respirometry helps to establish stress level, which translates into the costs of basic activity and maintenance in an organism (Heusner, 1991).

This project aims to establish the metabolic response of sacoglossans to projected climate change conditions. Specifically, the respiration rate of *E. stylifera* will be measured in conditions that are predicted to occur by the end of the 21st century (*i.e.*, increased ocean temperature, decreased food availability) to investigate sacoglossan physiological response to altered environmental conditions (IPCC, 2013; Sinutok et al., 2012). This study also considers how kleptoplasty contributes to the fitness of sacoglossans under these conditions by examining the respiration rate of *E. stylifera* when provided light (Douglas, 1994). In order to approximate net photosynthesis, *E. stylifera* will be provided light to induce photosynthesis, as well as deprived of light to inhibit photosynthesis. (Casalduero & Muniain, 2008). Under the elevated

temperature, *E. stylifera* is predicted to have an increased respiration rate. When deprived of food, *E. stylifera* will have a decreased respiration rate. In all treatments, slugs provided light are predicted to undergo photosynthesis, which will combat each stressor. By studying the respiration rate of *E. stylifera* under different environmental conditions, this will provide insight on how complex physiological interactions can be affected by fluctuating environmental conditions.

Materials & Methods

Study Site and Organism

This study took place along the northern coast of Mo'orea, French Polynesia. Mo'orea is a small (134 km^2) island surrounded by barrier reef. The study's focal species, *Elysia stylifera* (Jensen, 1997), occurs in Australia and French Polynesia, but has few documented observations (Allen, Krug & Marshall, 2009; Phuong, 2010). Though once placed in the genus *Elysiella* with its sister species *E. pusilla*, molecular phylogenetics confirm *E. stylifera* and *E. pusilla* belong to a basal group of *Elysia* spp. (Bergh, 1872; Gosliner, 1995). *E. stylifera* and *E. pusilla* co-occur on host algae in the genus *Halimeda*, but tend to have different host species preferences, where *E. stylifera* has been recorded to occur primarily on *H. distorta* and *H. opuntia* (Phuong, 2010). *E. stylifera* can be distinguished by rhinophore length, coloration, and parapodia size (Fig. 1).

Collection of E. stylifera

Specimens were obtained by collecting the host algae *H. distorta* and *H. opuntia* in Cook's Bay (17°29'25.09"S, 149°49'32.28"W), Mo'orea between October 15 and November 14, 2016. Cook's Bay is a shallow reef flat consisting of sandy substrate with live and dead coral. This lagoon is a turbid freshwater inlet with high freshwater flow during rainstorm events. Collection sites were adjacent to Gump Station (< 5 m depth). *Elysia stylifera* was identified with the help of Dr. Patrick Krug from California State University, Los Angeles. Field experiments were approved by the University of California, Berkeley to work on the University of California, Berkeley Gump Research Station.

Host algae was immediately transported back to the Gump Station wet lab and sorted through for *E. stylifera* by shaking algae in fresh sea water and collecting dislodged slugs, as well as picking through algae. The collected slugs were then relocated to sea water flow tanks that were partially shaded and exposed to 14:10 h natural light/dark cycles. Slugs were kept individually in modified 50 mL falcon tubes with 75 µm filter tops. Falcon tubes were manually re-filled with fresh sea water of appropriate temperature at least once every eight hours to assure clean oxygenated water. A total of 25 individuals were randomly selected to be maintained at ambient temperature (27 - 28°C), while 25 other individuals were subjected to elevated temperature treatments and maintained in aquaria at 29 - 30°C. *Elysia stylifera* were kept with *H. distorta* as a food source until 24 hours prior to respirometry. Individuals were then deprived of food after the first day of respirometry and measured approximately four days later.

