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Microzooplankton grazing, growth and gross growth efficiency are affected by pCO2 induced changes in phytoplankton biology

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Microzooplankton grazing, growth and gross growth efficiency are affected by pCO_2 induced changes in phytoplankton biology

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Kelly A. Still

May 2016

Microzooplankton grazing, growth and gross growth efficiency are affected by pCO_2 induced changes in phytoplankton biology

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By
Kelly Ann Still
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ABSTRACT

Accumulating evidence shows that ocean acidification (OA) alters surface ocean chemistry and, in turn, affects aspects of phytoplankton biology. However, very little research has been done to determine if OA-induced changes to phytoplankton morphology, physiology and biochemistry may indirectly affect microzooplankton, the primary consumers of phytoplankton. This is one of the first studies to explore how OA may indirectly affect microzooplankton ingestion, population growth and gross growth efficiency (GGE). I hypothesized 1) that the physiology, biochemistry and morphology of the phytoplankton *Rhodomonas* sp. would be directly affected by elevated pCO_2 and 2) that pCO_2-induced changes in *Rhodomonas* sp. would affect grazing, growth rates, and GGE in microzooplankton consumers. To test my first hypothesis, I cultured the ecologically important phytoplankton, *Rhodomonas* sp., semi-continuously for 17 days under three pCO_2 treatments (400ppmv, 750ppmv and 1000ppmv). During this time I characterized *Rhodomonas* sp. cell size, C:N, cellular total lipids, growth rate, cellular chlorophyll *a* concentrations and carbohydrates. *Rhodomonas* sp. cell bio-volume and total cellular lipids were the only aspects of *Rhodomonas* sp. found to be significantly affected by pCO_2. On average, *Rhodomonas* sp. cell bio-volume increased by ~60% and ~100% and total cellular lipids increased by 36% and 50% when cultured under moderate and high pCO_2 treatments, respectively, compared to the ambient treatment. To test my second hypothesis, the pCO_2-acclimated *Rhodomonas* sp. were fed to four microzooplankton species, two tintinnid ciliates (*Favella ehrenbergii* (recent name change to *Schmidingerella* sp.) and *Coxliella* sp.)

and two heterotrophic dinoflagellates (*Gyrodinium dominans* and *Oxyrrhis marina*). Two experimental designs were used to test whether microzooplankton grazing and growth are affected by OA through changes in prey state. My data confirm my hypothesis that microzooplankton grazing is affected by OA-induced changes to their prey. In three out of the four grazers tested, short term ingestion rates were either higher or non-linear when grazers fed on moderate and high pCO_2 acclimated *Rhodomonas* sp., compared to the ambient treatment cells. Using multiple linear regression models to test for the factors that explain the observed variation in microzooplankton short term ingestion rates across pCO_2 treatments, prey cell bio-volume explained 43, 82 and 88% of the variability in short term grazing rates for *O. marina, G. dominans* and *F. ehrenbergii,* respectively. In contrast to the short term grazing results, I found that during long term grazing experiments, *G. dominans* and *Coxliella* sp. grazed ambient pCO_2 acclimated *Rhodomonas* sp. significantly faster than moderate and high cultured cells. *O. marina* demonstrated a non-linear feeding response in both short and long term grazing experiments, where *O. marina* ingested moderate pCO_2 acclimated *Rhodomonas* sp. faster than ambient and high pCO_2 acclimated prey. Microzooplankton growth rates were higher for all microzooplankton species when feeding on *Rhodomonas* sp. cultured under moderate and high pCO_2 compared to ambient pCO_2 diets. *G. dominans* and *Coxliella* sp. were the only grazers that demonstrated a difference in GGE across treatments, showing increased GGE when feeding on prey cultured under elevated pCO_2. These findings validate my hypothesis that OA-induced changes in *Rhodomonas* sp. morphology and biochemistry affects microzooplankton grazing and growth. If the alteration of phytoplankton morphology and nutritional quality observed in

this study is wide spread across phytoplankton taxa under OA, and this, in turn, affects microzooplankton grazing and growth dynamics as seen here, it will serve as a mechanism to alter future biogeochemical processes in pelagic marine food webs.

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INTRODUCTION

Primary production by phytoplankton provides the bulk of the organic carbon that fuels marine biogeochemical cycles. Through photosynthesis, phytoplankton convert dissolved CO_2 in the surface ocean to organic and inorganic matter, generating ~50% of global primary production (Finkel et al. 2009). Microzooplankton, in particular heterotrophic dinoflagellates and ciliates, are the principal consumers of phytoplankton (Strom et al. 2001, Sherr and Sherr 2002, Calbet and Landry 2004, Sherr and Sherr 2007). Some microzooplankton are capable of growing as fast as or faster than their phytoplankton prey, resulting in tight temporal coupling between populations (Kuipers and Witte 1999, Strom 2002). Their high intrinsic growth rate and high biomass-specific ingestion rates allow them to consume an average of 60 to 75% of phytoplankton production (Levinsen and Nielsen 2002, Calbet and Landry 2004).

In addition to being the major consumers of marine primary production, microzooplankton play a pivotal role in ecosystem functioning as key components of the microbial loop (Sherr and Sherr 2002) and serving as an important link for the transfer of primary productivity to higher trophic levels such as copepods and larval fish (Calbet and Landry 2004, Tillmann 2004, Drira et al. 2010). Microzooplankton are the dominant nutrient remineralizers in the ocean because of their comparatively high biomass specific metabolic rates and their low gross growth efficiencies (GGE) (Sherr and Sherr 2002, Calbet and Landry 2004). Microzooplankton recycle ~ 40 to 63% of the nitrogen requirement for recycled primary production, in comparison to copepods that contribute only 14%

(Buitenhuis et al. 2010). In addition, sloppy feeding and fecal pellet production by microzooplankton substantially contribute to the pools of dissolved (DOM) and particulate organic matter (POM), important components of biogeochemical cycling. In grazing experiments Strom et al. (1997) found that microzooplankton released 16-37% of ingested algal carbon as DOM in comparison to phytoplankton that released 3 to 7% of cell organic carbon per day as DOM. Finally, microzooplankton's size, biochemical composition and swimming behavior can make them the preferred food for mesozooplankton (Calbet 2008).

Given microzooplankton's role as the ocean's dominant grazer, and the biogeochemical implications of this, considerable research has been done over the last few decades to elucidate microzooplankton grazing dynamics (e.g. Landry and Hassett 1982, Verity 1985, Strom 2002, Caron and Hutchins 2012). An important finding from this body of research was that microzooplankton, seemingly simple, single-celled protists, are not indiscriminate grazers, but instead are capable and active in selecting which prey to consume (Verity 1991, Strom 2002, Tillmann 2004) within a community of prey choices. Microzooplankton selectivity is shown to depend on certain characteristics of phytoplankton biology and physiology including. cell size and biochemical characteristics (C:N stoichiometry, total lipids) (e. g. John and Davidson 2001, Tillmann 2004, Strom 2002, Caron and Hutchins 2012).

Phytoplankton morphology is one characteristic that causes microzooplankton to feed selectively (Hansen 1994). A microzooplankton's feeding mechanism and feeding apparatus can constrain it's ingestion to prey cells within a defined size range (Hansen 1992, Hansen 1994, Tillmann 2004), thus making it more efficient at capturing and ingesting prey

of an optimal size (Jonsson 1986, Hansen 1992, Hansen 1994, Hansen 1996, Flynn et al. 1996). Feeding mechanisms and apparati differ among microzooplankton functional groups. As such, so too does the optimal prey size for different microzooplankton (Hansen 1994, Tillmann 2004). A review by Hansen (1994) showed that the optimal predator:prey size ratio for ciliates is ~ 8:1 , whereas athecate phagotrophic dinoflagellates are capable of ingesting phytoplankton approximately as large or larger than themselves, with ratios between 0.15:1 and 5.2:1 (Hansen 1994, Tillmann 2004).

In addition to phytoplankton morphology, biochemical recognition of prey by microzooplankton also induces selective grazing behavior. Microzooplankton can recognize and adjust feeding rates based on C:N stoichiometry, fatty acid content, carbohydrate and protein content (e. g. John and Davidson 2001, Urabe et al. 2003, Shannon et al. 2007, Wynn Edwards et al. 2014). For example, ciliates and dinoflagellates demonstrated higher ingestion rates on phytoplankton with low C:N and C:P ratios and high fatty acid content compared to prey cells with higher C:N and C:P and lower fatty acid content (e.g. John and Davidson 2001, Urabe et al. 2003, Shannon et al. 2007, Schoo et al. 2013). Thus, these phytoplankton physiological and biochemical properties are important in contributing to the grazing selection of prey by microzooplankton.

In addition to phytoplankton biochemistry (ie. the nutritional quality) being an important driver of microzooplankton grazing behavior, it also, in part, affects microzooplankton population growth rates and GGE (Urabe et al. 2003, Elser et al. 2003, Anderson et al. 2005, Hantzsche and Boersma 2010). For example, a significant reduction in the microzooplankton growth rates of *O. marina* (Hantzsche and Boersma 2010) and the

cladoceran growth rates of *Daphnia* sp. (Urabe et al. 2003), have been found when feeding on phytoplankton prey with elevated C:N and C:P ratios. Additionally, certain essential fatty acids are also important to the growth and reproductive success of heterotrophs (Kattner et al. 2007). Some fatty acids required for microzooplankton growth must be obtained from their diet and cannot be synthesized de novo in microzooplankton (Malzahn et al. 2007, Kattner et al. 2007, Leu et al. 2013). Therefore, it is energetically favorable for microzooplankton to be selective, and to choose prey with physiological, biochemical, and morphological characteristics that promote high growth rates and GGE.

The physiological and biochemical properties that determine the nutritional quality of phytoplankton for microzooplankton are governed mostly by bottom-up variables such as temperature (Harrison et al. 1990, Fu et al. 2007), irradiance (Renaud et al. 1991, Finkel et al. 2006), nutrient speciation and concentration (Harrison et al. 1990, Kilham et al. 1997), and surface ocean dissolved inorganic carbon speciation (e.g. Burkhardt and Riebesell 1997, Fu et al. 2007, Hutchins et al. 2007, Juneja et al. 2013). An emerging climate variable that will alter ocean chemistry and, as accumulating evidence shows, affects the physiology and biochemistry of phytoplankton is ocean acidification (OA) (Riebesell 2004, Rossoll et al. 2012, Schoo et al. 2013, Wynn-Edwards et al. 2014). OA results from the dissolution of rising atmospheric carbon dioxide (CO_2), which is increasing at geologically unprecedented rates, into the surface ocean. The dissolution of this CO_2 into surface ocean waters causes a series of CO_2 equilibrium reactions. These reactions result in an increase in carbonic acid (H_2CO_3), bicarbonate (HCO_3^-), pCO_2 and H^+, and a reduction in carbonate (CO_3^{2-}) (Millero

2013). The increase in H^+ causes the surface ocean pH to drop, and acidify the surface ocean (Millero 2013).

The extent to which phytoplankton may respond to increasing pCO_2 is likely dependent on the physiological mechanisms of inorganic carbon uptake and assimilation. Phytoplankton represent a phylogenetically diverse group, differing in their photosynthetic efficiency and carbon concentrating mechanisms (CCMs) (Giordano et al. 2005). The dark reactions of photosynthesis is catalyzed by the enzyme Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). Rubisco has intrinsically low affinity for pCO_2, achieving half saturation of carbon fixation at pCO_2 concentrations well above those present in surface sea water today (Badger et al. 1998, Riebesell 2004, Rost 2004). To overcome Rubisco's low affinity for CO_2, CO_2 must be either concentrated at the site of fixation, or its concentration increased by converting abundant HCO_3^{2-} to CO_2 using the enzyme carbonic anhydrase. However, both of these CCMs are costly to phytoplankton (Burkhardt et al. 2001), and may be affected by OA through the increased pCO_2. Phytoplankton species with effective CCM's will likely be less sensitive to increasing pCO_2 levels than those with less efficient CCM's (Burkhardt et al. 2001). There is evidence suggesting some phytoplankton species with less efficient CCMs assimilate dissolved inorganic carbon (DIC) more efficiently in elevated pCO_2 conditions (Hein 1997, Engel et al. 2005, Riebesell et al. 2007).

Growing evidence shows that OA affects aspects of phytoplankton biology that can, in turn, affect microzooplankton grazing behavior. For example, OA can alter phytoplankton cell size and shape. The calcifying phytoplankton, *Emiliania huxleyi* responded to OA conditions by increasing cell volume (Iglesias-Rodriguez et al. 2008, Wuori

2012, Jones et al. 2013, Kendall 2015). In addition, marine calcifying species *E. huxleyi* and *Gephyrocapsa oceanica* cultured under OA conditions had malformed coccoliths and incomplete coccospheres (Riebesell et al. 2000a). Tillmann (2004) suggests morphology of a prey cell including cell size and shape are the 1st order determinants of prey suitability for microzooplankton. Secondary constituents important to microzooplankton grazing, like phytoplankton physiology and biochemistry, are also affected by OA. For example, phytoplankton species cultured in OA conditions increased their rate of carbon uptake compared to uptake of N and P, resulting in high cellular C:N and C:P (e.g. Burkhardt and Riebesell 1997, Urabe et al. 2003, Riebesell et al. 2007, Schoo et al. 2013). Depending on species, OA also resulted in increasing (Schoo et al. 2013) or decreasing (Riebesell et al. 2000b, Rossoll et al. 2012) cellular fatty acid and carbohydrate content.

If the alteration of phytoplankton morphology and nutritional quality is wide-spread across phytoplankton taxa under OA, and this, in turn, affects microzooplankton grazing and growth dynamics, it will serve as a mechanism to alter future biogeochemical processes in pelagic marine food webs. Ultimately, grazing rate and GGE of microzooplankton determine the direction and magnitude of organic carbon flow in marine food webs (Caron and Hutchins 2012). If microzooplankton GGE decreases under OA conditions, this could serve to stimulate the microbial loop through increased POM/DOM production, or more carbon may be lost from the system by increased microzooplankton respiration. Alternatively, if microzooplankton GGE increases under OA this could lead to stronger coupling with higher trophic levels. Thus, OA-induced changes in phytoplankton biology that can alter microzooplankton feeding and population growth rates will be controlling

factors in how energy and biomass is transferred through the marine food web (Goldman et al. 1987, Anderson 2005, Caron and Hutchins 2012, Schoo et al. 2013).

To investigate how OA affects the trophic interactions between phytoplankton and microzooplankton, in this study I first characterized the physiology, morphology and biochemistry of an important and ecologically relevant phytoplankton species under elevated pCO_2. I then fed these pCO_2-acclimated phytoplankton cells to different species of microzooplankton to determine the effect that any OA-induced changes to prey had on microzooplankton ingestion and growth rates. The results of this study will help understand the response and trophic transfer efficiency of the base of the marine pelagic food web in a more acidified ocean.

METHODS

Hypotheses and Experimental Approach

To better understand how ocean acidification may affect the marine planktonic food webs, I tested two hypotheses:

Hypothesis 1: The physiology, biochemistry and morphology of the phytoplankton *Rhodomonas* sp. will be affected by elevated pCO_2.

Hypothesis 2: pCO_2 induced changes to the physiology, biochemistry and morphology of *Rhodomonas* sp. will affect grazing and growth rates in microzooplankton consumers.

To test these hypotheses, I first cultured the phytoplankton species *Rhodomonas* sp. semi-continuously under three target pCO_2 concentrations, an ambient and two elevated concentrations. Under these treatment conditions I measured a suite of phytoplankton physiological and biochemical characteristics (see sections below). After characterizing the *Rhodomonas* sp. response to OA, pCO_2 acclimated *Rhodomonas* cells were used to test whether microzooplankton grazing and growth are affected by OA through OA-induced changes to prey state. Two experimental designs were used to test this hypothesis. First, short term (ST) microzooplankton ingestion rates were estimated by measuring the accumulation rate of ingested *Rhodomonas* sp. cells inside of microzooplankton food vacuoles over a short time scale (mins.). Second, microzooplankton were acclimated to the

diet of pCO_2 cultured *Rhodomonas* sp. for 5 days before long term (LT) (24 h) microzooplankton ingestion and growth rates were measured and calculated using equations from Heinbokel (1978). From these rates, gross growth efficiency was calculated to determine if trophic transfer efficiency will be affected by OA.

pCO_2 Culturing System

The ocean acidification laboratory at Shannon Point Marine Center works by creating atmospheric CO_2 gas concentrations that are supplied to cultures contained inside near air-tight Plexiglas boxes. Gas exchange between the atmosphere and culture media serves as the mechanism by which media pCO_2 chemistry is maintained. CO_2 treatment atmospheres were produced by adding reagent grade CO_2 to air (previously scrubbed free of CO_2 with a Pure Gas CAS series CO_2 adsorber) using Sierra Smart-Trak 2 mass flow controllers. Once the CO_2 atmospheres were created, they were split and sent to several locations using a flow-regulated control panel (Fig. 1). One pathway for the treatment gases was to a darkened environmental incubator held at 15° housing 3 20L carboys. Prior to experiments the carboys were filled with autoclaved filtered seawater amended with f/50 nutrients. The f/50 media in the carboys was vigorously bubbled with CO_2 treatment gasses for at least 48 h and serves as pre-equilibrated pCO_2 treatment media. A second pathway for the CO_2 treatment atmospheres was a walk in environmental chamber (EC) that holds experimental cultures inside the Plexiglas boxes. The EC was held at constant temperatures and cultures were supplied with photosynthetically active radiation (PAR) at a given intensity and light:dark cycles. Plexiglas boxes were continuously supplied with CO_2 treatment atmospheres. A LiCor LI820 CO_2 sensor monitors concentrations of atmospheric

CO_2 in the inflow gas, in gas exiting the Plexiglas boxes, and in headspaces of the pre-equilibrating carboys.

Carbonate chemistry of the pre-equilibrated pCO_2 treatment media and experimental cultures was determined by measuring total alkalinity (TA) and total dissolved inorganic carbon (DIC). TA was measured with Gran titration using a Metrohm 888 Titrando titrator and approximately 0.1N HCl and NaCl titrant. For corrections of TA, a certified reference material (CRM) Batch 131 (2013) provided by Andrew Dickson (Scripps Institution of Oceanography, San Diego, USA) was titrated prior to the titration of samples. An Apollo SciTech DIC analyzer was used for DIC measurements and the CRM Batch 131 (2013) was used for calibration of the machine prior to sample measurements. The program CO2sys was used to calculate pCO_2 using DIC and TA as parameters (constants: Millero et al.; pH scale: seawater scale) (Lewis and Wallace 1998).