Respiration experiment

Elysia stylifera were not fed for 24 h before the experiment to minimize the effects of digestion on metabolism. Furthermore, experiments occurred during the day (6:00 – 16:00) to minimize difference in nighttime activity. For elevated temperature treatments, slugs were incubated for 48 h in seawater at 30°C. Each replicate had twelve 750 µL independent chambers on a microplate with oxygen sensor spots (PreSens Precision Sensing GmbH, Germany; SDR software v38). Six chambers were transparent and exposed to light and the other six were covered by black tape to block light. Each chamber was filled with filtered seawater (2 µm filter) at the appropriate temperature. For the selected treatment, five slugs were randomly placed into light chambers, five slugs were randomly placed into dark chambers, and the remaining two chambers had filtered treatment water with no slugs ('blanks'). The blanks served as a control for the natural variation in oxygen concentration due to the presence of microorganisms and temperature variation. Once slugs were placed in their individual chambers, the filtered seawater was again added to each well to replace displaced water; caps were then cautiously attached to avoid air bubbles. The microplate was placed into the resting chamber, which was temperature controlled (27.5 - 28°C or 29.5 - 30°C) with continuous flowing freshwater (Loligo 1421 resting chamber). A fluorescent lamp (28W 10,000K dual compact fluorescent/actinic aquarium light) was turned on and placed 4 cm away, parallel to the light-treated chambers to promote photosynthesis. Slugs were allotted 10 min to acclimate to the flowing chamber with the fluorescent light. Oxygen concentration was then measured every 30 s for 15 min under the fluorescent light. At the end of the incubation, individuals were placed into petri dishes with fresh sea water. Individuals were photographed under a stereoscope. Wet weight was also measured. The selected individuals were re-measured for respirometry under the same light/dark treatment following four additional days without food for the starvation treatment. Again, wet weight was measured.

Oxygen consumption rate was calculated by producing a regression line of oxygen concentration over time. The first 12-15 min of incubation were excluded from the regression to prevent initial stress effects on the slugs. There were also points where slug movement may have blocked the oxygen sensor and significantly altered the oxygen concentration reading within an unrealistic time frame. To control for these effects within each replicate, a 2-5 min interval where there were no substantial shifts in oxygen concentration was selected to produce the regression lines. For each replicate, the light and dark blanks showed comparable changes in oxygen concentration. Respiration rates were calculated for every individual after correcting for oxygen consumption rates in the light blank chamber during that trial. The respiration rates were divided by two and expressed as $\mu mol\ O_2\ min^{-1}$ (wet weight g)$^{-1}$.

Statistical analysis

Two two-way repeated measures analysis of variance (ANOVA) were conducted in R using the nlme and multcomp packages to test the significance of starvation period and temperature on the respiration rate of *E. stylifera* (R Developmental team; Pinheiro et al., 2016; Hothorn, Bretz & Westfall, 2008) under the a) dark treatment and b) light treatment. Each test was followed by a *post hoc* Tukey HSD test with the treatment (ambient or elevated temperature; starvation period)

as the predictor variable and oxygen consumption rate as the dependent variable to determine the nature of differences found by the ANOVA.

The difference in mean oxygen consumption rate between dark and light individuals showed the relative net photosynthesis. No statistical tests were done on net photosynthesis. Errors were displayed as confidence intervals. All graphs were produced in R using the ggplot2 package (Wickham, 2009).

Results

Respiration in dark

Experiments were run at 28°C under dark conditions for five days, with respiration rate assessed on day 1 (fed) and day 5 (starved). *Elysia stylifera* individuals showed a 67% decrease in respiration rate over the course of the five days, indicating significant metabolic suppression (two-way repeated measures ANOVA, $Z = -3.00$, $df = 39$, $p < 0.01$) (Fig. 2). Trials were also run at 30°C to mimic the impacts of rising ocean temperatures. After five days of starvation, the respiration rates of individuals increased by 18% and did not show a significant difference (two-way repeated measures ANOVA, $Z = 0.49$, $df = 39$, $p = 0.96$). Comparing between the temperature treatments, there were no statistically significant differences. After one day of starvation, individuals at 30°C had a respiration rate 41% lower than those at 28°C (two-way repeated measures ANOVA, $Z = -1.91$, $df = 39$, $p = 0.23$). After five days of starvation, individuals at 30°C had a respiration rate 53 % higher than those at 28°C (two-way repeated measures ANOVA, $Z = 1.67$, $df = 39$, $p = 0.34$).

In vivo photosynthesis

Respirometry was also run under the same conditions as described above with light to induce photosynthesis and there was minimal evidence of positive oxygen production (Fig. 3). The average oxygen consumption rates of the starved slugs at 28°C and 30°C were significantly different (two-way repeated measures ANOVA, $Z = 3.04$, $df = 35$, $p < 0.01$). However, these measurements were unable to differentiate photosynthesis from respiration rate. To account for respiration rate, the difference was taken between light and dark slugs to find net photosynthesis (Fig. 4). Photosynthesis was most productive day 1 at ambient temperature (28°C) and least productive day 5 at the elevated temperature (30°C). Under both temperature treatments, the effect of starvation decreased the average net photosynthesis. The net photosynthesis for slugs at 28°C decreased by 66% after five days of starvation. The net photosynthesis for slugs at 30°C decreased by 50% after five days of starvation. When comparing temperatures, net photosynthesis was 37% less efficient at 30°C on the first day of starvation (Fig. 4).