Experimental Organisms and Culture Methods

Phytoplankton

The unicellular flagellated cryptomonad, *Rhodomonas* sp. (Strain 755), was chosen for this study because it is considered an ecologically important resource (Klaveness 1988). *Rhodomonas* sp. is common, but rarely abundant, in coastal or estuarine marine waters (Graham et al. 2009). In colder and deeper waters, such as the North Sea, *Rhodomonas* sp. can contribute significantly to primary productivity during the winter and early spring months (Graham et al. 2009). They are an important food source for many

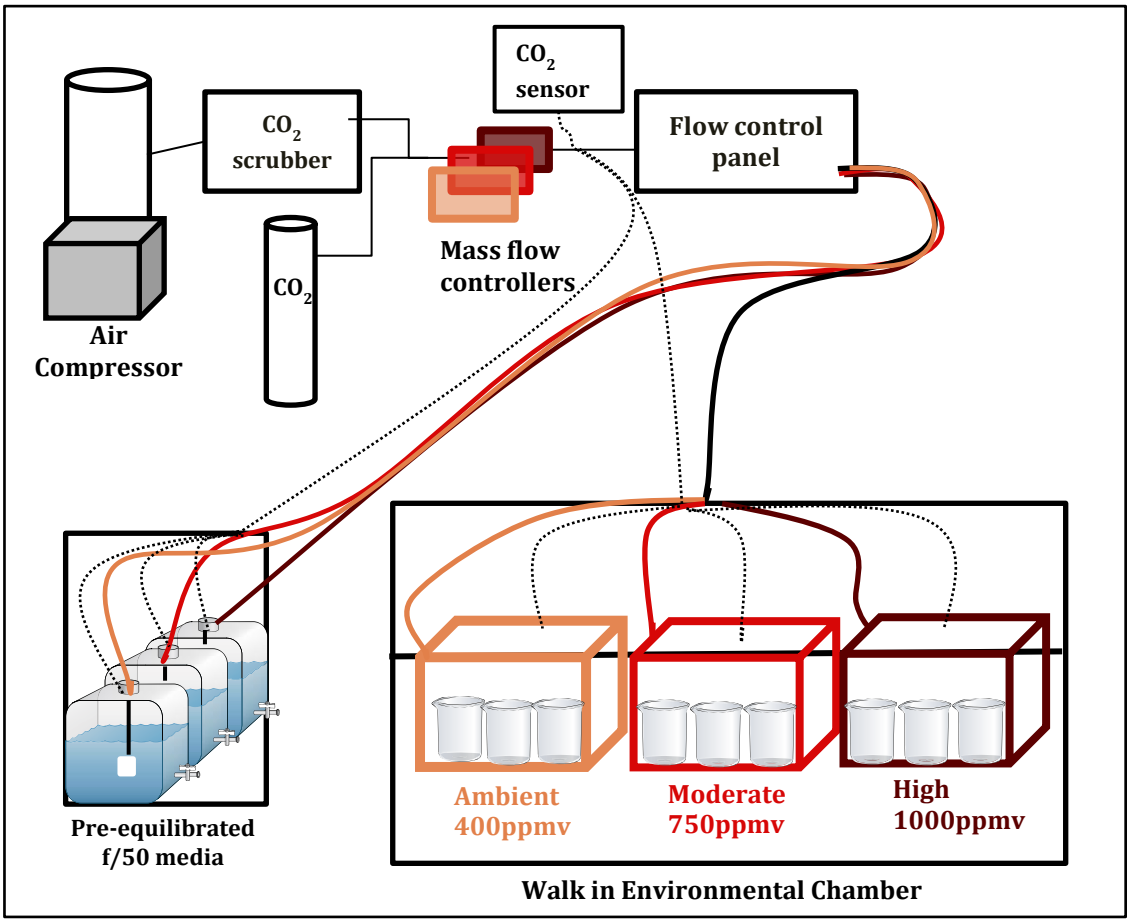


Figure 1 Diagram of SPMC's ocean acidification culturing system. See text for details.

microzooplankton, including ciliates and dinoflagellates, because they are readily ingested and digested, lack toxins, and contain relatively high proportions of two essential, highly unsaturated fatty acids (HUFAS) (Graham et al. 2009). As such, it is an ideal model phytoplankton to study the effects of elevated pCO_2 on planktonic food web ecology. *Rhodomonas* sp. strain 755 was isolated from Long Island Sound in the North Atlantic. Maintenance cultures of *Rhodomonas* sp. were maintained in batch cultures in autoclaved filtered seawater amended with f/50 nutrient levels in the EC.

Microzooplankton

The microzooplankton used in this study included two tintinnid ciliates and two dinoflagellate species. The two tintinnid ciliates used were *Favella ehrenbergii* (SPMC 150). (recent name change to *Schmidingerella taraikaensis* (Agatha & Strüder-Kypke 2012)) and *Coxliella* sp. (SPMC 160). Both were isolated from northern Puget Sound, Washington, USA. The two heterotrophic dinoflagellates used were *Gyrodinium dominans*, isolated from Skagerrak between Denmark and Sweden (Hansen & Daugbjerg 2004), and *Oxyrrhis marina* (SPMC 107), isolated from Puget Sound, Washington, USA. Stock microzooplankton cultures were maintained on prey mixtures, which included *Rhodomonas* sp., in 0.2 µm autoclaved filtered seawater (~30 psu) amended with a dilute trace metal mixture (ciliate medium, Gifford 1985). Stock *Rhodomonas* sp. was grown in f/2, without added Si. Microzooplankton and phytoplankton cultures were maintained at 15 °C under a 14:10 light:dark cycle. All culture media was prepared from seawater collected from the Puget Sound.

Semi-continuous Culturing of *Rhodomonas* sp.

During experiments, *Rhodomonas* sp. was cultured semi-continuously under the target pCO_2 concentrations of ambient (400ppmv), moderate (750ppmv) and high (1000ppmv). These pCO_2 concentrations were chosen because they include the current atmospheric CO_2 concentration and fall within the range of predicted atmospheric CO_2 concentrations for the end of this century (IPCC 2007). Maintenance culture of *Rhodomonas* sp. in mid exponential growth was used to inoculate 500 ml of pre-equilibrated media in three replicate 1L polycarbonate experimental bottles per pCO_2 treatment. These bottles were subsequently placed in Plexiglas boxes inside the EC. The EC was held at 15°C and experimental bottles were incubated under a 14:10 light: dark cycle at ~ 66.40 µmol photons sec^{-1} m^{-2}. After inoculation, *Rhodomonas* cultures were allowed to grow for 4 days to a density of approximately ~50,000 cells ml^{-1}.

For semi-continuous culturing, subsamples from *Rhodomonas* sp. experimental bottles were taken daily to determine cell concentrations. These cell concentrations were then used to calculate the required volume to be removed, and replaced with new pre-equilibrated media to bring cell concentrations of *Rhodomonas* sp. down to ~25,000 cells ml^{-1}. This density was determined in preliminary experiments to be adequate to maintain pCO_2 near target treatment concentrations in experimental cultures. To ensure that target pCO_2 concentrations were maintained in experimental bottles during the experiments, subsamples were taken for TA and DIC from the volume removed from the experimental bottles during daily dilutions (dilution volume). TA samples were taken every other day, preserved with $HgCl_2$ and stored at 4°C until analysis. DIC samples were prepared for

analysis by filtering experimental water through a 0.2µm nylon syringe filter the morning of each experimental day. DIC samples were stored in air tight vials and kept at 4°C until analysis within 60 days. Semi-continuous experiments lasted for a total of 16 to 17 days, during which samples were taken for the analysis of the following *Rhodomonas* sp. parameters: intrinsic growth rate (d^{-1}), cellular chlorophyll *a* (pg Chl *a* $cell^{-1}$), cell biovolume (μm^3), cellular carbohydrate mass (pg $cell^{-1}$), cellular carbon and nitrogen (pg $cell^{-1}$), and total cellular lipid mass (pg $cell^{-1}$) (see table 1). Four *Rhodomonas* sp. semi-continuous experiments were conducted and *Rhodomonas* sp. cells from each separate experiment served as prey for the different microzooplankton grazing experiments (Table 1).

Phytoplankton Characterization

Bio-volume

Subsamples from each treatment replicate experimental bottle were taken and fixed with Lugol's acid to preserve cells for cell bio-volume analysis. For analysis, an Olympus CH30 compound microscope networked to a CoolsnapCF Photometrics camera was used to image individual *Rhodomonas* sp. cells under 400X magnification. Using RSI image software 50 cells were haphazardly selected from each treatment replicate and were photographed (150 images per pCO_2 treatment). ImageJ software was used to measure *Rhodomonas* sp. cell length and width. *Rhodomonas* sp. is described as having a prolate spheroid shape. Therefore, cell bio-volume was calculated as:

$$V_{prolate} = \left(\frac{4}{3}\right)\pi a^2 b \quad (1)$$

Where *a*= ½ width and *b*= ½ length of the *Rhodomonas* sp. cell.

Table 1. Description of each experiment, including microzooplankton species used in short term (ST) and long term (LT) grazing experiments and day the grazing experiment was done, *Rhodomonas* sp. prey physiological and biochemical attributes assessed, and the duration (days) of semi-continuous culturing for each experiment. GR: growth rate (d^{-1}); CBIO: cell bio-volume (μm^3); CARB: carbohydrate (pg $cell^{-1}$); POC: particulate organic carbon (pg $cell^{-1}$); PON: particulate organic nitrogen (pg $cell^{-1}$); TLIPIDS: total lipids (pg $cell^{-1}$), Chl a: cellular chlorophyll *a* (pg Chl *a* $cell^{-1}$). The subscript by each prey attribute represents the number of times each parameter was assessed (*n)* during the duration of each experiment.

Expt. #	**ST Grazing**	**LT Grazing**	**Prey Attributes Assessed**	**Duration (days)**
1	*G. dominans* and *F. ehrenbergii* Day 9	N/A	GR_{10}, $CBIO_{10}$, Chl a_1, $CARB_1$, POC_1, PON_1	10
2	*O. marina* Day 9	*O. marina* Day 16	GR_{17}, $CBIO_7$, Chl a_2, $CARB_2$, POC_2, PON_2	17
3	*Coxliella* sp. Day 9	*Coxliella* sp. Day 15	GR_{17}, $CBIO_9$, Chl a_2, $CARB_2$, POC_2, PON_2, $TLIPIDS_1$	16
4	N/A	*G. dominans* Day 16	GR_{16}, $CBIO_{10}$, Chl a_2, $CARB_2$, POC_2, PON_2, $TLIPIDS_1$	17

Cellular C and N content

Samples for *Rhodomonas* sp. particulate cellular carbon and nitrogen (pg cell^{-1}) were taken by gently vacuum filtering 100 mL from each pCO_2 treatment replicate onto 21mm muffled GF/F filters. After filtration, filters were removed and placed in tin boats. Samples and controls (media blanks, filter blanks and capsule blanks) were placed in a drying oven for 24 h at 50-60 ° C, after which time they were removed and placed in a desiccator until analysis. Tin boats containing the filters and controls were folded into pellets, and then combusted using a Micro Cube elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer at the UC Davis Stable Isotope Facility. *Rhodomonas* sp. cellular nitrogen and carbon content (pg) were normalized to cell^{-1} and μm^{-3}.

Total Cellular Lipid Mass

Samples for total cellular lipid mass were taken and analyzed using an adaption of Bligh and Dyer (1959) and Ryckebosch et al. (2012). Samples were gently vacuum filtered on to muffled GF/F filters. Sample volume varied between 250-300 mL depending on experimental volume available. After filtration filters were wrapped in muffled aluminum foil and flash frozen by placing the filters on top of dry ice. After flash freezing, samples were stored at -80 °C until analysis. All glass culture tubes, foil pieces, and Pasteur pipets used for laboratory extraction were muffled for a full 8 h prior to analysis. For extraction, filters were transferred from the freezer into a clean tissue grinder containing 1.9 mL of 1:2 v/v $CHCl_3$:MeOH and 0.125 mL DI water. Filters were homogenized into the solution and then transferred to a clean glass culture tube and vortexed for 1 minute. Samples were then sonicated in a water bath at room temperature for 10 minutes and then centrifuged at

2000rpm for 10 minutes to remove particulate matter. The supernatant was then transferred to another glass culture tube and 0.625 mL of $CHCl_3$ and 0.625 ml DI water were added and then centrifuged for an additional 10 minutes. After centrifugation, the samples were separated into two phases and the bottom organic phase was carefully collected using a Pasteur pipet and transferred to a pre-weighed glass culture tube. After transfer, the samples were evaporated using nitrogen gas. The weight of the dried samples was used to determine total cellular lipid mass. Data were normalized to $cell^{-1}$, μm^{-3}, and pg C^{-1}.

Intrinsic Growth Rate

Subsamples from semi-continuous cultures of *Rhodomonas* sp. were taken once daily in the morning after dilutions (Table 1). From this, cell density was measured using a Beckman Z2 coulter counter and served as population size at T_1. After 24h of growth, samples were taken the following morning were used to determine population size at T_2. *Rhodomonas* sp. specific growth rate was then calculated according to the exponential growth equation:

$$K = \ln[\frac{C_2 - C_1}{T_2 - T_1}] \quad (2)$$

where C_2 and C_1 are the concentrations of *Rhodomonas* sp. at times T_2 and T_1 respectively.

Chlorophyll *a*

Cell suspensions from each treatment replicate bottle on day 10 and the final day of each semi-continuous experiment were taken for Chl *a* analysis (Table 1). For this, 10 mL

was filtered onto glass fiber filters (GF/F). Filters were immediately folded and placed in test tubes containing 6 mL of 90% v/v acetone and stored at -20 °C for 24 h. After 24 h samples were warmed to room temperature in the dark, filters were removed and the test tubes were centrifuged before fluorometrically analyzed using a Turner Designs Trilogy fluorometer. Raw fluorescence pre- and post-addition of 10% HCl was used to calculate Chl *a* according to the acidification method of Parsons (1984). *Rhodomonas* sp. Chl *a* content (pg) was normalized to cell $^{-1}$ and μm^{-3}.

Cellular Carbohydrate Content

Samples for analysis of cellular carbohydrate mass were taken by gravity filtering 30 mL of experimental culture through 2.5 cm muffled GF/F filters. Filters were folded and wrapped in muffled aluminum foil and stored at -80°C until analysis. Upon analysis, the filters were first extracted in 1.0 mL of 95% H_2SO_4 and 1.0 mL of nanopure water in a sonication bath for 30 minutes, and then for 20 h at room temperature. After extraction samples were centrifuged at 10,000 rpm for 6 minutes. Of the pooled extract 1.6 mL was transferred to fresh test tubes. Concentrated H_2SO_4 (4 mL) and 10% phenol (0.8 mL) were introduced quickly to each vial of 1.6 mL sample and allowed to react for 30 minutes at room temperature. Samples were analyzed colormetrically using a Spec20D+ spectrophotometer at 485nm. S tandards of known dextrose concentrations (1, 2, 4, and 8 mg L^{-1}) were used to calculate cellular carbohydrate mass. Carbohydrate content in the sample was scaled to total extract volume and cellular carbohydrate content (pg $cell^{-1}$) was found using calculated cell densities. Carbohydrate mass was further normalized to *Rhodomonas* sp. biovolume (μm^3) and carbon (pg C $cell^{-1}$).

Microzooplankton Ingestion and Growth Rates

Measuring Ingestion Rates of Microzooplankton Using Epifluorescence Microscopy

In order to calculate ST microzooplankton ingestion rates I used a method that differentiates between the autofluorescing signatures of microzooplankton and their ingested prey. Autotrophic phytoplankton cells fluoresce red or orange under blue light excitation, whereas microzooplankton grazers fluoresce semi-transparent green. Both can be easily visualized under epifluorescence microscopy (Strom et al. 2007), and individuals can be easily differentiated by DAPI-induced (4', 6-diamidino-2-phenylindole) blue fluorescence of individual nuclei. Because microzooplankton cells are semi-transparent, the red auotofluorescing phytoplankton cells can easily be visualized and counted inside microzooplankton food vacuoles.

To accurately count the small individual phytoplankton cells within the microzooplankton food vacuoles, samples were analyzed under high magnification (400X). When quantifying the number of phytoplankton cells within microzooplankton food vacuoles using this method it is first necessary to reduce background fluorescence within microzooplankton food vacuoles originating from their maintenance food. Without doing so it is difficult to impossible to discern individual prey cells of interest. To achieve this, grazers are typically removed from their maintenance prey prior to an experiment. Physical separation of grazer from prey is challenging, and the appropriate method for removing background food depends on the ratio of microzooplankton to phytoplankton size. If the difference in cell size between the grazer and prey is large, mechanical separation can be done using a mesh screen. If the cell size ratio of the grazer and prey is small, then

maintenance food is eliminated by allowing microzooplankton to graze down prey concentrations to near zero prior to the start of an experiment. Once prey is removed, care must be taken to ensure starvation of grazers does not elicit a state of feeding dormancy.

Once grazer food vacuoles are cleared of background fluorescence, grazers are typically placed in new media, and treatment prey cells are added. This point serves as time zero of an experiment. Over the course of min. to hrs., samples from experimental flasks are taken at several pre-determined time points. The chosen time points should show an incremental increase in ingested phytoplankton over time, but be short enough to prevent microzooplankton food vacuoles from becoming saturated with prey, a situation that makes it impossible to identify individual phytoplankton cells or calculate a feeding rate. The selection of these time points is thus specific to each microzooplankton species' feeding rate. For experiments, the number of phytoplankton cells in the food vacuoles of individual microzooplankton are counted at each time point. Then, the average number of prey cells in microzooplankton food vacuoles is plotted against each respective sampling time point. Microzooplankton ingestion rate is then determined as the slope of ingested cells regressed over time.

Microzooplankton Short Term Ingestion Rate Experiment

ST grazing experiments were done during each semi-continuous *Rhodomonas* sp. characterization experiment (Table 1). These experiments were done after at least 8 days of semi-continuous culturing to ensure *Rhodomonas* sp. achieved physiological steady-state in their respective pCO_2 treatments (Wuori 2012, Kendall 2015). Prior to the experiments, maintenance food was removed from the grazers, and the method to achieve this differed

depending on the grazer tested. The ciliates, *F. ehrenbergii* (150 µm length, Jörgensen 1924) and *Coxliella* sp. (85 µm length), which are much larger than *Rhodomonas* sp. (~8 µm in length), were physically separated by gentle reverse filtration through a 20µm mesh screen. This allowed the gentle capture of microzooplankton but allowed *Rhodomonas* sp. to pass through the filter. After sieving, *F. ehrenbergii* and *Coxliella* sp. were re-suspended in ciliate medium ~20 h and ~48 h prior to experiments, respectively. Because these ciliates do not attain high density, sieving had the added benefit of concentrating the grazer population, decreasing the amount of time needed to search for ciliate cells under microscopy during sample analysis. For the dinoflagellates, which are closer in size (~25 µm in length) to *Rhodomonas* sp., it was not possible to remove background food by sieving. Instead, *G. dominans* and *O. marina* were allowed to graze down the background food present in stock cultures to very low densities. This was done by placing the cultures under low light for 11 and 7 days for *G. dominans* and *O. marina*, respectively, prior to experiments. Low light served to inhibit the growth of the phytoplankton prey cells.

Once microzooplankton were cleared of background fluorescence, microzooplankton were dispensed into media pre-equilibrated to the three respective pCO_2 treatments. To ensure that there were at least 100 replicate microzooplankton cells to count on a slide, microzooplankton were inoculated to achieve a density of 20-50 cells ml^{-1} (Table 2). pCO_2-acclimated *Rhodomonas* sp. culture was pooled from each of the three pCO_2 treatment replicates. From this, cells were added to the experimental bottles containing microzooplankton. Bio-volume of *Rhodomonas* sp. cells from each pCO_2 treatment were measured the day prior to short term grazing experiments for each pCO_2

treatment. The volume of *Rhodomonas* sp. culture added to experimental bottles was then set to achieve saturating food concentrations of 400 µg C l^{-1}, based on the relationship between POC and bio-volume for protist plankton <3,000 μm^3 described by Menden-Deuer and Lessard (2000). The resulting prey concentrations were expected to have equal carbon content and approximately equal total bio-volume, rather than equal cell numbers. However, because *Rhodomonas* sp. POC density was greater than expected from the Menden-Deuer and Lessard (2000) relationship overall densities were 150 to 200% of carbon saturating conditions. In addition *Rhodomonas* sp. POC density varied across each pCO_2 treatment, resulting in different prey carbon saturating concentrations in each pCO_2 treatment (Table 2). If *Rhodomonas* sp. was not the optimal food for the microzooplankton, an optimal diet treatment was added to serve as positive controls. If microzooplankton grazing on the optimal diet treatments were positive (i.e. the slope of prey cells ingested versus time > 0), grazers were deemed physiologically healthy. For *O. marina* and *F. ehrenbergii*, optimal diet prey were *Isochrysis galbana* and *Heterocapsa triquetra*, respectively. No optimal diet treatments were used for *G. dominans* and *Coxliella* sp. because *Rhodomonas* sp. was their primary maintenance food in stock cultures.