Discussion

Respiration rate under stress

Metabolism is a useful physiological measurement for understanding how organisms perform. When organisms are exposed to stressors, oxygen is usually depleted more rapidly (Miller et al., 2014). The average respiration rate of *E. stylifera* individuals in the dark served as a metabolic proxy to investigate the metabolism of *E. stylifera* under stressful conditions (altered temperature, food depletion) (Green et al., 2000). These proxies inform on performance, including growth, activity, and reproduction (Sokolova, 2013).

Temperature has been shown to have a significant effect on invertebrate metabolic rates (Marsden, Newell & Ahsanullah, 1973; Gillooly, Brown & West, 2001). Studies have shown that the survival of some tropical species is jeopardized just 2°C above present seawater (Nguyen et al., 2011). In this experiment, the respiration rate of slug individuals at the higher temperature had a slightly lower average respiration rate (Fig. 2). Generally, the metabolic rate of invertebrates increases with temperature (Marsden, Newell & Ahsanullah, 1973; Gillooly, Brown & West, 2001). There are several possible explanations for this decrease. *Elysia stylifera* may have been past the upper limits of its optimal temperature (Huey & Stevenson, 1979). When invertebrates are their optimal temperature, performance drops relatively quickly (Huey & Stevenson, 1979). This suggests that the performance of *E. stylifera* may have been at risk. Alternatively, individual variation may have been lower overall for individuals at 30°C.

Starvation appeared to have a greater impact on *E. stylifera* than temperature. The reduction in respiration rate by 67% after five days of starvation at 28°C suggests there was a substantial physiological response by *E. stylifera* (Fig. 2) (Guppy & Withers, 1999). Starvation has been shown to induce metabolic suppression in other invertebrates including crabs, insects, and mollusks (Guppy & Withers, 1999; Wallace, 1972; Marsden, Newell & Ahsanullah, 1973; Bennett, Kukal & Lee, 1998; Maas et al., 2011). During metabolic suppression, certain metabolic processes are slowed or altered to sustain energy (Hand & Hardewig, 1996). Such processes are the downregulation of protein synthesis and depression of macromolecular turnover (Hand & Hardewig, 1996). For *E. stylifera,* food shortages may trigger the shut down or slowing of important physiological processes, decreasing the momentary fitness of the individuals. If individuals are under these stressful conditions for too long, their performance may be jeopardized (*i.e.*, survival, growth, reproduction). Although respiration rate was measured after five days, the effect of starvation may have been apparent sooner, and may show a stronger signal with longer starvation time.

The combined effect of stressors (elevated temperature and food deprivation) did not seem to trigger metabolic suppression. Instead, the respiration rate increased by 18%, indicating physiological activity was potentially upregulated under these stressors (Fig. 2). It is not clear what metabolic activity changed; however, observations lead to postulations about altering energy for protection or reproduction. During mass measurements, it was noted that the individuals at 30°C tended to produce more mucus. It was also noted that some slugs laid eggs within only the five days of starvation. The kleptoplasts are stored in mucus-producing tissues,

which are a form of protection and are known to have a high metabolic turnover (Trench, Greene & Bystrom, 1969). *Elysia stylifera* may have produced more mucus or induced a reproductive state as a stress response.

In vivo photosynthesis

Photosynthesis via kleptoplasty is a unique adaptation for sacoglossans and was predicted to combat stressful conditions. The difference in the respiration rate between individuals in the light and dark treatments under ambient conditions (28°C day 1) demonstrates that photosynthesis was likely occurring (Fig. 4). When provided light, oxygen was released as a photosynthetic product and counteracted *E. stylifera* respiration, resulting in a decrease in respiration rate. Net photosynthesis for each stressor was calculated (Dark respiration – Light respiration) so that kleptoplasty response could be characterized (Fig. 4). In all cases, net photosynthesis was positive.