Grazing sampling time points for each microzooplankton are shown in Table 2. Sampling consisted of dispensing 20 mL samples into bottles previously filled with 0.5% glutaraldehyde and DAPI. These samples were stored at 4°C for 12 h to allow enough time for DAPI to stain microzooplankton and *Rhodomonas* sp. nuclei. Samples for the ciliates were filtered onto 20 µm and 10 µm pore size polycarbonate (PC) filters for *F. ehrenbergii* and *Coxliella* sp., respectively. Samples for the dinoflagellates were filtered onto 5 µm pore

Table 2. Experimental design for short term microzooplankton grazing experiments. T_0 grazer (grazer inoculation density (cells ml^{-1})); T_0 prey (prey inoculation density (cells ml^{-1})) with the equivalent prey (µg C L^{-1}) values below in parentheses; A: ambient; M: moderate, H: high pCO_2 treatments. Bio-volume of *Rhodomonas* sp. from each pCO_2 treatment was measured the day prior to short term grazing experiments and used to determine the prey densities to inoculate each pCO_2 experimental treatment bottle to achieve saturating food concentrations (400 µg C l^{-1}, Menden-Deuer and Lessard 2000).

Grazer	Sample Time Points (min)	T_0 Grazer	T_0 Prey		
			A	M	H
G. dominans	15, 45, 90	40	18,000 (844)	16,800 (774)	16,500 (779)
F. ehrenbergii	15, 30, 45	20	18,000 (844)	16,800 (774)	16,500 (779)
O. marina	30, 60, 90, 120	50	17,400 (774)	13,300 (624)	13,000 (618)
Coxliella sp.	15, 30, 45	20	20,600 (1056)	14,000 (755)	13,000 (715)

size PC filters. Filters were slide mounted by placing the filter on a microscope slide, immersing it in immersion oil, and then covering it with a microscope cover slip. Slides were kept at -20°C until analysis, and all slides were analyzed within 3 months of preparation.

For each sampling time point, the number of *Rhodomonas* sp. cells in the food vacuoles of the first 100 haphazardly encountered microzooplankton was counted. A value of 0 was used to represent microzooplankton with empty food vacuoles. As described above, for each treatment replicate, the average number of prey cells in microzooplankton food vacuoles were plotted against each respective sampling time point. A single microzooplankton ingestion rate for each ST ingestion rate experiment was found by fitting a linear regression to the number of cells ingested over the entire time elapsed. The percentage of the microzooplankton population found to be feeding was determined as the number of microzooplankton found with *Rhodomonas* sp. cells in the food vacuoles divided by the total number of microzooplankton counted.

Microzooplankton Long-term Ingestion and Growth Rate Experiment

To determine whether patterns observed in ST ingestion rates were conserved over longer time periods, a second set of experiments was conducted. The design of these experiments allowed me to simultaneously measure ingestion rates over longer time periods, and the growth rates of microzooplankton as a function of diet pCO_2 treatment. Prior to measuring microzooplankton ingestion and growth rates, grazers were allowed to acclimate for 5 days to the pCO_2-acclimated prey diet. When measuring microzooplankton feeding rates in response to changes in food quality, long incubation periods (minimum 24

h) are recommended to allow for feeding rates to stabilize (Jakobsen & Strom 2004, Meunier et al. 2011, Calbet et al. 2013). Therefore, the purpose of the acclimation period was to ensure microzooplankton achieved physiological steady-state to any OA-induced changes in the prey biology. pCO_2 chemistry and prey concentration were maintained during the acclimation period by refreshing the microzooplankton and *Rhodomonas* sp. treatment cultures (microzooplankton treatment bottles) with semi-continuous *Rhodomonas* sp. culture or pre-equilibrated media.

Acclimation period

The 5 day acclimation period for long term grazing experiments began on day 11 of each semi-continuous experiment, following the ST ingestion rate experiments and the characterization of *Rhodomonas* sp. biology (Table 1 and 3). For acclimation, pCO_2-acclimated *Rhodomonas* sp. were pooled from semi-continuous treatment replicates and used to inoculate five 1 L replicate microzooplankton treatment bottles at prey concentrations that achieved saturating food concentrations of 400 µg C l^{-1}, based on the relationship between POC and bio-volume for protist plankton <3,000 μm^3 described by Menden-Deuer and Lessard (2000) as was done in ST grazing experiments (Table 3). Microzooplankton were inoculated at varying densities depending on the species (Table 3). To control for the influence of microzooplankton nutrient regeneration, ammonium chloride (1 µM) was added to the semi-continuous *Rhodomonas* sp. cultures and the microzooplankton treatment bottles. During the acclimation period, both microzooplankton treatment bottles and the semi-continuous *Rhodomonas* sp. cultures

Table 3. Time zero (T_0) concentrations (cells mL^{-1}) of microzooplankton and *Rhodomonas* sp., with the equivalent prey (µg C L^{-1}) values below in brackets, at the start of the acclimation period and during long term (LT) grazing and growth rate experiments. Bio-volume of *Rhodomonas* sp. from each pCO_2 treatment was measured the day prior to LT grazing experiments and used to determine the prey densities to inoculate each pCO_2 experimental treatment bottle to achieve saturating food concentrations (400 µg C l^{-1}, Menden-Deuer and Lessard 2000).

	Acclimation	**Acclimation T_0 prey**			**LT**	**LT T_0 prey**		
Grazer	**T_0 grazer**	**A**	**M**	**H**	**T_0 grazer**	**A**	**M**	**H**
O. marina	100	16,500 (1,071)	13,000 (823)	12,600 (879)	200	29,000 (1,882)	22,300 (1,412)	17,200 (1,201)
Coxliella sp.	60	20,597 (1,069)	13,947 (869)	12,829 (758)	15	60,000 (3,114)	36,000 (2,243)	33,000 (1,950)
G. dominans	300	19,500 (1,207)	13,000 (854)	11,000 (834)	640	20,000 (1,238)	10,000 (657)	8,000 607)

were incubated in their respective atmospheric pCO_2 conditions. In order to keep microzooplankton treatment and semi-continuous cultures of *Rhodomonas* sp. populations in steady state over the course of the 5 day acclimation period, it was necessary to adjust volumes within bottles daily. Over a 24 h period, concentrations of microzooplankton and *Rhodomonas* sp. changed due to grazing and growth. Therefore, daily subsamples from microzooplankton treatment bottles and semi-continuous *Rhodomonas* sp. cultures were taken to establish prey cell densities. Cell densities of *Rhodomonas* sp. in microzooplankton treatment bottles were then used to calculate how much volume needed to be removed from microzooplankton treatment bottles and replaced with either pre-equilibrated media (f/50 medium without 1µm NH_4Cl) (if grazing rates did not exceed *Rhodomonas* sp. growth rates) or culture from the semi-continuous *Rhodomonas* sp. bottles to bring prey concentrations back to the calculated 400 µg C L^{-1} densities (Menden-Deuer and Lessard 2000). After prey densities were adjusted in microzooplankton treatment bottles, *Rhodomonas* sp. densities in the semi-continuous *Rhodomonas* sp. cultures were diluted with respective pre-equilibrated pCO_2 media to approximately match prey concentrations in the microzooplankton treatment bottles to keep *Rhodomonas* sp. in steady state. To ensure that target pCO_2 concentrations were maintained in microzooplankton treatment bottles, subsamples were taken for TA and DIC.

24h Microzooplankton Ingestion Rate Experiment

The 24 h (LT) microzooplankton ingestion rate experiment began after the 5 day acclimation period. In preparation for this, subsamples from microzooplankton treatment bottles and semi-continuous *Rhodomonas* sp. cultures were taken to determine cell

concentrations of *Rhodomonas* sp. Microzooplankton treatment bottles were adjusted to bring prey concentrations to 400 µg C L^{-1} by either adding pre-equilibrated media or culture from semi-continuous *Rhodomonas* bottles as described above. The semi-continuous *Rhodomonas* sp. cultures were diluted so that *Rhodomonas* sp. cell densities in these bottles were the same as in the microzooplankton treatment bottles and then served as control bottles for the 24 h experiment. Subsamples were then taken from microzooplankton treatment and semi-continuous *Rhodomonas* sp. bottles to determine initial (T_0) cell concentrations for *Rhodomonas* sp. and microzooplankton grazers. The semi-continuous *Rhodomonas* sp. cultures and microzooplankton treatment bottles were incubated in their respective atmospheric pCO_2 conditions for 24 h. After 24 h, subsamples for the same counts were taken again and served as T_f abundances. Microzooplankton grazing, ingestion and clearance rates were calculated according to Heinbokel (1978).

For *Coxliella* sp. it was not possible to adjust prey concentrations back to values closer to 400 µg C L^{-1} in grazer bottles prior to the start of the 24 h ingestion rate experiment measurement. As a result, prey carbon concentrations were greater in all pCO_2 treatments in comparison to the other microzooplankton 24 grazing experiments done (Table 3). The ciliate densities were very low in microzooplankton treatment bottles by day 5 of the acclimation period. This was a result of *Rhodomonas* sp. growth rates exceeding the ciliate's ingestion rate in the microzooplankton treatment bottles. As such, dilution to bottles containing *Coxliella* sp. with pre-equilibrated media was required each day during the acclimation period to keep *Rhodomonas* sp. near 400 µg C L^{-1}. To avoid diluting *Coxliella* sp. to densities too low to measure ingestion rates during the grazing experiments,

microzooplankton treatment and *Rhodomonas* sp. semi-continuous culture bottles were not manipulated, and, consequently densities were not adjusted on the last day of the acclimation period. Therefore, prey concentrations during the LT ingestion rate experiment were higher than 400 µg C L^{-1} for *Coxliella* sp. in comparison to the other microzooplankton (Table 3).

24 h Microzooplankton Growth Rate

During the 24 h (LT) ingestion rate experiments, microzooplankton growth rates were also measured. Manual cell counts for microzooplankton were done using microscopy and a Sedgewick Rafter Chamber from the subsamples taken from microzooplankton treatment bottles at T_0 and at T_f that had been fixed with Lugol's acid.

Description of Heinbokel (1978) Equations

The growth constant (*k*) for *Rhodomonas* sp. was calculated from the grazer-free semi-continuous *Rhodomonas* sp. cultures using:

$$k = \ln[\frac{C_2 - C_1}{T_2 - T_1}] \quad (3)$$

Where C_2 and C_1 are the concentrations of prey (cells mL^{-1}) at times T_2 and T_1 respectively. Growth rates of the microzooplankton (*g*) (d^{-1}) in the experimental bottles was calculated by:

$$g = \ln[\frac{M_2 - M_1}{T_2 - T_1})]$$

(4)

where M_2 and M_1 are the concentrations of microzooplankton at times T_2 and T_1 respectively.

Microzooplankton grazing rate (G) (day^{-1}) of the microzooplankton was calculated as:

$$G = k - \frac{(\ln\left(\frac{C_2}{C_1}\right)}{(T_2 - T_1)}$$

(5)

Where C_2 and C_1 are the concentrations of prey within microzooplankton treatment bottles at times T_2 and T_1 respectively.

Time-averaged *Rhodomonas* sp. concentration with microzooplankton present (<*C*>) (*Rhodomonas* sp. cells mL^{-1}) during the time interval T_2-T_1 was calculated by:

$$< C > = C_1 * [\frac{e^{(k-G)(T_2-T_1)} - 1}{(T_2 - T_1)(k - G)}]$$

(6)

Where C_1 is the concentration of *Rhodomonas* sp. in microzooplankton treatment bottles at T_1.

Because dinoflagellates and ciliates are capable of growth over 24 h, the average microzooplankton concentration ($\bar{M}$) (cells mL^{-1}) was calculated as:

$$\bar{M} = (\frac{M_2 - M_1}{(\ln(M_2) - \ln(M_1))})$$

(7)

Where M_2 and M_1 are the concentrations of microzooplankton at times T_2 and T_1 respectively.

Microzooplankton clearance rate (F) (mL microzooplankton^{-1} day^{-1}) was calculated from:

$$F = \frac{G}{\overline{M}}$$

(8)

And microzooplankton ingestion rate (I) (*Rhodomonas* sp. cells microzooplankton^{-1} day^{-1}) is calculated from:

$$I = < C > * F$$

(9)

Microzooplankton Gross Growth Efficiency

Gross growth efficiency (%) of the different microzooplankton species was determined by dividing the amount of C ingested (pg C ingested grazer^{-1}day^{-1}) by the total C produced by microzooplankton (pg C production grazer^{-1}day^{-1}).

Microzooplankton carbon production was calculated by multiplying the increase in microzooplankton abundance over 24 h (ΔM, grazer cells ml^{-1}day^{-1}) with then C mass values (POC_M, pg C grazer cell^{-1}) previously reported for each microzooplankton species used in this study (*Coxliella* sp. (Verity 1985), *G. dominans* (Nakamura et al. 1995) and *O. marina* (Montagnes et al. 2011)).

Both ST- and LT-term microzooplankton ingestion rates (I, prey cells grazer^{-1}day^{-1}) and the *Rhodomonas* sp. POC cell^{-1} results (POC_R, pg C *Rhodomonas* sp. cell^{-1}) were used to calculate *Rhodomonas* sp. carbon specific ingestion. The average microzooplankton concentration $\bar{M}$ (grazer cells ml^{-1}day^{-1}) was used to determine pg *Rhodomonas* sp. C ingested microzooplankton^{-1} day^{-1}.

$$GGE = \frac{(\Delta M * POC_M)}{(I * POC_R * \bar{M})} \quad (10)$$

Statistical Methods

Statistical analyses were done using IBM SPSS Statistics 20 software. *Rhodomonas* sp. characterization, microzooplankton ingestion and growth rates data were tested with analysis of variance (ANOVA). If a treatment effect was found, a Tukey's post-hoc comparison was performed. A Levene's test was used to ensure equal variance across treatments. If this assumption was not met, the data were transformed. Data that were natural log transformed include: Expt. 2 day 10 and Expt. 4 day 16 *Rhodomonas* sp. cell bio-volume. *Coxliella* sp. GGE values were square root and common log transformed to meet homogeneity. *G. dominans* GGE values calculated using microzooplankton ST ingestion rate values could not be normalized with transformation. As such, treatment effects were tested using the non-parametric Kruskal-Wallis test. Microzooplankton percent feeding data were tested with repeated measures ANOVA (ANOVAR) and significant differences were defined as $p<0.05$ for all statistical analyses.

Stepwise multiple linear regression models (MLR) were used to determine what predictive biochemical and physiological factors accounted for the pCO_2 related variability in ingestion of moderate and high pCO_2 cultured *Rhodomonas* sp. by the microzooplankton *O. marina*, *F. ehrenbergii* and *G. dominans*. The model included the predictive *Rhodomonas* sp. variables: cell bio-volume, POC $cell^{-1}$, PON $cell^{-1}$, Chl *a* $cell^{-1}$and carbohydrates $cell^{-1}$. The model evaluated each variable by using a p value of 0.05 to enter and 0.10 to exit, and eliminated variables stepwise if they were not significant predictors of the observed increased ingestion rates.

RESULTS

pCO_2 Chemistry

Target pCO_2 concentrations for ambient, moderate and high treatments were 400ppm, 750ppm and 1000ppm. Average pCO_2 of pre-equilibrated media for each experiment (n=4) was found by averaging the pCO_2 treatment replicate values (n=3) that were found at the beginning of each experiment from each pCO_2 treatment carboy holding pre-equilibrated media. Average pCO_2 concentrations of pre-equilibrated media across all experiments (n=4) were 379 ± 16, 733 ± 27 and 969 ± 43 (ppmv) for ambient, moderate and high, respectively.

Similarly, average pCO_2 of semi-continuous cultures of *Rhodomonas* sp. was found for each experiment by averaging three pCO_2 treatment replicate values that were calculated for each experimental day, then these daily average values were used to calculate a mean across the entire experiment (n= # of days [total measurements per treatment]: Expt. 1, n=5[15]; Expt. 2: n=17[51]; Expt. 3: n=16[48]; Expt. 4: n=17[51]) (Table 4, Figure 2). Average pCO_2 in semi-continuous *Rhodomonas* sp. culture was slightly lower than the pre-equilibrated media due to photosynthetic drawdown by the phytoplankton during experiments. The average percent decrease in pCO_2 between pre-equilibrated media and semi-continuous cultures of *Rhodomonas* sp. for the 4 experiments were 20.6%, 21.4% and 13.9% for ambient, moderate and high, respectively.

Average daily pCO_2 concentrations of experimental cultures of microzooplankton and *Rhodomonas* sp. was found for each LT grazing experiment by averaging the pCO_2

treatment replicate values (n=5) (that were calculated and included each day of the 5 day acclimating feeding period and the 24 h LT grazing experiment). Then these daily average values were used to calculate a mean across the entire experiment (n= # of days [total measurements per treatment: LT grazing *O. marina*, n=7[35]; LT grazing *Coxliella* sp., n=6[30]; LT grazing *G. dominans*, n=6[30]) (Table 5, Figure 3). During LT grazing experiments, the drawdown by photosynthesis of the phytoplankton was partially counteracted by respiration related to the microzooplankton. The average percent decrease in pCO_2 between pre-equilibrated media and cultures of mixed phytoplankton and microzooplankton for the three LT grazing experiments were ~ 10%, 12% and 8% for ambient, moderate and high, respectively.

Phytoplankton Characterization

Cell Bio-volume

A significant treatment effect of elevated pCO_2 on *Rhodomonas* sp. cell bio-volume was observed on days of ST ingestion rate experiments (Table 6 and 7, Figure 4). *Rhodomonas* sp. prey cells semi-continuously cultured during Expt 1 were used for ST ingestion rate experiments with both *G. dominans* and *F. ehrenbergii* (Table 1). For ST ingestion rate experiments with *G. dominans* (Expt. 1), *F. eherenbergii* (Expt. 1) and *Coxliella* sp. (Expt. 3), I found a significant stepwise increase in *Rhodomonas* sp. cell bio-volume with increasing pCO_2 (Tables 6 and 7). For *O. marina* ST ingestion rate experiment (Expt. 2), *Rhodomonas* sp. prey cells in high pCO_2 were significantly larger than

Table 4. Mean of calculated daily average pCO_2 concentrations (avg ppmv ± SD) of *Rhodomonas* sp. cultures in treatments ambient (A), moderate (M) and high (H) during semi-continuous experiments 1 n=5[15], 2 n=17[51], 3 n=16[48] and 4 n=17[51] (n= # of days [total measurements per treatment]. The average percent difference (%) between the pre-equilibrated media and semi-continuous *Rhodomonas* sp. cultures is on the right of pCO_2 values.

	Media	**Expt. 1**	**%**	**Expt. 2**	**%**	**Expt. 3**	**%**	**Expt. 4**	**%**
A	379 ± 16	310 ± 20	20.8	311 ± 39	19.7	290 ± 34	26.6	325 ± 33	15.3
M	733 ± 27	582 ± 16	23.0	579 ± 67	23.5	606 ± 49	19.0	599 ± 52	20.1
H	969 ± 43	874 ± 21	10.3	849 ± 72	13.2	820 ± 83	16.7	831 ± 83	15.3

Table 5. Mean of calculated daily average pCO_2 concentrations (avg ppmv ± SD) in microzooplankton and *Rhodomonas* sp. experimental cultures in pCO_2 treatments ambient (A), moderate (M) and high (H) during the acclimation period (5 days) and long term ingestion and growth rate experiments for *O. marina* n=7[35], *Coxliella* sp. n=6[30] and *G. dominans* n=6[30] (n= # of days [total measurements per treatment]). The average percent difference (%) between the pre-equilibrated media and microzooplankton and *Rhodomonas* sp. cultures is on the right side of all pCO_2 values in the table.

		Expt. 2		**Expt. 3**		**Expt. 4**	
	Media	***O. marina***	**%**	***Coxliella* sp.**	**%**	***G. dominans***	**%**
A	379 ± 16	338 ± 42	11.4	314 ± 47	18.8	377 ± 52	0.5
M	733 ± 27	670 ± 68	9.0	602 ± 51	19.6	683 ± 98	7.1
H	969 ± 43	928 ± 58	4.3	843 ± 74	13.9	910 ± 75	6.3

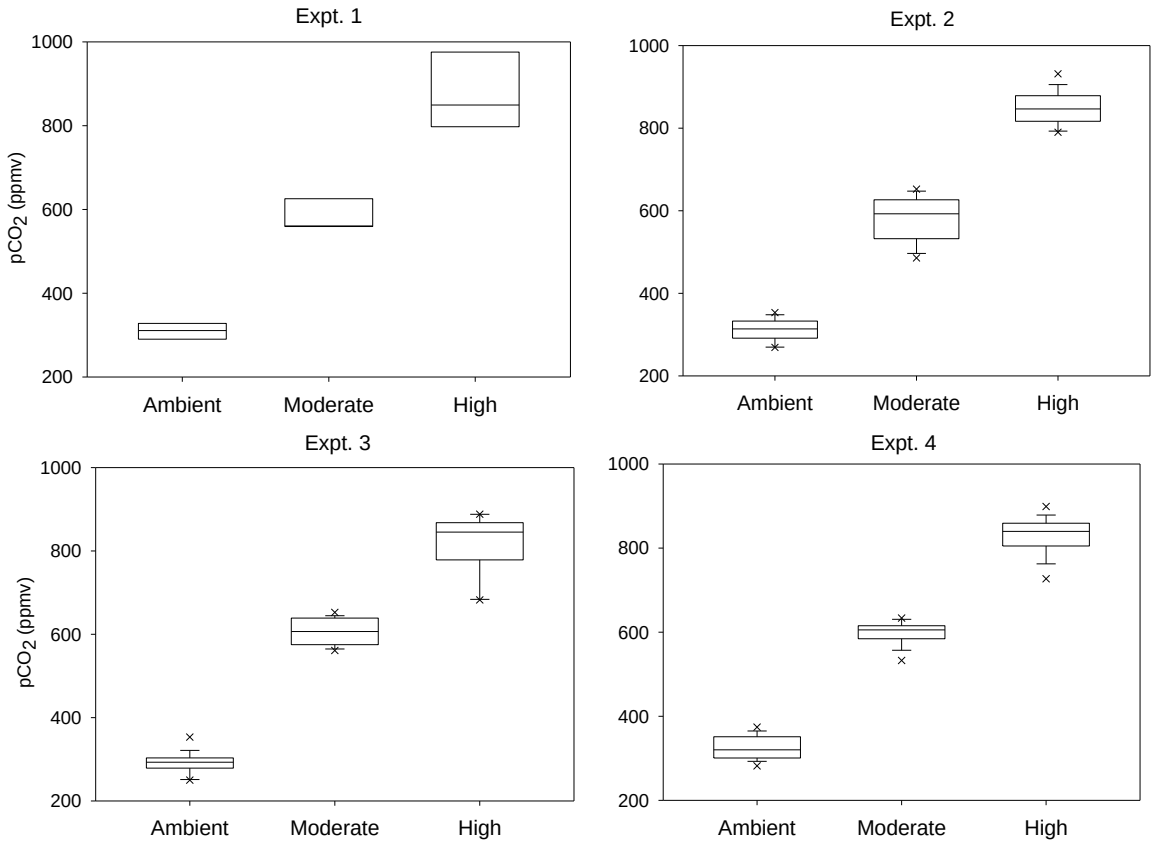


Figure 2.Average pCO_2 (ppmv) chemistry during semi-continuous culturing of *Rhodomonas* sp. in ambient, moderate and high pCO_2 treatments (n=# of days [total measurements per treatment]:Expt. 1 (n=5[15]), Expt. 2 (n=17[51]), Expt. 3 (n=16[48]), Expt. 4 (n=17[51]). The solid horizontal line represents the median and the top and bottom of the boxes represent the upper and lower quartile (25% of the data is greater or less than this value, respectively). The whiskers indicate the 90^{th} and 10^{th} percentiles. Outlying pCO_2 values are displayed with an X. Whiskers are not shown for Expt. 1 boxplot because n<10.

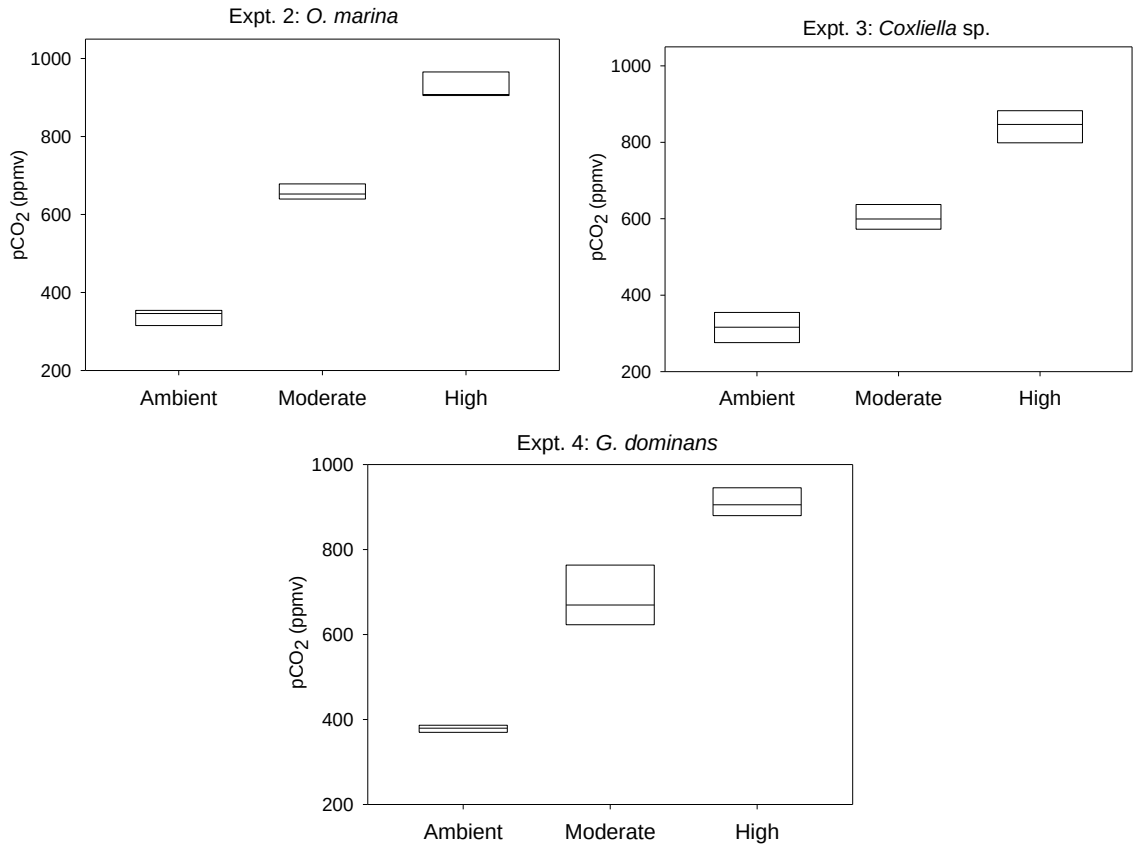


Figure 3. Average pCO_2 (ppmv) chemistry in experimental cultures of microzooplankton feeding on *Rhodomonas* sp. in ambient, moderate and high pCO_2 treatments for days 11-16/17 of experiments with the microzooplankton *O. marina* (n=# of days [total measurements per treatment]: (n=7[35]), *G. dominans* (n=6[30]) and *Coxliella* sp. (n=6[30]). The solid horizontal line represents the median and the top and bottom of the boxes represent the upper and lower quartile (25% of the data is greater or less than this value, respectively). Whiskers are not shown in the boxplots because n<10.

Table 6. *Rhodomonas* sp. physiological parameters (avg ±SD, n=3) measured from semi-continuous cultures in pCO_2 treatments ambient (A), moderate (M) and high (H) on day 10 of semi-continuous culturing during ST grazing experiments *G. dominans* and *F. ehrenbergii* (Expt. 1), *O. marina* (Expt. 2), *Coxliella* sp. (Expt. 3). No ST grazing experiment was done during Expt. 4. The symbol * indicates a ln transformation of data was done in order to meet homogeneity assumptions. Bold values indicate a significant pCO_2 treatment effect (ANOVA) and letters (a, b, c) denote treatments that are significantly different from each other (Tukey's post hoc).

Parameter			**Expt. 1** *G. dominans* & *F. ehrenbergii*	**Expt. 2** *O. marina*	**Expt. 3** *Coxliella* sp.	**Expt. 4** N/A
Biovolume (μm^3)		A	**137.31 ± 11.46$_A$**	**144.58 ± 10.29*$_A$**	**134.30± 14.15$_A$**	**154.46 ± 36.70$_A$**
		M	**170.88 ± 6.82$_B$**	**211.37 ± 16.77*$_B$**	**256.92 ± 8.84$_B$**	**256.41 ± 26.08$_B$**
		H	**219.42 ± 5.67$_C$**	**260.58 ± 72.62*$_B$**	**314.18 ± 12.24$_C$**	**317.46 ± 27.07$_B$**
POC	pg C $cell^{-1}$	A	46.89 ± 2.21	44.53 ± 1.22	51.34 ± 9.80	51.94 ± 2.48
		M	46.07 ± 1.70	46.92 ± 5.10	53.91 ± 3.20	51.65 ± 3.88
		H	47.21 ± 1.46	47.45 ± 2.03	54.97 ± 5.30	56.94 ± 2.91
	pg C μm^{-3}	A	**0.34 ± 0.04$_A$**	**0.27 ± 0.02$_A$**	**0.39 ± 0.10$_A$**	**0.35 ± 0.10$_A$**
		M	**0.27 ± 0.01$_B$**	**0.19 ± 0.02$_B$**	**0.21 ± 0.02$_B$**	**0.20 ± 0.01$_B$**
		H	**0.22 ± 0.01$_C$**	**0.16 ± 0.02$_B$**	**0.18 ± 0.02$_B$**	**0.18 ± 0.02$_B$**
POC:PON Elemental ratio		A	8.58 ± 0.13	5.17 ± 0.30	5.48 ± 0.25	**3.36 ± 0.06$_A$**
		M	8.77 ± 0.82	5.21 ± 0.42	4.89 ± 0.36	**4.34 ± 0.64$_{AB}$**
		H	8.78 ± 0.54	5.16 ± 0.48	5.21 ± 0.09	**4.89 ± 0.79$_B$**
Growth rate (d^{-1})		A	**0.53 ± 0.02$_{AB}$**	0.54 ± 0.03	0.44 ± 0.16	0.54 ± 0.07
		M	**0.58 ± 0.02$_A$**	0.53 ± 0.05	0.54 ± 0.06	0.53 ± 0.03
		H	**0.50 ± 0.02$_B$**	0.56 ± 0.10	0.58 ± 0.06	0.53 ± 0.02
Chlorophyll *a*	pg $cell^{-1}$	A	1.30 ± 0.10	**0.97 ± 0.04$_A$**	**1.16 ± 0.11$_A$**	0.40 ± 0.12
		M	1.25 ± 0.15	**0.73 ± 0.03$_B$**	**0.92 ± 0.02$_B$**	0.51 ± 0.01
		H	1.17 ± 0.30	**0.69 ± 0.08$_B$**	**0.87 ± 0.02$_B$**	0.50 ± 0.04
	pg μm^{-3}	A	0.0095 ± 0.0013	**0.0060 ± 0.0005$_A$**	**0.0086 ± 0.0005$_A$**	0.0003 ± 0.0010
		M	0.0074 ± 0.0012	**0.0035 ± 0.0002$_B$**	**0.0036 ± 0.0001$_B$**	0.0020 ± 0.0001
		H	0.0053 ± 0.0013	**0.0027 ± 0.0004$_C$**	**0.0028 ± 0.0001$_B$**	0.0020 ± 0.0003
	pg chl a pg C^{-1}	A	0.028 ± 0.001	**0.022 ± 0.001$_A$**	0.024 ± 0.009	0.008 ± 0.004
		M	0.027 ± 0.004	**0.016 ± 0.001$_B$**	0.017 ± 0.001	0.010 ± 0.001
		H	0.025 ± 0.006	**0.015 ± 0.002$_B$**	0.016 ± 0.002	0.010 ± 0.001

Table 6. (continued)

Carbohydrates	pg fructose $cell^{-1}$	A	2.24 ± 0.71	25.05 ± 10.39	11.96 ± N/A	18.49 ± 3.94
		M	2.17 ± 0.77	24.73 ± 7.61	17.84 ± 9.56	22.51 ± 0.06
		H	2.37 ± 0.42	24.22 ± N/A	9.84 ± 4.70	23.95 ± 2.92
	pg fructose μm^{-3}	A	0.017 ± 0.006			0.130 ± 0.060
		M	0.014 ± 0.003			0.090 ± 0.009
		H	0.010 ± 0.001			0.080 ± 0.010
	pg fructose pg C^{-1}	A	0.05 ± 0.13			0.36 ± 0.08
		M	0.05 ± 0.11			0.44 ± 0.05
		H	0.05 ± 0.01			0.42 ± 0.04

Table 7. Analysis of Variance (ANOVA) and Tukey's post-hoc test results for *Rhodomonas* sp. physiological parameters which were found to be statistically significant on day 10 of semi-continuous culturing during ST grazing experiments *G. dominans* and *F. ehrenbergii* (Expt. 1), *O. marina* (Expt. 2), *Coxliella* sp. (Expt. 3).

			ANOVA		Tukey's post-hoc p-values		
Parameter		**Expt.**	**F value**	**P value**	**A-M**	**A-H**	**M-H**
Bio-volume (μm^3)		1	73.686	<0.0001	0.006	<0.0001	0.001
		2	9.295	0.015		0.013	
		3	177.218	<0.0001	<0.0001	<0.0001	0.003
		4	22.105	0.002	0.015	0.001	
POC (μm^{-3})		1	20.231	0.002	0.0250	0.001	0.025
		2	21.835	0.002	0.007	0.002	
		3	19.291	0.002	0.007	0.003	
POC:PON elemental ratio		4	5.185	0.049		0.044	
Growth rate (d^{-1})		1	10.708	0.01			0.009
Chlorophyll *a*	(pg $cell^{-1}$)	2	14.804	0.008	0.016	0.008	
		3	6.015	0.037		0.040	
	(pg μm^{-3})	2	146.424	<0.0001	<0.0001	<0.0001	0.007
		3	47.508	<0.0001	0.001	<0.0001	
	(pg chl a pg C^{-1})	2	16.819	0.006	0.012	0.006	

moderate and ambient pCO_2 treatment prey (Table 7). Average cell bio-volume across all ST grazing experiments was 142.7 ± 9.0, 223.8 ± 42 and 277.9 ± 47 μm^3 for ambient, moderate and high pCO_2, respectively. On average across all ST experiments, *Rhodomonas* sp. cells were 57 and 95% larger when cultured in moderate and high pCO_2 than in ambient pCO_2, respectively. While the size of ambient pCO_2 *Rhodomonas* sp. remained similar across experiments, the size of the treatment effect for moderate and high pCO_2 *Rhodomonas* sp. varied by experiment (Table 6).

Similarly, a significant treatment effect of elevated pCO_2 on *Rhodomonas* sp. cell bio-volume was observed on days of LT ingestion rate experiments (Table 8 and 9). Tukey's Post Hoc comparison revealed cell bio-volume of *Rhodomonas* sp. prey used for LT grazing experiments to be significantly larger in moderate and high pCO_2 treatments than ambient, except during LT grazing experiment with *O. marina* (Expt. 2) (Table 9). For *O. marina,* a significant stepwise increase in *Rhodomonas* sp. prey cell bio-volume was found with increasing pCO_2 (Tables 8 and 9). Average cell bio-volume of *Rhodomonas* sp. across all LT grazing experiments was 145.7 ± 12, 231.4 ± 15 and 289.7 ± 16 (μm^3) for ambient, moderate and high pCO_2, respectively. On average across LT grazing experiments, *Rhodomonas* sp. cells were 59 and 99% larger in moderate and high pCO_2 than in ambient pCO_2, respectively.

Cellular C and N content

Rhodomonas sp. POC and PON were measured on the days after microzooplankton grazing experiments (Day 10 and 16 or 17). In general, *Rhodomonas* sp. cultured under high pCO_2 had more particulate organic carbon (POC) $cell^{-1}$ than *Rhodomonas* sp. cultured under

Table 8. *Rhodomonas* sp. physiological parameters (avg ±SD, n=3) measured from semi-continuous cultures in pCO_2 treatments ambient (A), moderate (M) and high (H) on the last day of semi-continuous culturing during LT grazing experiments *O. marina* (Expt. 2), *Coxliella* sp. (Expt. 3) and *G. dominans* (Expt. 4). Bold values indicate a significant pCO_2 treatment effect (ANOVA) and letters (a, b, c) denote treatments which are significantly different from each other (Tukey's post hoc test).

Parameter			Expt. 2 *O. marina*	Expt. 3 *Coxliella* sp.	Expt. 4 *G. dominans*
Biovolume (μm^3)		A	**158.56 ± 18.33$_A$**	**143.3± 8.3$_A$**	**135.13 ± 5.03*$_A$**
		M	**215.68 ± 10.73$_B$**	**234.5 ± 18.6$_B$**	**244.46 ± 17.37*$_B$**
		H	**305.32 ± 18.33$_C$**	**291.7 ± 37.5$_B$**	**272.74 ± 39.51*$_B$**
POC	pg C $cell^{-1}$	A	64.92 ± 1.13	**51.91 ± 3.85$_A$**	61.91 ± 0.56
		M	63.33 ± 4.82	**62.27 ± 2.34$_B$**	65.66 ± 7.74
		H	69.80 ± 4.22	**59.06 ± 0.10$_B$**	75.88 ± 2.51
	pg C μm^{-3}	A	0.34 ± 0.20	**0.36 ± 0.03$_A$**	**0.46 ± 0.01$_A$**
		M	0.29 ± 0.04	**0.27 ± 0.01$_B$**	**0.27 ± 0.03$_B$**
		H	0.23 ± 0.02	**0.20 ± 0.01$_C$**	**0.22 ± 0.09$_B$**
POC:PON Elemental ratio		A	7.09 ± 0.79	7.60 ± 0.24	3.24 ± 0.84
		M	6.63 ± 0.48	8.43 ± 1.28	3.59 ± 1.43
		H	5.93 ± 0.41	8.09 ± 2.26	3.18 ± 0.87
Growth rate (d^{-1})		A	**0.51 ± 0.04$_A$**	0.49 ± 0.01	0.41 ± 0.07
		M	**0.64 ± 0.05$_B$**	0.69 ± 0.34	0.38 ± 0.24
		H	**0.61 ± 0.03$_B$**	0.69 ± 0.25	0.43 ± 0.10
Chlorophyll *a*	pg $cell^{-1}$	A	**1.38 ± 0.22$_A$**	**0.55 ± 0.01$_A$**	1.21 ± 0.06
		M	**1.82 ± 0.24$_{AB}$**	**0.72 ± 0.04$_B$**	1.20 ± 0.07
		H	**2.06 ± 0.12$_B$**	**0.61 ± 0.03$_B$**	1.09 ± 0.10
	pg μm^{-3}	A	0.0090 ± 0.0010	**0.0039 ± 0.0001$_A$**	**0.0090 ± 0.0004$_A$**
		M	0.0080 ± 0.0007	**0.0031 ± 0.0003$_B$**	**0.0050 ± 0.0008$_B$**
		H	0.0070 ± 0.0004	**0.0021 ± 0.0002$_C$**	**0.0040 ± 0.0006$_B$**
	pg chl a pg C^{-1}	A	0.033 ± 0.020	0.012 ± 0.001	0.020 ± 0.002
		M	0.029 ± 0.005	0.012 ± 0.001	0.018 ± 0.003
		H	0.030 ± 0.001	0.010 ± 0.001	0.014 ± 0.003
Carbohydrates	Pg fructose $cell^{-1}$	A	51.68 ± 2.24	4.45 ± 3.66	
		M	27.83 ± N/A	6.27 ± 4.37	
		H	63.50 ± 5.81	9.84 ± 6.13	
	pg fructose μm^{-3}	A		0.030 ± 0.026	
		M		0.030 ± 0.016	
		H		0.030 ± 0.015	
	pg fructose pg C^{-1}	A		0.08 ± 0.07	
		M		0.10 ± 0.07	
		H		0.17 ± 0.10	

Table 9. Analysis of Variance (ANOVA) and Tukey's post-hoc statistical test results for *Rhodomonas* sp. physiological parameters which were found to be statistically significant on the last day of semi-continuous culturing during LT grazing experiments with *O. marina* (Expt. 2), *Coxliella* sp. (Expt. 3) and *G. dominans* (Expt. 4).