Temperature had a negative effect on photosynthesis, with kleptoplasts 37% less efficient at the elevated temperature. Since sacoglossans are ectothermic, they are unable to maintain their own temperature. Consequently, sacoglossan and kleptoplast activity are highly dependent on environmental temperatures (Huey & Stevenson, 1979; Clark et al., 1981). The chloroplasts of *Halimeda* are vulnerable to heat stress, with reduced photochemical efficiency of photosystem II at 32°C, indicating a loss in efficiency (Sinutok et al., 2012). *Elysia stylifera* individuals are also at risk under heat stress. Thermal fitness curves of ectotherms are nonlinear and asymmetric, such that when animals are past the upper limits of their optimal temperature, performance drops relatively quickly (Huey & Stevenson, 1979). The reduced metabolism (Fig. 2) and photosynthesis (Fig. 4) observed after a shift of 2°C provide evidence that *E. stylifera* may have been near its peak performance and may be particularly susceptible to rising seawater temperatures (Schulte et al., 2011).

Starvation also seemed to negatively affect photosynthetic efficiency. At ambient temperature, net photosynthesis was reduced by 67% after five days of starvation. Photosynthesis may have declined due to kleptoplast degradation over time (Evertsen et al., 2007). This is because chloroplast function depends on the maintenance of proteins, enzymes, and pigments that are encoded in the algal nucleus, which is not retained within sacoglossans (Eberhard, Finazzi & Wollman, 2008; Serôdio et al., 2014; Rumpho et al., 2001). The level of degradation is variable among sacoglossan species and determines the length sacoglossans can retain chloroplasts without replacing them (Händeler et al., 2009; de Vries et al., 2013). *Elysia stylifera* is a short-term retaining sacoglossan (< 2 weeks), so it is not surprising that kleptoplasts lost efficiency over a short period of time (Phuong, 2010).

The combined effect of stressors (elevated temperature and food deprivation) had the greatest impact on net photosynthesis. Kleptoplasts may have been at their least efficient and most degraded state under the multi-stress treatment (Fig. 4). These findings suggest that kleptoplasty provided marginal benefit to *E. stylifera* when facing temperature stress and periods of intermittent starvation.

Respirometry as a tool

Kleptoplasty is known to provide additional energy to sacoglossans when exposed to light, suggesting that light could influence sacoglossan response to stressors. This study used respirometry to measure the energetic response of *E. stylifera* under different conditions and deduced photosynthetic benefit. Respirometry is not the conventional technique used for establishing photosynthetic activity in sacoglossans because oxygen concentration measurements are affected by respiration from the slug, the chloroplasts, mitochondria, and microbes (Evertsen et al., 2007). Measurements could have been influenced by behavioral traits displayed by slugs (opening vs. closing parapodia, locomotive vs. stationary) (Händeler et al., 2009). To account for this, all individuals were maintained under the same conditions and photosynthetic efficiency was only approximated relative to dark-treated slugs. One common method that can more precisely investigate kleptoplast functionality under these stressful conditions is pulse amplitude modulated (PAM) fluorometry (Wägele and Johnsen, 2001). This technique measures chlorophyll a autofluorescence derived from photosystem II and provides a relative value of photosystem II efficiency. Another method that measures photosynthesis and its energy contribution is carbon isotopic analysis. This technique demonstrates energetic contribution to sacoglossans (Trench, Trench & Muscatine, 1972; Hinde, 1978; Raven at al., 2001).

Elysia stylifera in future conditions

With ocean acidification and warming, *E. stylifera* will face physiological changes and an altered habitat. Its specialized food source, *Halimeda spp.,* is a calcifying macroalgae that has been shown to have decreased photosynthesis and calcification under predicted future conditions (Sinutok et al., 2012). When exposed to a combined elevated pCO_2 and elevated temperature (32°C), there was a 50 - 70% decrease in efficiency and 70 - 80% decrease in O_2 production (Sinutok et al., 2012). *Halimeda* species are predicted to be severely affected by climate change conditions and may be greatly reduced in abundance and distribution. These changes indicate a shifting habitat for *E. stylifera,* which may have severe impacts on the population depending on their capacity to modify their food source.