			ANOVA		Tukey's post-hoc p-values		
Parameter		**Expt.**	**F value**	**P value**	**A-M**	**A-H**	**M-H**
Bio-volume μm^3		2	46.978	<0.0001	0.033	<0.0001	0.003
		3	27.694	0.001	0.009	0.001	---------
		4	47.994	<0.0001	0.001	<0.0001	
POC	pg C $cell^{-1}$	3	11.907	0.008	0.007	0.038	
	μm^{-3}	3	69.953	<0.0001	0.001	<0.0001	0.008
		4	76.263	<0.0001	0.001	<0.0001	
Total lipids pg $cell^{-1}$		4	8.393	0.018		0.017	
Growth rate d^{-1}		2	9.082	0.015	0.015	0.049	
Chlorophyll *a*	pg $cell^{-1}$	2	8.912	0.016		0.014	
		3	9.113	0.015	0.013		
	pg μm^{-3}	3	52.393	<0.0001	0.009	<0.0001	0.003
		4	48.320	<0.0001	0.001	<0.0001	

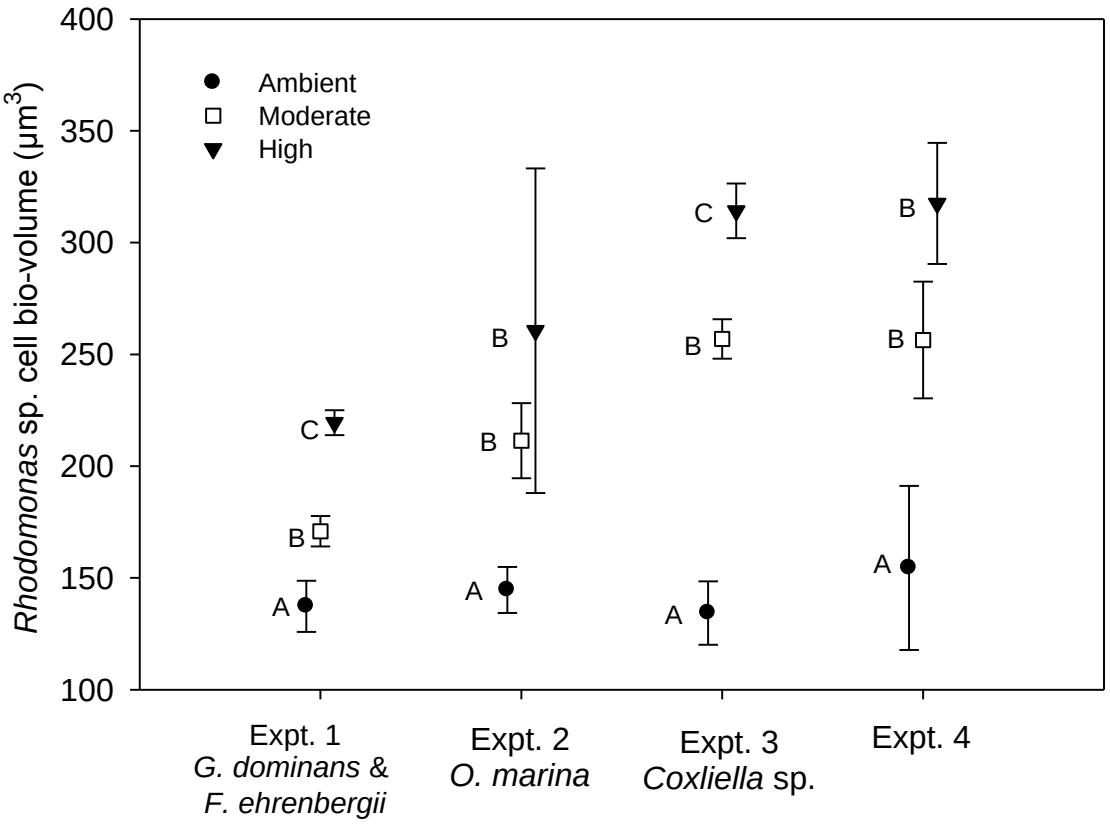


Figure 4. *Rhodomonas* sp. cell bio-volume (average ±SD, n=3) for pCO_2 treatments ambient, moderate and high on day 10 of semi-continuous culturing during ST grazing experiments with *G. dominans* and *F. ehrenbergii* (Expt. 1), *O. marina* (Expt. 2) and *Coxliella* sp. (Expt. 3). No ST grazing experiment was done during Expt. 4. Letters next to the data points represent treatments that were significantly different within a given experiment (Tukey's post hoc analysis). Refer to Table 6 for data.

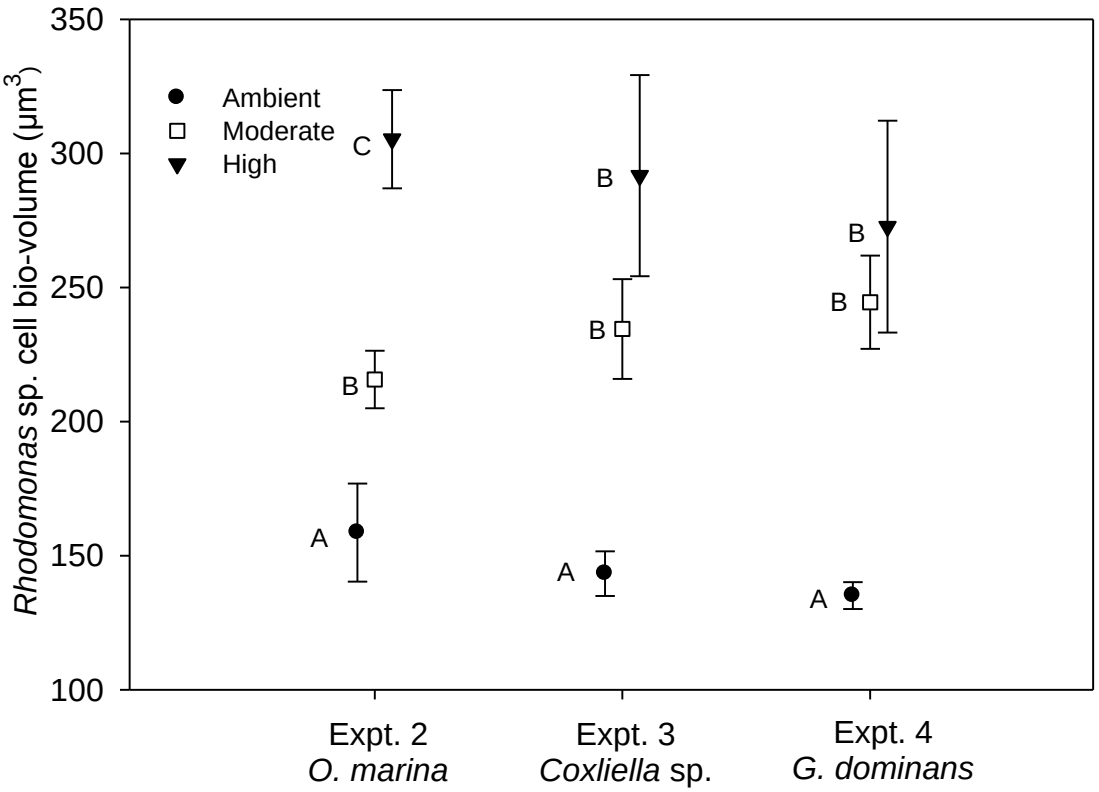


Figure 5. *Rhodomonas* sp. cell bio-volume (average ±SD, n=3) for pCO_2 treatments ambient, moderate and high on the last day of semi-continuous culturing during LT grazing experiments with *O. marina* (Expt. 2), *Coxliella* sp. (Expt. 3) and *G. dominans* (Expt. 4). Letters next to the data points represent treatments that were significantly different (Tukey's post hoc analysis). Refer to table 8 for data.

ambient and moderate pCO_2 (Table 6 and 8, figures 6 and 7). Though this trend was consistently present, it was also not significant in most cases, with the exception of prey used during the LT grazing experiment with *Coxliella* sp. (Expt. 3) (Table 9, Figure 7). In that case, moderate and high pCO_2 *Rhodomonas* sp. prey cells had significantly more POC $cell^{-1}$ than ambient (Table 9).

Rhodomonas sp. cellular carbon density (POC μm^{-3}) decreased with increasing pCO_2 (Table 6 and 8, Figures 8 and 9). The significant increase in cell bio-volume found in elevated pCO_2 treatments did not coincide with a similar increase in POC $cell^{-1}$. As a result, there was significantly less POC μm^{-3} in cells of *Rhodomonas* sp. in moderate and high pCO_2 treatments compared to ambient pCO_2 treatment. For *G. dominans* and *F. ehrenbergii* ST grazing experiments (Expt. 1) a stepwise decrease in cellular POC μm^{-3} occurred in ambient, moderate and high pCO_2 *Rhodomonas* sp. prey cells (Table 7). Similarly, for *Coxliella* sp. LT grazing experiments (Expt. 3) a stepwise decrease in cellular POC μm^{-3} occurred in ambient, moderate and high pCO_2 *Rhodomonas* sp. prey cells (Table 9). For *O. marina* (Expt. 2) and *Coxliella* sp. (Expt. 3) ST grazing experiments, moderate and high pCO_2 *Rhodomonas* sp. prey cells had significantly less cellular POC μm^{-3} than ambient (Table 7). This was also true for *G. dominans* (Expt. 4) LT grazing experiment, moderate and high pCO_2 *Rhodomonas* sp. prey cells had significantly less cellular POC μm^{-3} than ambient (Table 9).

Rhodomonas sp. POC: PON elemental ratio varied greatly for all pCO_2 treatments depending on the semi-continuous experiment and day of measurement (Table 6 and 8, Figures 10 and 11). Variability in POC: PON was mainly driven by varying cellular particulate

organic nitrogen values of *Rhodomonas* sp. (Figures 12 and 13). No consistent trend in POC: PON of *Rhodomonas* sp. was observed with increasing pCO_2.

Total Lipids

Rhodomonas sp. cellular total lipid content was measured on the days of *Coxliella* sp. (Expt. 3) and *G. dominans* (Expt. 4) LT grazing experiments. For the LT grazing experiment with the microzooplankton *G. dominans* (Expt. 4), significantly more cellular total lipids were found in high pCO_2 *Rhodomonas* sp. prey cells than ambient pCO_2 *Rhodomonas* sp. prey cells (Tables 9 and 10; Figure 14). This pattern of high pCO_2 *Rhodomonas* sp. having more cellular total lipids was present as well for *Rhodomonas* sp. prey used in LT grazing experiment with *Coxliella* sp. (Expt. 3), but not significant. The consistent increase in total lipids $cell^{-1}$ of high and moderate pCO_2 *Rhodomonas* sp. cells scaled with the significant increase in cell bio-volume with increasing pCO_2 and no significant difference in *Rhodomonas* sp. total lipids density was found across pCO_2 treatments (Table 10, Figure 15). In addition no significant difference in *Rhodomonas* sp. cellular total lipids per picogram carbon was found with increasing pCO_2 (Table 10, Figure 16).

Growth Rate

Growth rate of *Rhodomonas* sp. was measured daily during semi-continuous experiments (Table A1, Figures A1, A2, A3 and A4). In four experiments, average growth rates (average ± SD) varied between 0.38 ± 0.24 and 0.69 ± 0.3 (d^{-1}) across all pCO_2 treatments (Table 6 and 8). For ST grazing experiments with *G. dominans* and *F. ehrenbergii*

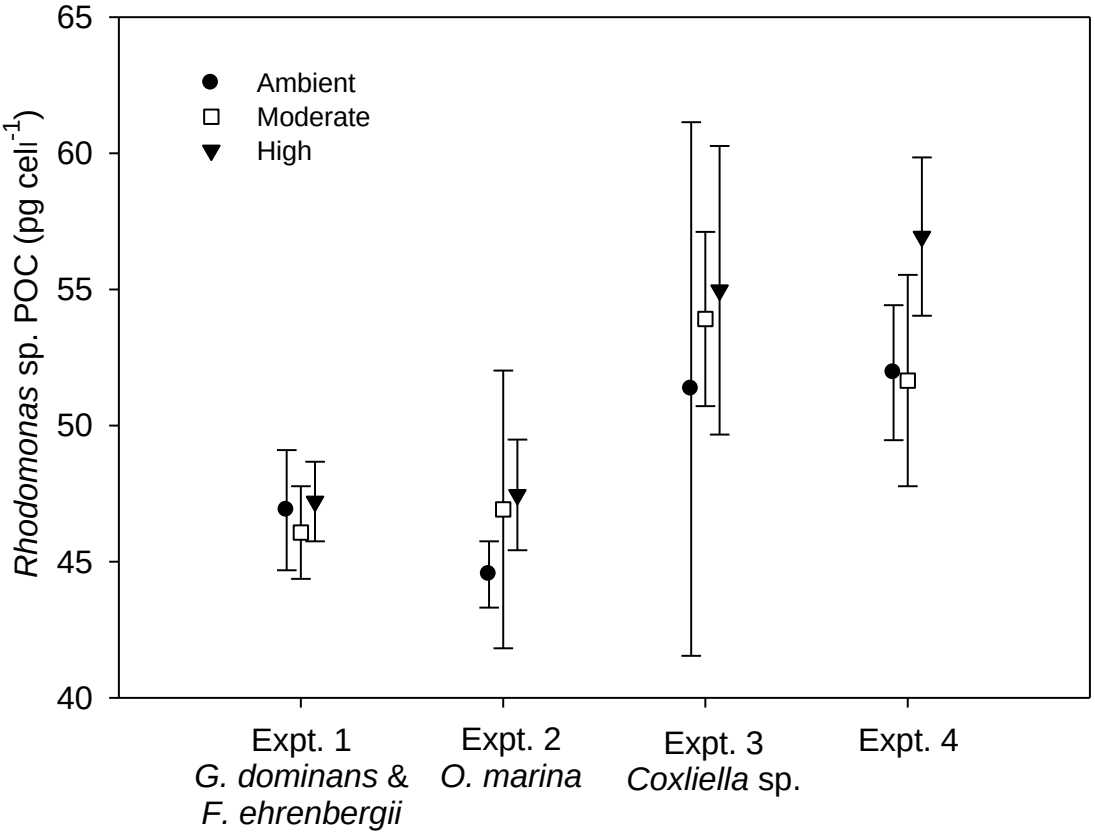


Figure 6. *Rhodomonas* sp. cellular particulate organic carbon (pg cell^{-1}) (average ± SD, n=3) for pCO_2 treatments ambient, moderate and high on day 10 of semi-continuous culturing during ST grazing experiments with *G. dominans* and *F. ehrenbergii* (Expt. 1), *O. marina* (Expt. 2) and *Coxliella* sp. (Expt. 3). No ST grazing experiment was done during Expt. 4. Refer to Table 6 for data.

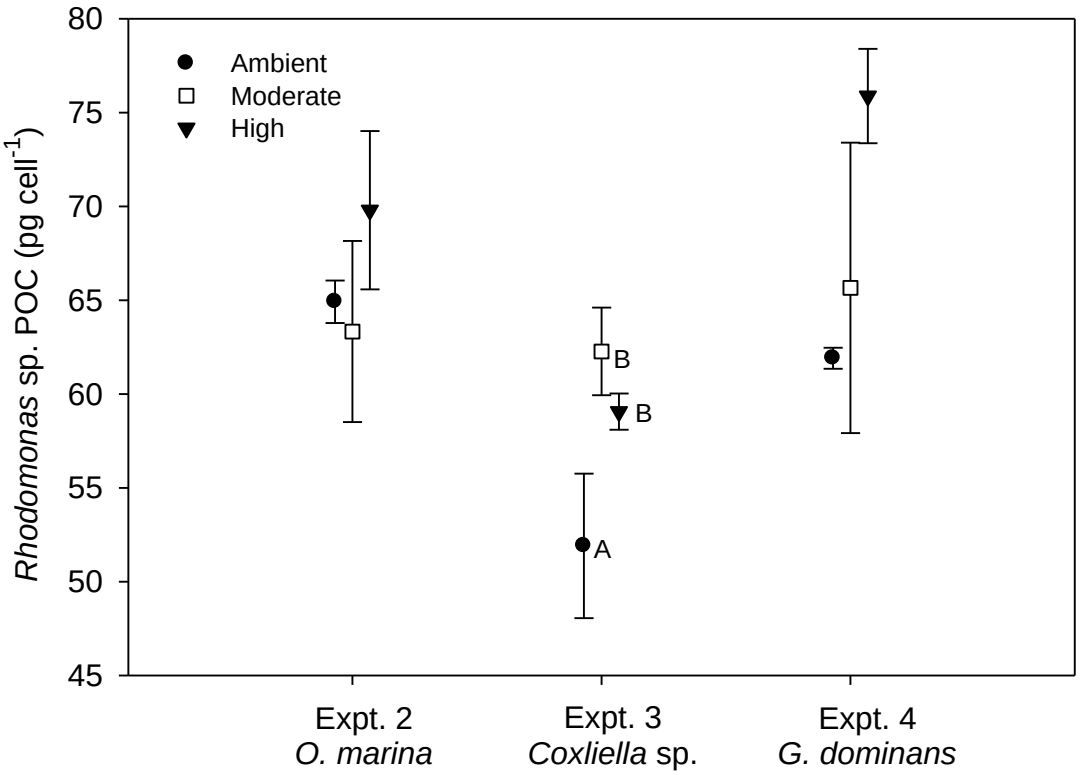


Figure 7. *Rhodomonas* sp. cellular particulate organic carbon (pg cell^{-1}) (average ± SD, n=3) for pCO_2 treatments ambient, moderate and high on the last day of semi-continuous culturing during LT grazing experiments with *O. marina* (Expt. 2), *Coxliella* sp. (Expt. 3) and *G. dominans* (Expt. 4). Letters next to the data points represent treatments that were significantly different (Tukey's post hoc analysis). Refer to Tables 8 and 9 for data.

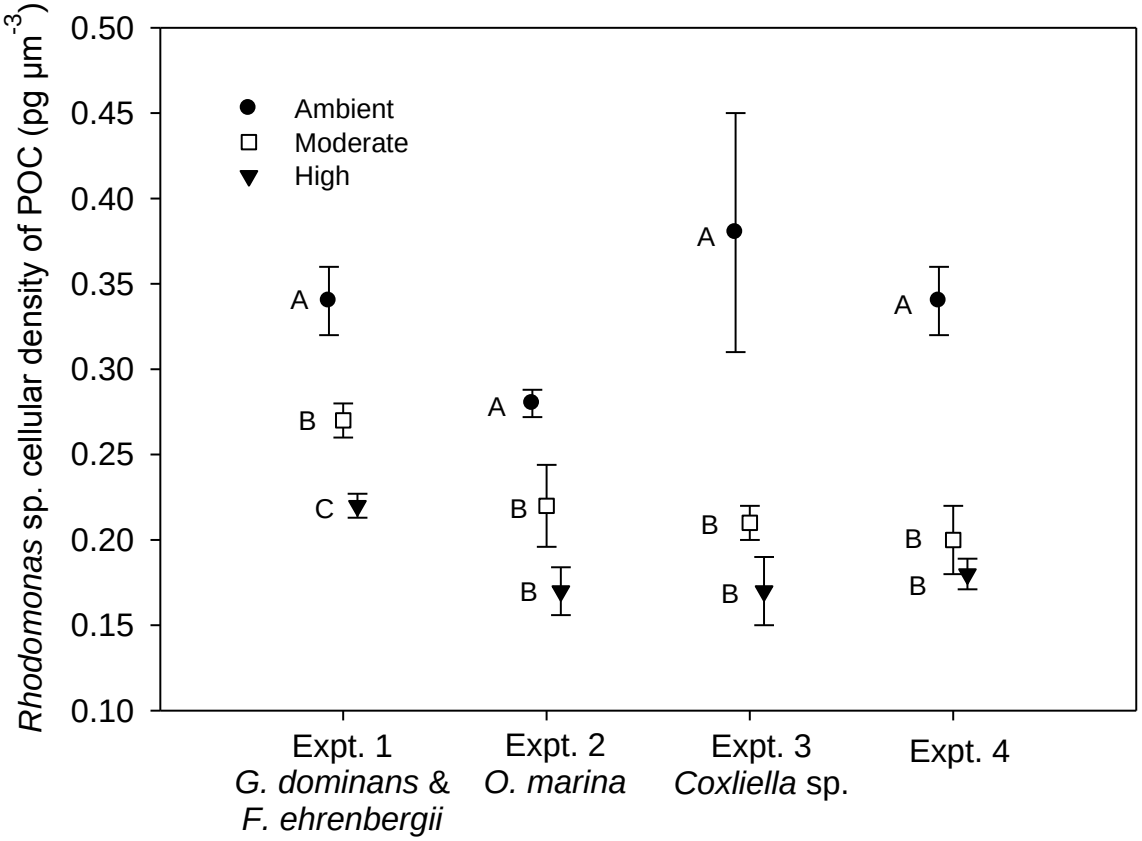


Figure 8. *Rhodomonas* sp. cellular particulate organic carbon density (POC μm^{-3}) (average ± SD, n=3) for pCO_2 treatments ambient, moderate and high on day 10 of semi-continuous culturing during ST grazing experiments with *G. dominans* and *F. ehrenbergii* (Expt. 1), *O. marina* (Expt. 2) and *Coxliella* sp. (Expt. 3). No ST grazing experiment was done during Expt. 4. Letters next to the data points represent treatments that were significantly different (Tukey's post hoc analysis). Refer to Tables 6 and 7 for data.

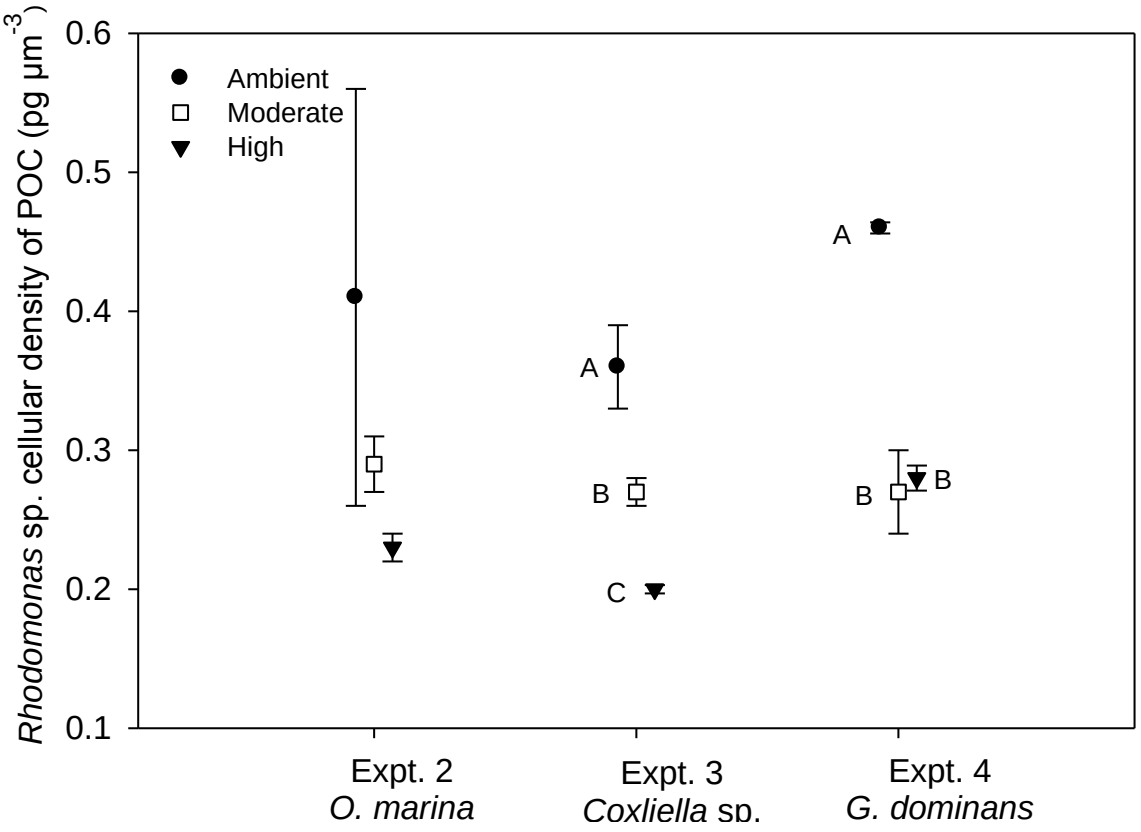


Figure 9. *Rhodomonas* sp. cellular particulate organic carbon density (POC μm^{-3}) (average ± SD, n=3) for pCO_2 treatments ambient, moderate and high on the last day of semi-continuous culturing during LT grazing experiments with *O. marina* (Expt. 2), *Coxliella* sp. (Expt. 3) and *G. dominans* (Expt. 4). Letters next to the data points represent treatments that were significantly different (Tukey's post hoc analysis). Refer to Tables 8 and 9 for data.

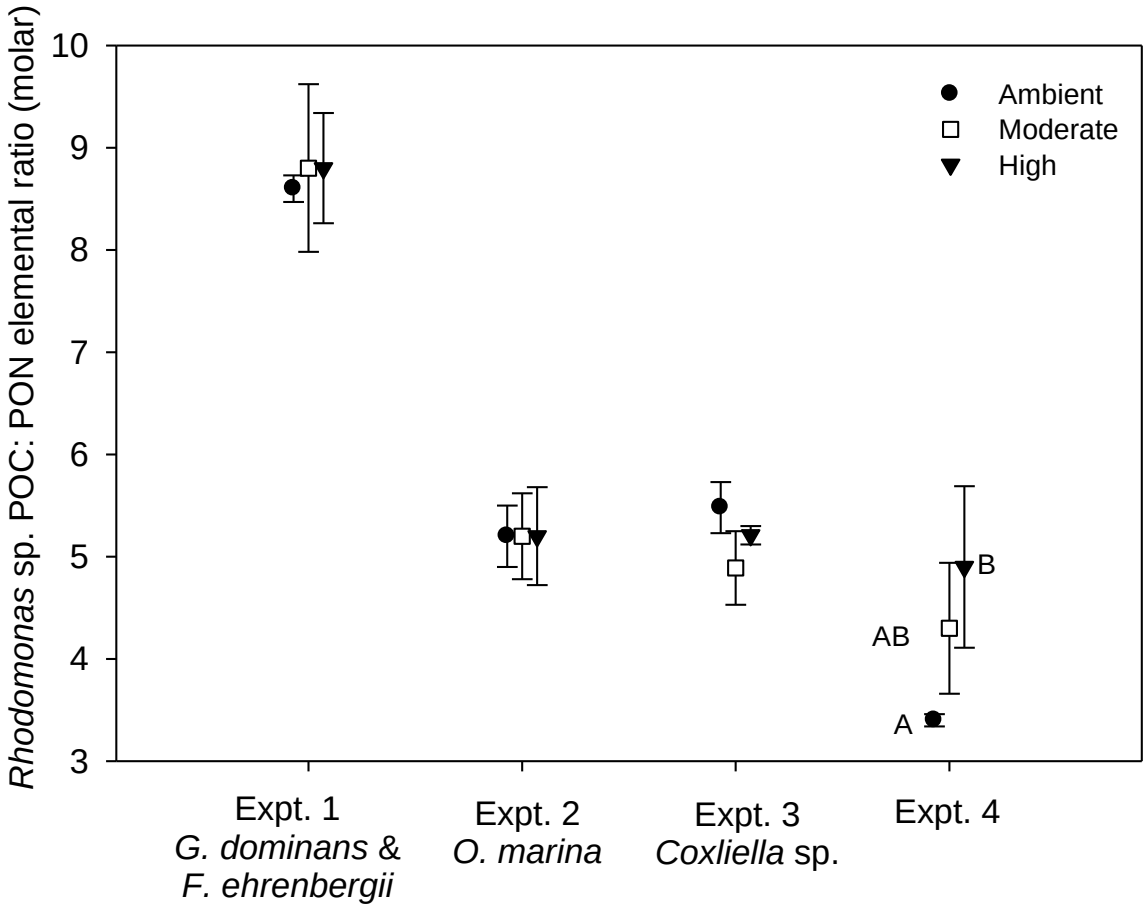


Figure 10. *Rhodomonas* sp. cellular particulate organic carbon:particulate organic nitrogen (POC:PON) (average ± SD, n=3) for pCO_2 treatments ambient, moderate and high on day 10 of semi-continuous culturing during ST grazing experiments with *G. dominans* and *F. ehrenbergii* (Expt. 1), *O. marina* (Expt. 2) and *Coxliella* sp. (Expt. 3). No short term grazing experiment was done during experiment 4. Letters next to the data points represent treatments that were significantly different (Tukey's post hoc analysis). Refer to Tables 6 and 7 for data.

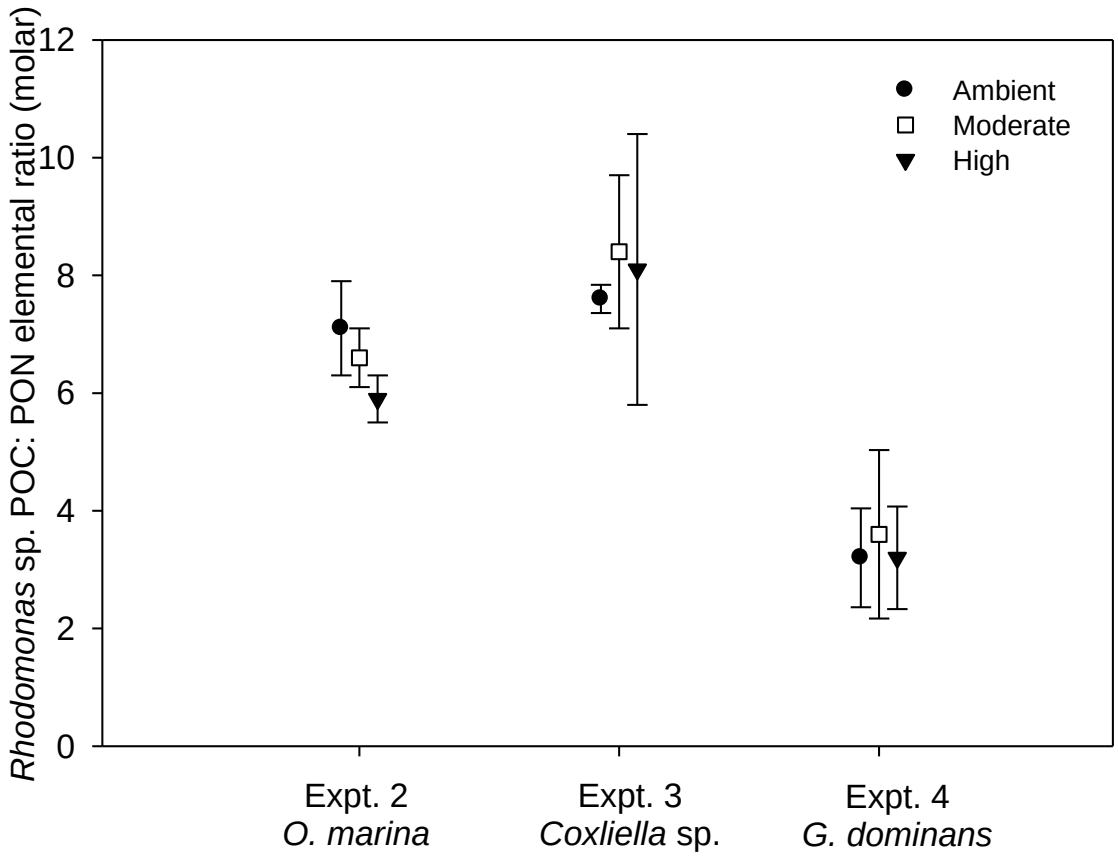


Figure 11. *Rhodomonas* sp. cellular particulate organic carbon:particulate organic nitrogen (POC:PON) average ± SD, n=3) for pCO_2 treatments ambient, moderate and high on the last day of semi-continuous culturing during LT grazing experiments with *O. marina* (Expt. 2), *Coxliella* sp. (Expt. 3) and *G. dominans* (Expt. 4). Refer to Tables 8 and 9 for data.

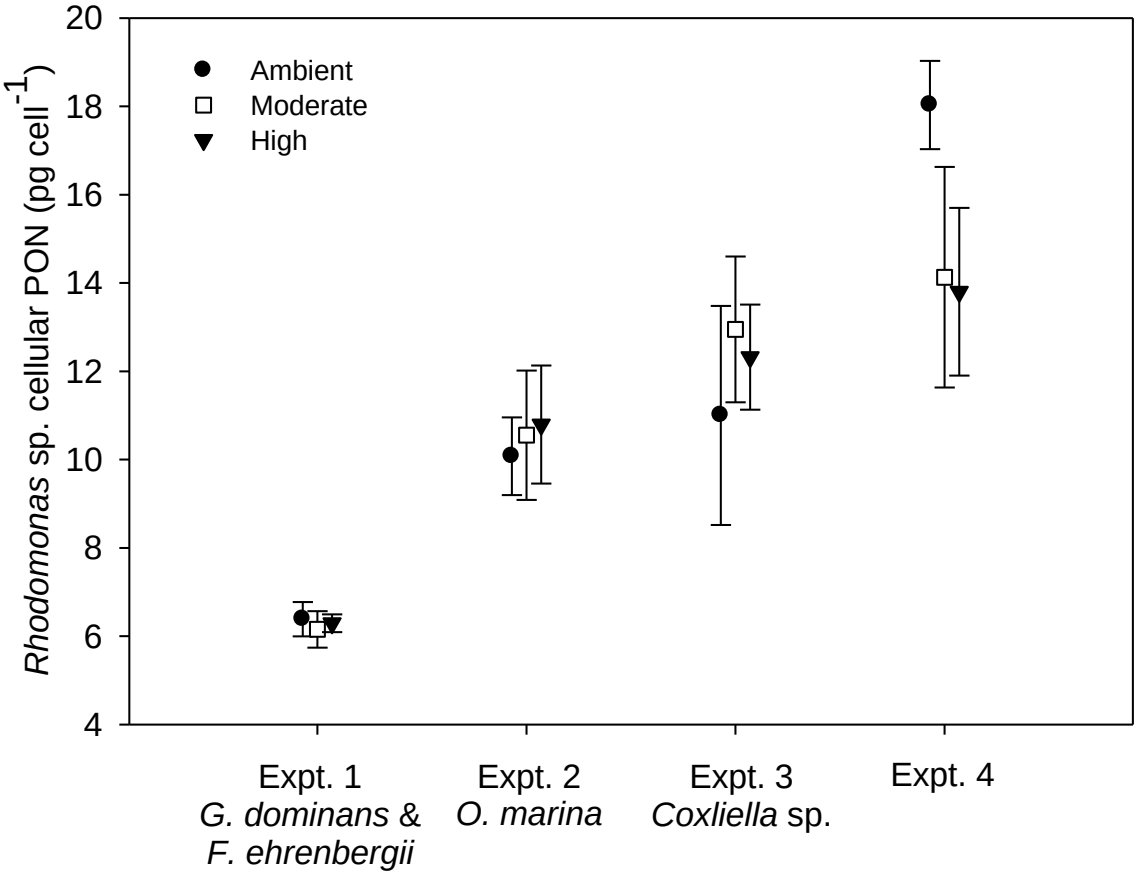


Figure 12. Cellular particulate organic nitrogen (pg N $cell^{-1}$) (average ± SD, n=3) for pCO_2 treatments ambient, moderate and high on day 10 of semi-continuous culturing during ST grazing experiments with *G. dominans* and *F. ehrenbergii* (Expt. 1), *O. marina* (Expt. 2) and *Coxliella* sp. (Expt. 3). No short term grazing experiment was done during experiment 4. Refer to Table 6 for data.

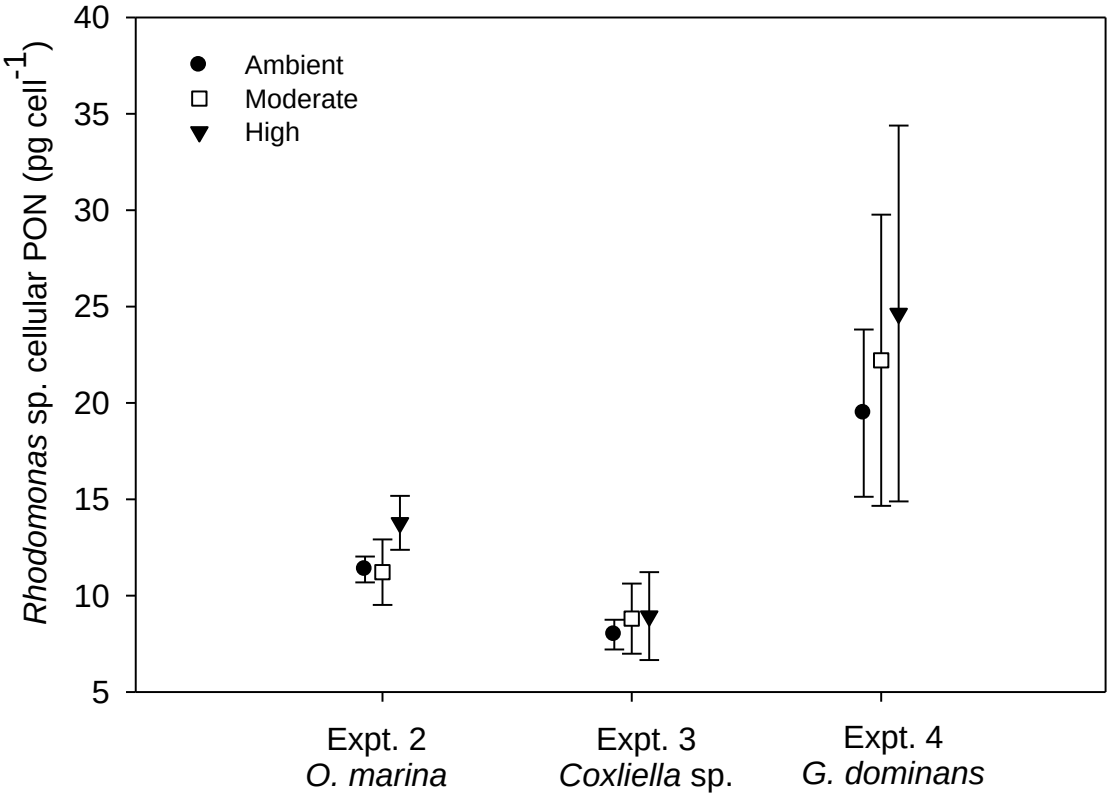


Figure 13. Cellular particulate organic nitrogen (pg N $cell^{-1}$) (average ± SD, n=3) for pCO_2 treatments ambient, moderate and high on the last day of semi-continuous culturing during LT grazing experiments with *O. marina* (Expt. 2), *Coxliella* sp. (Expt. 3) and *G. dominans* (Expt. 4). Refer to Table 8 for data.