This investigation provides insights into sacoglossan physiology and response to future environmental ocean conditions. It suggested that a sacoglossan species with short-term kleptoplast retention may not benefit metabolically under elevated temperatures or limited food availability. In addition, kleptoplasty may not aid sacoglossans in overcoming environmental shifts because plastids may degrade or lose function more rapidly. These findings show the sensitivity of such an interactive system, and add to the uncertainty regarding the ecological function of kleptoplasty in short-term retention species. Further investigation in other species, including long-term retention sacoglossans, is necessary to understand the ecological value of kleptoplasts to sacoglossans, especially in a transforming environment.

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Figure 1

Elysia stylifera.

Elysia stylifera is generally < 1 cm in length with long knobby rhinophores, large parapodia that can open, and occasionally rosy patches of color.

Figure 2(on next page)

Dark respiration rate.

Effect of starvation on respiration rate of *E. stylifera* at 28°C (n=12) and 30°C (n=14) in the dark treatment. Asterisks designate the significance of starvation on oxygen consumption rate at 28°C (two-way repeated measures ANOVA, $Z = -3.00$, $df = 39$, $p < 0.01$).

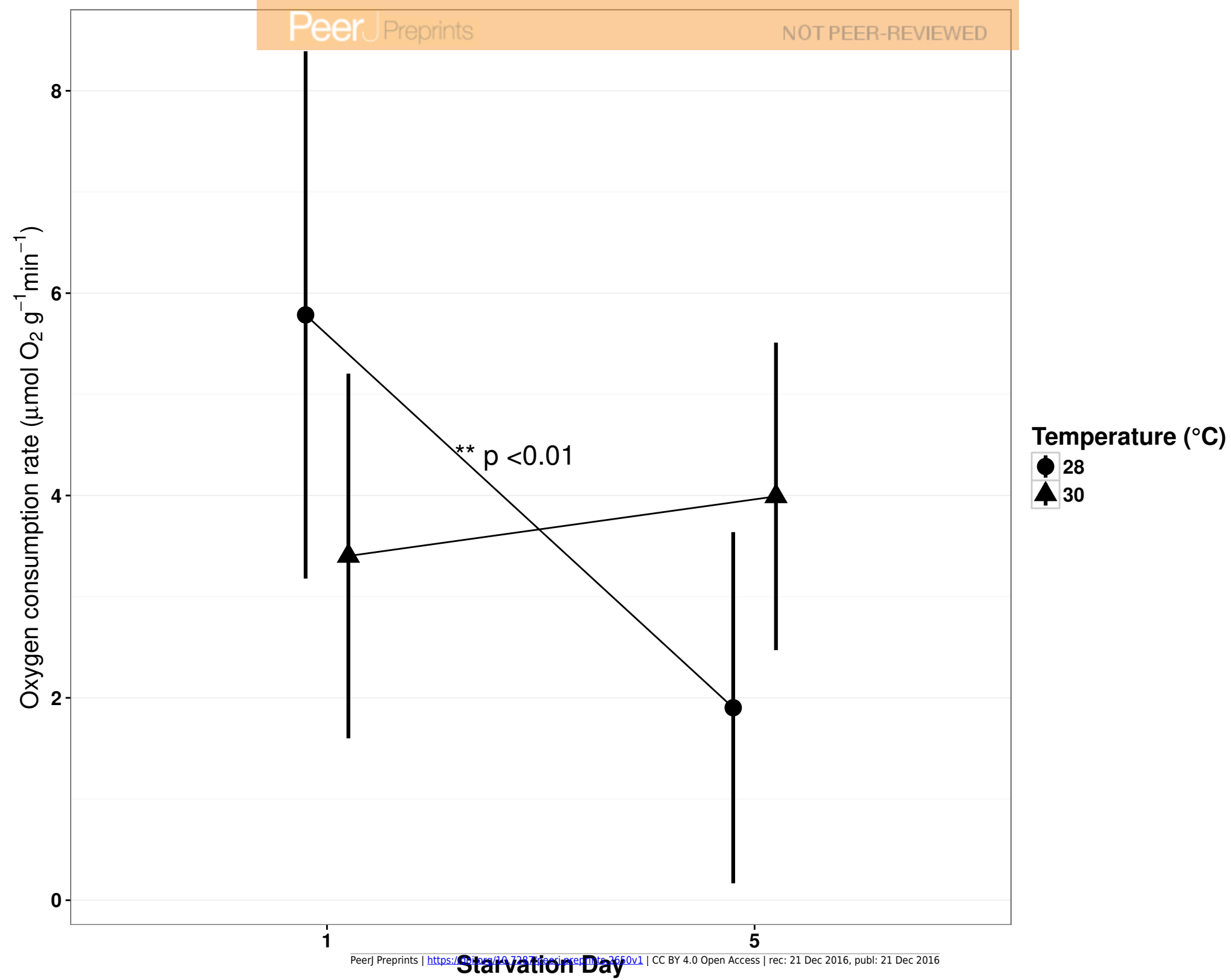

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Figure 3(on next page)

Light respiration rate.

Effect of starvation on respiration rate and photosynthetic rate of *E. stylifera* at 28°C (n=11) and 30°C (n=13) in the light treatment. Asterisks designate the significance of temperature and starvation day on oxygen consumption rate (two-way repeated measures ANOVA, $Z = 3.04$, $df = 35$, $p < 0.01$).

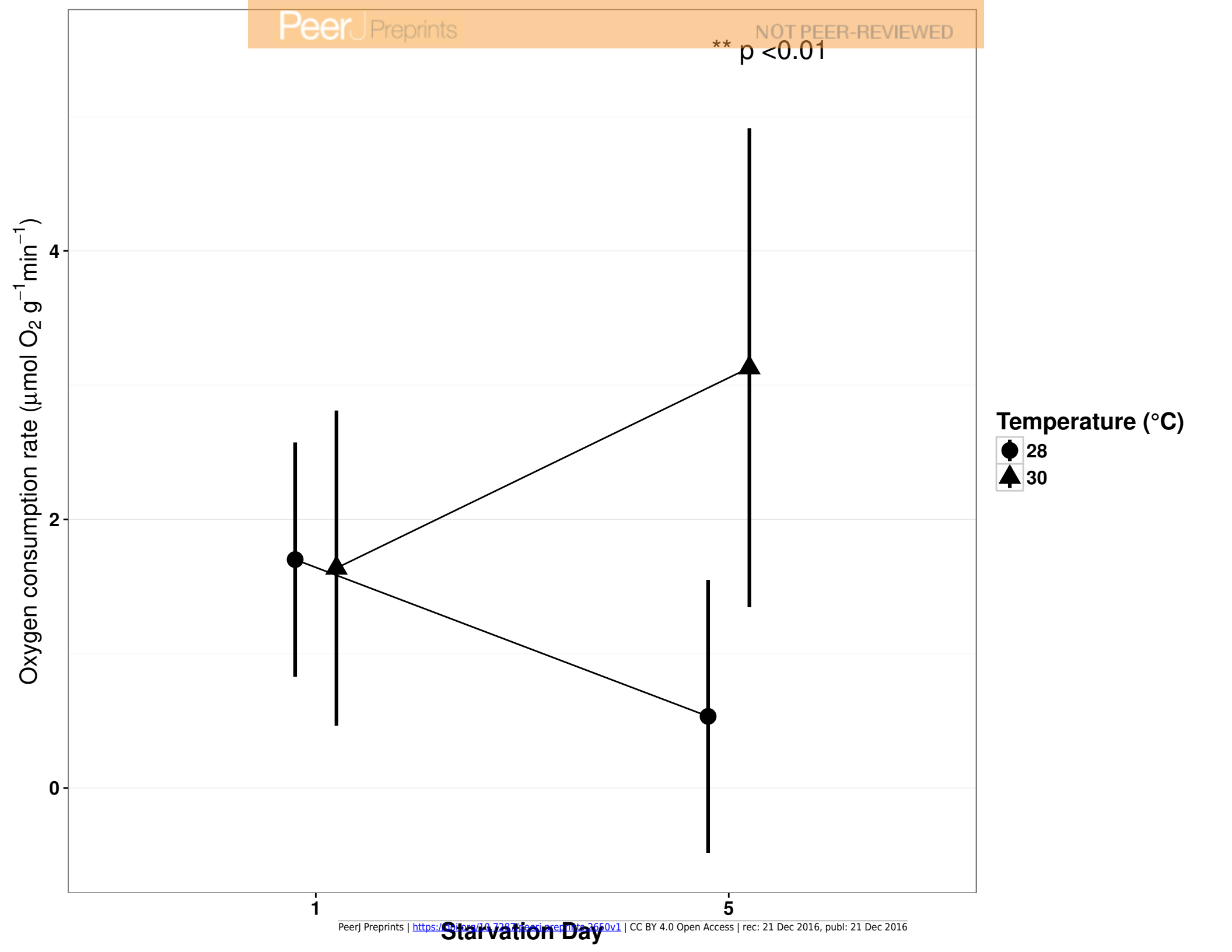