Table 10. *Rhodomonas* sp. total lipids (pg cell^{-1}, pg lipids µm^{-3}, pg lipids pg C^{-1}) (avg ±SD, n=3) measured from semi-continuous cultures in pCO_2 treatments ambient (A), moderate (M) and high (H) on the last day of semi-continuous culturing during LT grazing experiments *O. marina* (Expt. 2), *Coxliella* sp. (Expt. 3) and *G. dominans* (Expt. 4). Bold values indicate a significant pCO_2 treatment effect (ANOVA) and letters (A, B, C) denote treatments which are significantly different from each other (Tukey's post hoc test).

Parameter			Expt. 3 *Coxliella* sp.	Expt. 4 *G. dominans*
Total lipids	pg cell^{-1}	A	13.9 4 ± 2.86	**14.65 ± 1.89*$_A$**
		M	19.03 ± 3.25	**19.98 ± 1.43*$_{AB}$**
		H	19.90 ± 0.76	**22.21 ± 3.26*$_B$**
	pg µm^{-3}	A	0.097 ± 0.020	0.095 ± 0.015
		M	0.080 ± 0.007	0.084 ± 0.005
		H	0.069 ± 0.010	0.074 ± 0.010
	pg lipids pg C^{-1}	A	0.27 ± 0.06	0.24 ± 0.03
		M	0.31 ± 0.06	0.31 ± 0.03
		H	0.32 ± 0.02	0.29 ± 0.05

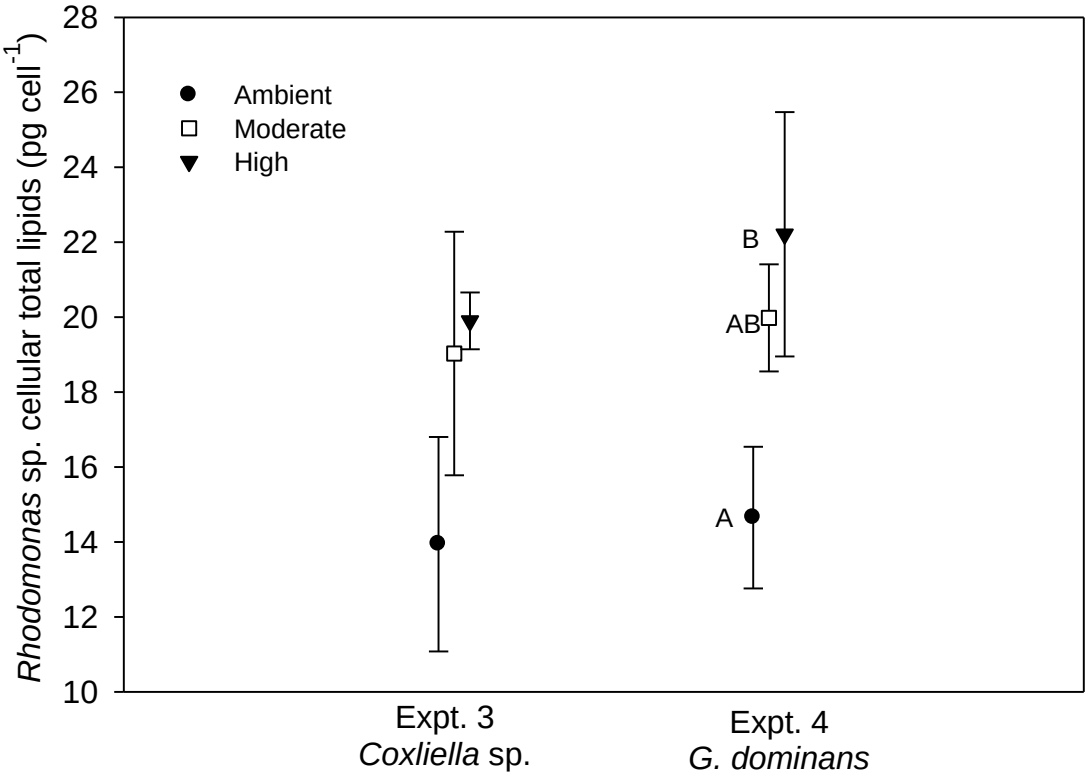


Figure 14. *Rhodomonas* sp. cellular total lipids (pg cell^{-1}) (average ±SD, n=3) for pCO_2 treatments ambient, moderate and high on the last day of semi-continuous culturing during LT grazing experiments with *Coxliella* sp. (Expt. 3) and *G. dominans* (Expt. 4). Letters next to the data points represent treatments that were significantly different (Tukey's post hoc analysis). Refer to Tables 10 and 11 for data.

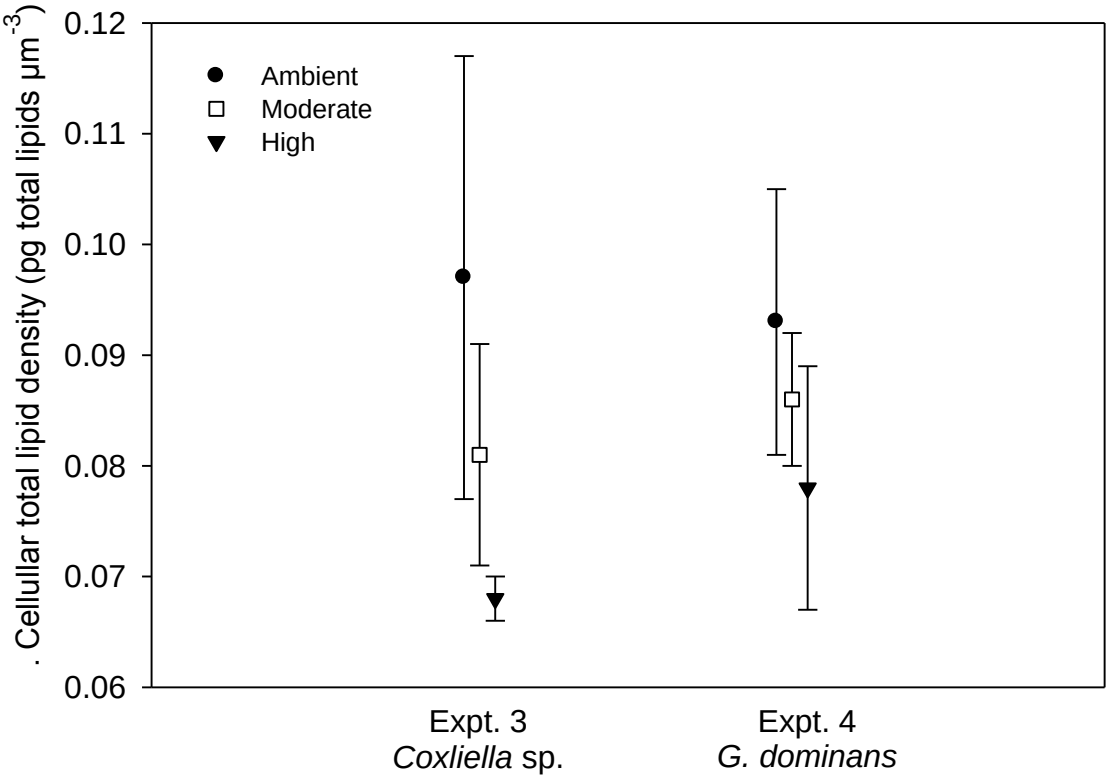


Figure 15. Rhodomonas sp. cellular total lipid density (pg μm^{-3}) (average ±SD, n=3) for pCO_2 treatments ambient, moderate and high on the last day of semi-continuous culturing during LT grazing experiments with Coxliella sp. (Expt. 3) and G. dominans (Expt. 4). Refer to Table 10 for data.

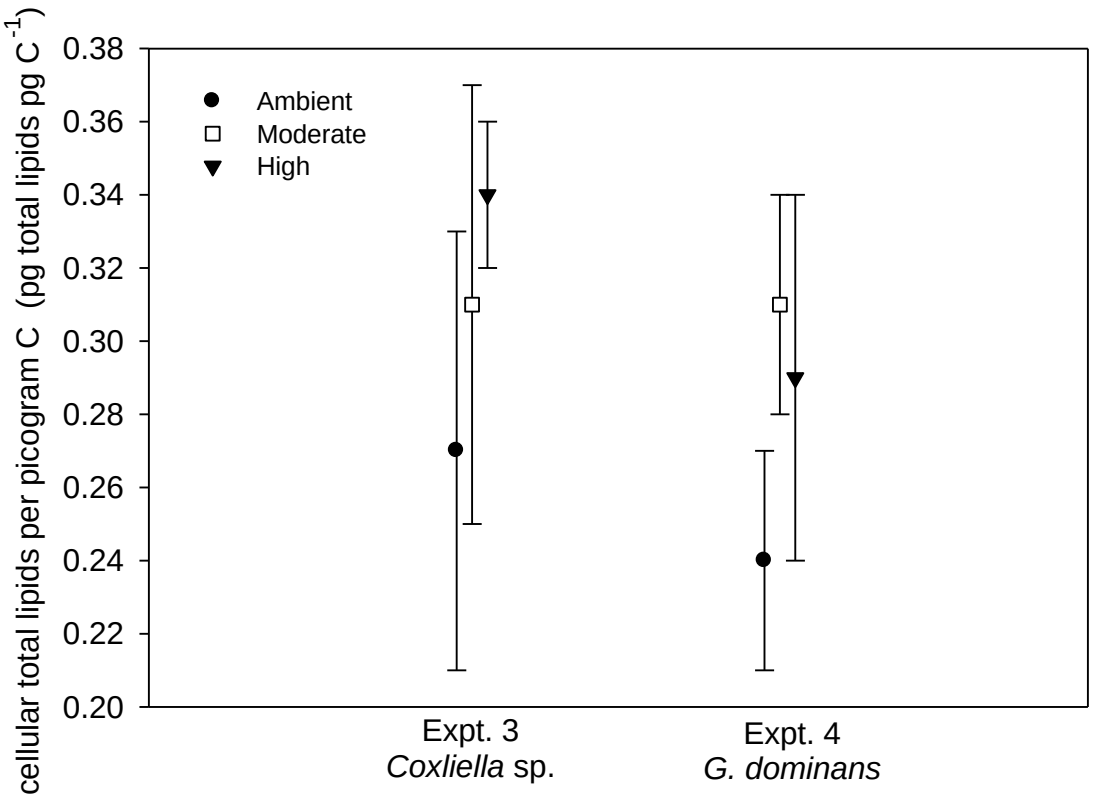


Figure 16. *Rhodomonas* sp. total lipids per cellular POC (pg POC^{-1}) (average ±SD, n=3) for pCO_2 treatments ambient, moderate and high on the last day of semi-continuous culturing during LT grazing experiments with *Coxliella* sp. (Expt. 3) and *G. dominans* (Expt. 4). Refer to Table 10 for data.

(Expt. 1), *Rhodomonas* sp. growth rate was significantly higher in moderate pCO_2 compared to high pCO_2 (Tables 6 and 7). For LT grazing experiment with *O. marina* (Expt. 2) I also observed *Rhodomonas* sp. growth rate to be significantly higher in moderate pCO_2 in comparison to ambient and high pCO_2 treatments (Tables 8 and 9). Overall, no strong or consistent relationship in *Rhodomonas* sp. growth rate was observed with elevated pCO_2. Day to day variation in growth rate of *Rhodomonas* sp. existed (Figures A1, A2, A3 and A4), but no distinct patterns of variability were found.

Chlorophyll *a*

Rhodomonas sp. cellular chlorophyll *a* (Chl *a*) (pg $cell^{-1}$) content was measured on the day after microzooplankton grazing experiments (Day 10 and 16 or 17). The effect of pCO_2 on *Rhodomonas* sp. cellular Chl *a* (pg $cell^{-1}$) was not consistent over time or across experiments (Tables 6 and 8, Figures A9 and A10). Chl *a* (pg $cell^{-1}$) was greatest in the ambient treatment in two instances, in moderate in one instance, high in one instance and no significant differences were found three instances. Specifically, for ST grazing experiments with *O. marina* (Expt. 2) and *Coxliella* sp. (Expt. 3) there was significantly more cellular Chl *a* (pg $cell^{-1}$) in ambient pCO_2 *Rhodomonas* sp. cells than moderate and high pCO_2 (Table 7). No significant pCO_2 effect on cellular Chl *a* (pg $cell^{-1}$) was found between pCO_2 treatments for *Rhodomonas* sp. prey used during ST grazing experiments with *G. dominans* and *F. ehrenbergii* (Expt. 1) (Table 6). During *O. marina* LT grazing experiment (Expt. 2) ambient pCO_2 *Rhodomonas* sp. prey had significantly more cellular chl *a* (pg $cell^{-1}$) than high pCO_2 *Rhodomonas* sp. (Table 9). For the LT grazing experiment with *Coxliella* sp. (Expt. 3)

moderate pCO_2 *Rhodomonas* sp. prey had significantly more cellular chl *a* (pg $cell^{-1}$) than ambient pCO_2 *Rhodomonas* sp. (Table 9).

Because Chl *a* per *Rhodomonas* sp. cell did not scale with increased cell bio-volume found in elevated pCO_2 treatments, a consistent pattern of lower Chl *a* density in moderate and high pCO_2 *Rhodomonas* sp. cells relative to ambient was found (Table 6 and 8, Figures A11, A12). For the ST grazing experiment with *O. marina* (Expt. 2), *Rhodomonas* sp. prey cells had significantly lower chl *a* density with increasing pCO_2 treatments (Table 7). During *Coxliella* sp. ST grazing experiment (Expt. 3), ambient and moderate pCO_2 *Rhodomonas* sp. prey cells had significantly more chl *a* density than high pCO_2 *Rhodomonas* sp. prey cells (Expt. 3) (Table 7). For the LT grazing experiment with *Coxliella* sp. (Expt. 3), *Rhodomonas* sp. prey cells had significantly lower chl *a* density with increasing pCO_2 treatments (Table 9). *G. dominans* LT grazing experiment (Expt. 4), *Rhodomonas* sp. prey cells cultured in ambient and moderate pCO_2 treatments had significantly more chl *a* density than high pCO_2 (Table 9).

Rhodomonas sp. cellular chlorophyll *a* per picogram carbon did not vary significantly between pCO_2 treatments, with the exception of one experimental measurement (Table 6 and 8, Figures A13, A14). *Rhodomonas* sp. prey used in ST grazing experiment with *O. marina* (Expt. 2) had significantly more cellular chl *a* POC^{-1} in ambient pCO_2 than moderate and high (Table 7).

Carbohydrates

Rhodomonas sp. cellular carbohydrate content was not affected by elevated pCO_2 (Table 6 and 8, Figures A15 and A16). Results varied greatly between days and experiments.

The variability in *Rhodomonas* sp. carbohydrate values was a result of measurement effects such as inconsistent extraction from filters. No trend or pattern in this variability was found. *Rhodomonas* sp. cellular carbohydrate density (as pg fructose equivalents μm^{-3}) and cellular carbohydrate POC^{-1} was not affected by elevated pCO_2 either.

Microzooplankton Grazing

Microzooplankton Short Term Ingestion Rates

A linear regression was fit to the cells ingested over time elapsed for each treatment replicate (n=3) and these slopes were then averaged to find a single short term microzooplankton ingestion rate value for each pCO_2 treatment (Table 11, appendix B, Table B1, Figures B1, B2, B3 and B4). All time points were included in the regressions except for *Coxliella* sp. and *O. marina.* For *O. marina*, the linear regression was only fit to total cells ingested up to 90 minutes because after this time the # of prey cells ingested by *O. marina* no longer followed a linear pattern (Figure B1). *Coxliella* sp. fed on *Rhodomonas* sp. in each pCO_2 treatment during the first 15 minutes of the short term grazing experiment and after this time the number of cells ingested by *Coxliella* sp. no longer increased linearly (Figure B4). Therefore, the slopes of the lines, total prey cells ingested versus time, between time 0 and 15 min were used as ingestion rates to compare between pCO_2 treatments for this microzooplankton. R^2 values of the regressions were >0.90 with only two high pCO_2 treatment replicates for *O. marina* being <0.90 (Table B1).

The microzooplankton ST ingestion rates show cells of *Rhodomonas* sp. cultured in elevated pCO_2 conditions were eaten at a faster rate by microzooplankton than

Rhodomonas sp. cultured in ambient conditions, with the exception of *Coxliella* sp. *O. marina* ingested moderate pCO_2 *Rhodomonas* sp. (1.88 ± 0.05 cells grazer^{-1} h^{-1}) significantly faster than ambient pCO_2 *Rhodomonas* sp. (1.56 ± 0.024 cells grazer^{-1} h^{-1}) (Table 12; Figure 17A). *G. dominans* ingestion of *Rhodomonas* sp. increased significantly in a stepwise fashion with increasing pCO_2 treatments (A: 3.20 ± 0.19; M: 3.50 ± 0.02; H: 4.03 ± 0.06 cells grazer^{-1} h^{-1}) (Table 12; Figure 17B). *F. ehrenbergii* ingested *Rhodomonas* sp. cultured in high pCO_2 (28.4 ± 0.92 cells grazer^{-1} h^{-1}) significantly faster than moderate (23.4 ± 1.8 cells grazer^{-1} h^{-1}) and ambient (20.8 ± 0.92 cells grazer^{-1} h^{-1}) (Table 12; Figure 17C). No pCO_2 effect on *Coxliella* sp. ingestion of *Rhodmonas* sp. was found after 15 minutes of feeding (Table 11; Figure 17D).

Percent Feeding

During ST ingestion rate experiments, the portion of the microzooplankton population feeding was determined at each sample time point (Figure 18). The two dinoflagellates, *G. dominans* and *O. marina,* were the only species to have a significant pCO_2 treatment effect on the portion of population feeding during the ST ingestion rate experiments (Figure 18A). A significantly greater percentage of *G. dominans* fed on moderate and high pCO_2 *Rhodomonas* sp. in comparison to ambient pCO_2 *Rhodomonas* sp. during the experiment (Table 13). With time, the difference in percent feeding across treatment for *G. dominans* lessened in all pCO_2 treatments (Figure 18A), with ~7, 5 and 2% more of the *G. dominans* population actively feeding in moderate and high pCO_2 treatments than ambient at the sample time points 15, 45 and 90 minutes, respectively. But no significant pCO_2*time effect on *G. dominans* percent feeding was found (Table 13). For the

other dinoflagellate, a significantly greater percentage of *O. marina* were feeding on the moderate and high pCO_2 *Rhodomonas* sp. in comparison to ambient pCO_2 *Rhodomonas* sp. during the experiment (Table 13; Fig. 18B). No significant pCO_2*time effect on *O. marina* percent feeding was found (Table 13).