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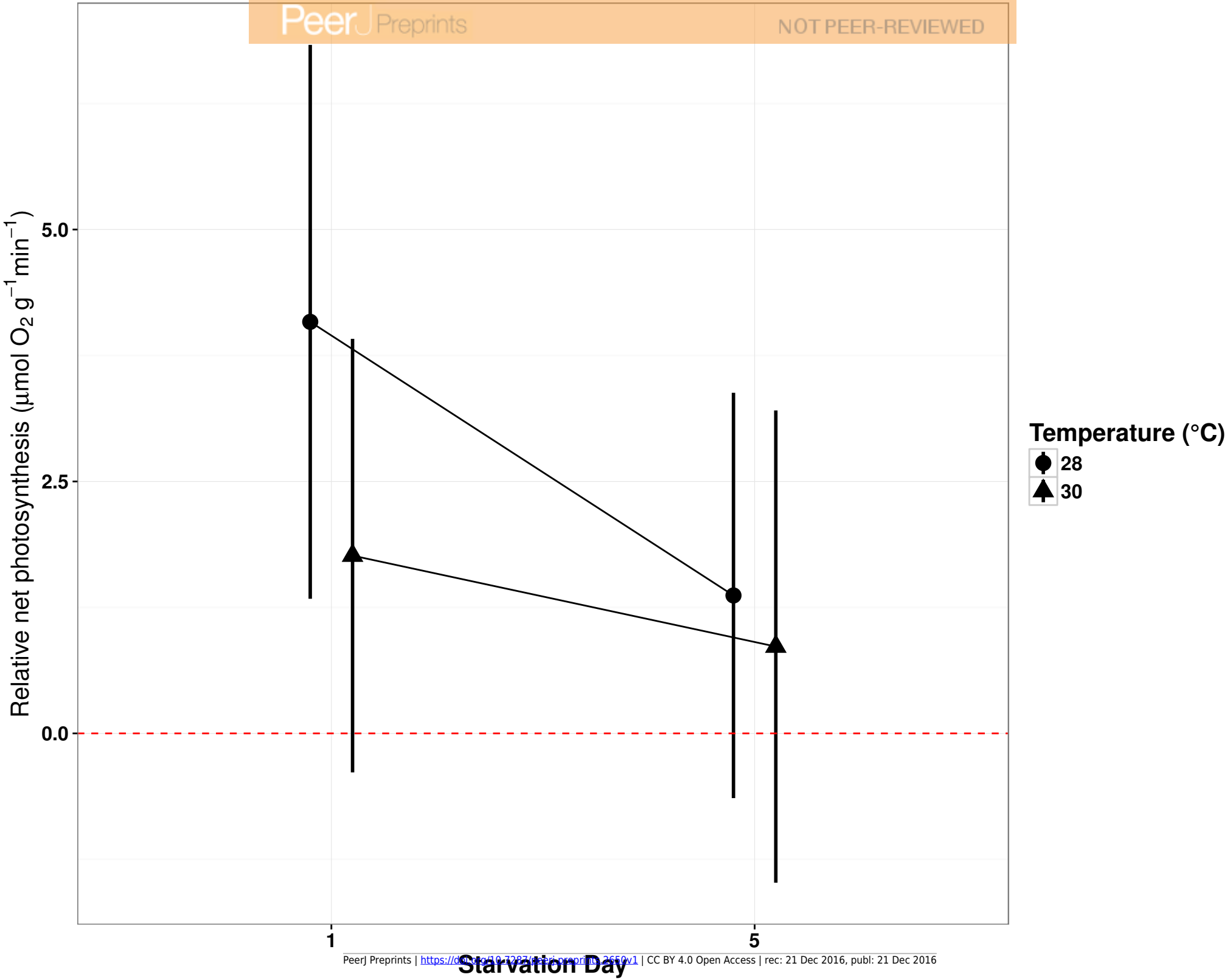
Figure 4(on next page)

Net photosynthesis (Dark respiration rate – light respiration rate).

Data below the dashed red line at 0 µmol O_2 g^{-1} min^{-1} indicates where photosynthesis is not occurring.



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