In contrast, for the ciliate *F. ehrenbergii*, the initial percent feeding on *Rhodomonas* sp. was the same between pCO_2 treatments, but as time passed more *F. ehrenbergii* appeared to be actively grazing on *Rhodomonas* sp. in the ambient compared to high treatments (Figure 18C). While there was no significant pCO_2 effect found on *F. ehrenbergii* percent population feeding, there was a significant pCO_2*time effect on percent *F. ehrenbergii* feeding over the ST ingestion rate experiment (Table 13; Fig. 18C). In general, for the other ciliate, *Coxliella* sp., the percentage of population feeding across all treatments was low (<45%) (Figure 18D). No pCO_2 effect was found on the portion of *Coxliella* sp. feeding across pCO_2 treatment, but a significant pCO_2*time effect was found (Table 13; Figure 18D). Initially, there was a trend towards a greater percentage of *Coxliella* sp. feeding on high pCO_2 *Rhodomonas* sp. than ambient. But as time passed in the experiment, significantly more *Coxliella* sp. were actively feeding on ambient pCO_2 *Rhodomonas* sp. than moderate and high pCO_2 *Rhodomonas* sp.

Optimal Diet

During ST ingestion rate experiments an additional treatment, optimal diet, was added for the microzooplankton *F. ehrenbergii* and *O. marina.* Stock cultures of *F. ehrenbergii* and *O. marina* required a mixed diet of phytoplankton prey for growth maintenance. In comparison, the other microzooplankton in this study, *G. dominans* and

Coxliella sp., were maintained and grew on a monospecific diet of *Rhodomonas* sp. prey. For *F. ehrenbergii* and *O. marina* optimal diet prey were *Heterocapsa triquetra* and *Isochrysis galbana*, respectively. This optimal diet treatment was added for *F. ehrenbergii* and *O. marina* to serve as a positive control, i.e. to assess if grazing responses in pCO_2 treatments were due to the physiological health of the grazer prior to the start of the experiment or pCO_2 induced treatment effects on the prey. For both *F. ehrenbergii* and *O. marina*, ingestion rates on the optimal diet treatments were positive (i.e. the slope of the prey cells ingested >0), and so these grazers were deemed physiologically healthy (Figures B5 and B7). For a more detailed description of these data and comparison of microzooplankton ingestion of the optimal diet treatment to *Rhodomonas* sp. cultured in pCO_2 treatments refer to appendix B.

Multiple Linear Regression Model

Stepwise Multiple Linear Regression Models (MLR) were used to determine if the *Rhodomonas* sp. physiological and biochemical factors examined explained the significant variation in microzooplankton ingestion rates. The model included the predictive *Rhodomonas* sp. variables: cell bio-volume, POC $cell^{-1}$, PON $cell^{-1}$, Chl *a* $cell^{-1}$and carbohydrates $cell^{-1}$. Results of the MLRs and Pearson Correlation Matrices, showed that ST ingestion rates of *O. marina*, *G. dominans* and *F. ehrenbergii* could be predicted by and were highly correlated to cell biovolume of *Rhodomonas* sp. in each pCO_2 treatment (Table 14). *Rhodomonas* sp. cell bio-volume accounted for 43, 82 and 88 % of the variability in *O. marina, G. dominans,* and *F. ehrenbergii* ST ingestion rates, respectively (Table 14).

Table 11. Microzooplankton short term (ST) and long term (LT) ingestion and growth rates when feeding on *Rhodomonas* sp. cultured semi-continuously in ambient (A), moderate (M), and high (H) pCO_2. Gross growth efficiency (GGE) was calculated using both short and long term ingestion rates. LT grazing experiments were not done with *F. ehrenbergii.* Bold values indicate statistical significance. (ANOVA, α=0.05). The symbol * indicates a natural log transformation of data was done in order to meet homogeneity assumptions. The symbol ~ indicates a non-parametric test Kruskal-Wallis was used because data could not be transformed to meet assumptions.

Microzooplankton	pCO_2	ST ingestion (cells grazer^{-1} h^{-1})	LT ingestion (cells grazer^{-1} d^{-1})	Growth rate (day^{-1})	GGE (%)	
					ST	LT
O. marina	A	**1.56 ± 0.02**$_A$	**9.23 ± 6.78**$_A$	**0.56 ± 0.05**$_A$	10.9 ± 2.21	36.8 ± 30.1
	M	**1.88 ± 0.05**$_B$	**24.67 ± 5.48**$_B$	**0.75 ± 0.08**$_{AB}$	12.2 ± 3.05	24.0 ± 9.9
	H	**1.73 ± 0.18**$_{AB}$	**14.49 ± 1.43**$_A$	**0.84 ± 0.05**$_B$	13.7 ± 1.83	40.3 ± 6.6
G. dominans	A	**3.20 ± 0.19**$_A$	**9.16 ± 1.70***$_A$	**0.33 ± 0.03**$_A$	**~3.57 ± 0.86**$_A$	**33.0 ± 11.3**$_A$
	M	**3.50 ± 0.02**$_B$	**5.83 ± 0.55***$_B$	**0.66 ± 0.06**$_B$	**~5.90 ± 1.28**$_B$	**84.6 ± 12.7**$_B$
	H	**4.03 ± 0.06**$_C$	**4.50 ± 0.49***$_C$	**0.65 ± 0.02**$_B$	**~4.41 ± 0.36**$_A$	**95.4 ± 9.4**$_B$
Coxliella sp.	A	3.34 ± 0.06	**1248.36 ± 278.32***$_A$	**0.13 ± 0.04**$_A$	35.6 ± 14.9$_A$	2.45 ± 1.08$_A$
	M	3.29 ± 0.22	**193.14 ± 96.10***$_B$	**0.43 ± 0.12**$_B$	66.9 ± 32.5$_{AB}$	33.2 ± 18.0$_B$
	H	3.43 ± 0.23	**226.32 ± 194.78***$_B$	**0.59 ± 0.07**$_B$	87.8 ± 25.5$_B$	56.7 ± 48.6$_B$
Favella ehrenbergii	A	**20.8 ± 0.92**$_A$				
	M	**23.4 ± 1.80**$_{AB}$				
	H	**28.4 ± 0.92**$_C$				

Table 12. Analysis of Variance (ANOVA) and Tukey's post-hoc test results for Microzooplankton short term (ST), long term (LT), growth rate and gross growth efficiency (GGE) which were found to be statistically significant.

			ANOVA		Tukey's post-hoc p-values		
Parameter	**Microzooplankton**	**Expt.**	**F value**	**P value**	**A-M**	**A-H**	**M-H**
ST ingestion rate	*O. marina*	2	6.995	0.027	0.023		
	G. dominans	1	37.602	<0.0001	0.047	<0.0001	0.004
	F. ehrenbergii	1	27.293	0.001		0.001	0.007
LT ingestion rate	*O. marina*	2	11.765	0.002	0.002		0.016
	G. dominans	4	33.094	<0.0001	0.001	<0.0001	0.025
	Coxliella sp.	3	14.877	0.001	0.001	0.001	
Growth rate	*O. marina*	2	4.350	0.044		0.026	
	G. dominans	4	4.766	0.030	0.001	0.001	
	Coxliella sp.	3	8.534	0.005		0.002	
GGE_{ST}	*Coxliella* sp.	3	6.251	0.014		0.012	
GGE_{LT}	*G. dominans*	4	37.009	<0.0001	<0.0001	<0.0001	
	Coxliella sp.	3	24.870	<0.0001	<0.0001	<0.0001	

Table 13. Repeated Measures Analysis of Variance (ANOVA) and Tukey's post-hoc test results for microzooplankton percent feeding during microzooplankton short term (ST) ingestion rate experiments which were found to be statistically significant.

			ANOVAR pCO_2 *time		ANOVAR pCO_2		Tukey's post-hoc p-values		
Parameter	**Microzooplankton**	**Expt.**	**F value**	**P value**	**F value**	**P value**	**A-M**	**A-H**	**M-H**
Percent Feeding	*O. marina*	2	1.734	0.170	10.531	0.011	0.026	0.013	
	G. dominans	1	2.488	0.099	19.747	0.002	0.006	0.003	
	F. ehrenbergii	1	14.948	<0.0001	1.956	0.222			
	Coxliella sp.	3	3.575	0.038	2.922	0.130			

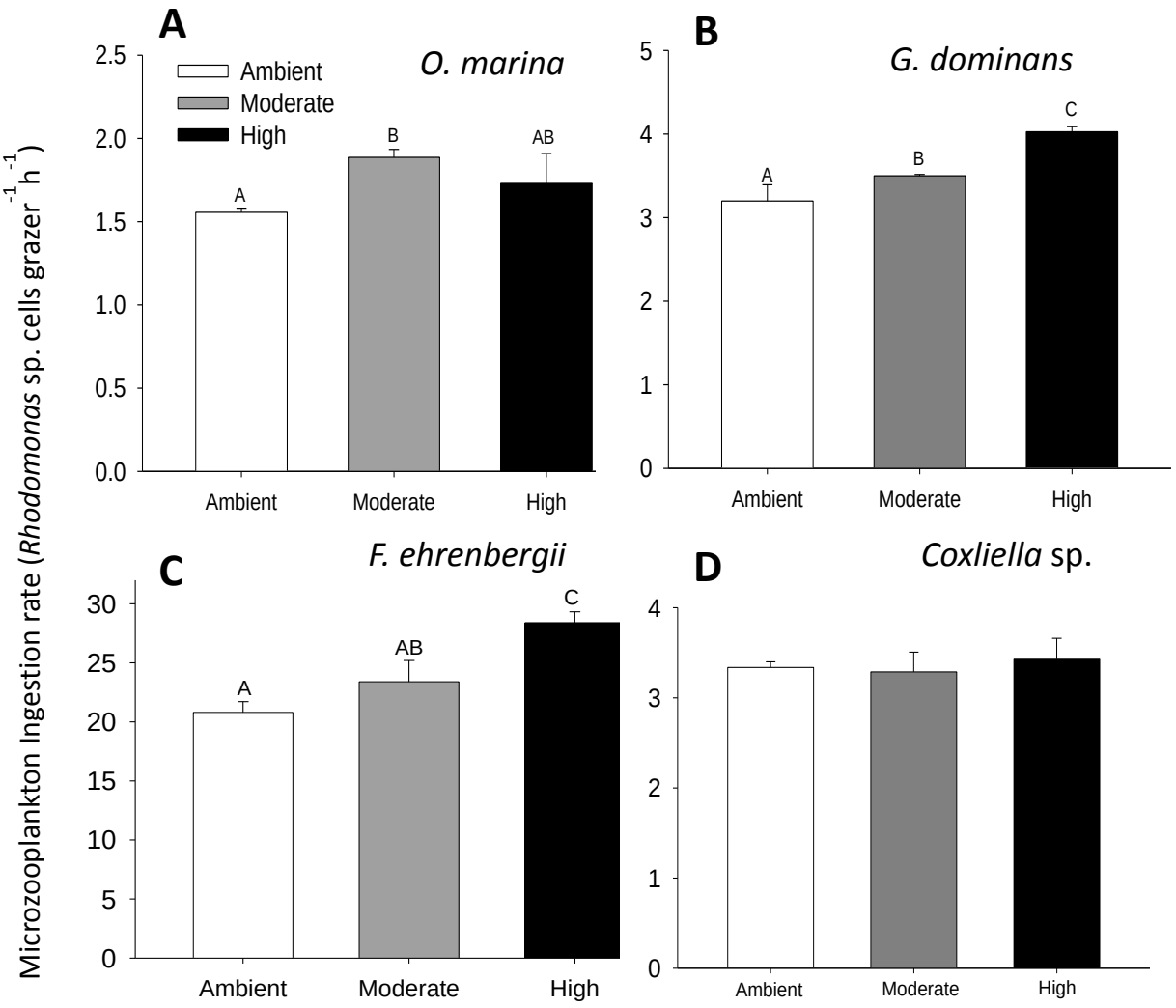


Figure 17. Short term ingestion rate of A) *Oxyrrhis marina* B) *G. dominans* C) *F. ehrenbergii* and D) *Coxliella* sp. (average ± SD, n=3) feeding on *Rhodomonas* sp. cultured semi-continuously in pCO_2 treatments ambient moderate and high. Shared letters (A, B, C) on the graph indicate rates that did not differ significantly (Tukey's post-hoc test, $p>0.05$). Refer to Tables 11 and 12 for data.

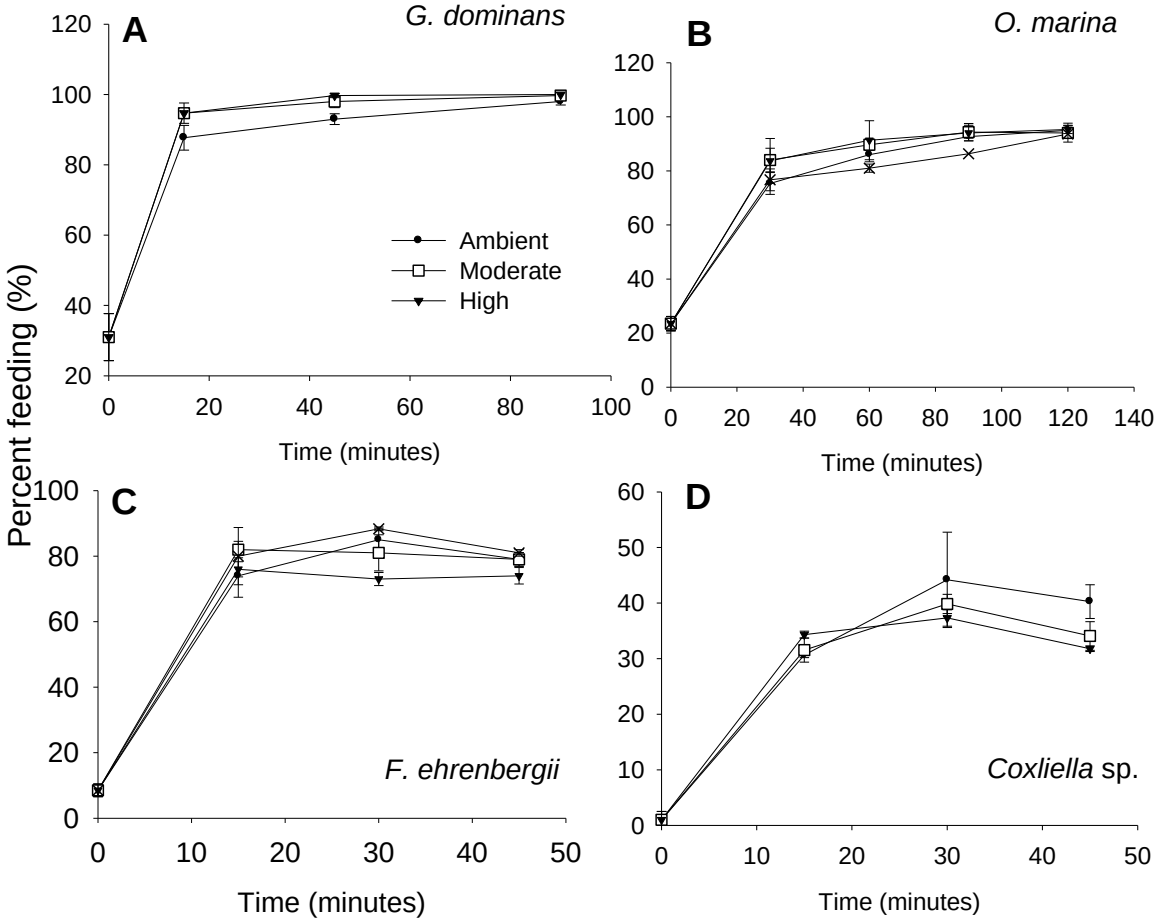


Figure 18. Percentage of A) *Gyrodinium dominans* B) *Oxyrrhis marina* C) *Favella ehrenbergii* and D) *Coxliella* sp. feeding on *Rhodomonas* sp. (average ± SD, n=3) cultured semi-continuously in pCO_2 treatments ambient moderate and high during a short term ingestion rate experiment over time (min). Percentage of *Oxyrrhis marina* and *Favella ehrenbergii* feeding on the optimal prey, *Isochrysis galbana* and *Heterocapsa triquetra* , respectively, were measured simultaneously as a positive control. Refer to Table 13 for ANOVAR results.

Table 14. Multiple linear regression models included the predictive *Rhodomonas* sp. variables: cell bio-volume, POC cell^{-1}, PON cell^{-1}, Chl *a* cell^{-1}and carbohydrates cell^{-}1. The regression model eliminated all variables except cell bio-volume as the predictive factor that explained variation in short term ingestion rate. IR (Microzooplankton ST ingestion rate), BV (*Rhodomonas* sp. bio-volume).

	Pearson's correlation				
Microzooplankton	P	p-value	R^2	Adj. R^2	Equation
O. marina	0.710	0.031	0.508	0.438	IR = 3.227 *10^{-5} + 0.022(BV)
F. ehrenbergii	0.947	<0.0001	0.898	0.883	IR = 0.002 + 0.135(BV)
G. dominans	0.919	<0.0001	0.845	0.823	IR = 0 + 0.32 (BV)

Long Term Microzooplankton Ingestion Rate Experiments

Microzooplankton LT ingestion rates of *Rhodomonas* sp. were affected by the pCO_2 treatment of their prey cells (Tables 11 and 12, Figures 22A, B and C). The pCO_2 trend in ingestion rates found in LT grazing experiments contrasted with that found in ST experiments for *G .dominans* and *Coxliella* sp. but not for *O. marina* (Table 11). It was not possible to do a LT grazing experiment *with F. ehrenbergii* because a monospecific diet of *Rhodomonas* sp. was not sufficient for maintaining population growth for this microzooplankton.

Similar pCO_2 effects on *O. marina* ingestion of *Rhodomonas* sp. were found in ST and LT ingestion rate experiments (Table 11, Figures 17A and 22A). *O. marina* ingested *Rhodomonas* cultured in moderate pCO_2 significantly faster than ambient and high pCO_2 cultured *Rhodomonas* sp. (Table 12). Average LT ingestion rates for each pCO_2 treatment ambient, moderate and high were: 9.2 ± 6.8, 24.7 ± 5.5 and 14.2 ± 1.4 cells grazer^{-1} day^{-1} respectively. High variability existed between the replicate ingestion rates found in ambient and moderate pCO_2 treatments, with values ranging from 4 to 19 and 17 to 32 cells grazer^{-1} d^{-1}, respectively. The ingestion rate values found for *O. marina* from short term ingestion rate experiments were faster than calculated LT ingestion rates.

G. dominans LT ingestion rates of *Rhodomonas* sp. suggest a different pCO_2 effect compared to ST ingestion rate findings (Table 11, Figures 17B and 22B). *G. dominans* average LT ingestion rates of *Rhodomonas* sp. were 9.2 ± 1.7, 5.8 ± 0.55 and 4.5 ± 0.49 cells grazer^{-1} day^{-1} for ambient, moderate and high pCO_2 respectively. *G. dominans* ingested *Rhodomonas* sp. significantly slower with increasing pCO_2 treatments (Table 12). *G.*

dominans ingestion rate of ambient pCO_2 *Rhodomonas* sp. was most variable, with values ranging from 7 to 12 cells grazer^{-1} d^{-1}. ST ingestion rates for *G. dominans* were also faster than LT ingestion rates.

No significant pCO_2 effect was found for the ingestion of *Rhodomonas* sp. by *Coxliella* sp. in ST grazing experiments. But, in the LT grazing experiment, *Coxliella* sp. ingested ambient pCO_2 *Rhodomonas* sp. significantly faster than moderate and high (Tables 11 and 12, Figures 17D and 22C). Average *Coxliella* sp. LT ingestion rates of ambient, moderate and high pCO_2 *Rhodomonas* sp. were: 1248.4 ± 278.3, 193.1 ± 96 and 226.3 ± 194.8 cells grazer^{-1} d^{-1}, respectively. *Coxliella* sp. ingestion rates of *Rhodomonas* sp. were highly variable between replicates in each pCO_2 condition. *Coxliella* sp. ingestion rates of ambient *Rhodomonas* sp. were most variable with values ranging from 946 to 1585 cells grazer^{-1} d^{-1}. Ingestion rates of moderate and high pCO_2 *Rhodomonas* sp. were much less than ambient with replicate values ranging from 45 to 298 and 66 to 530 cells grazer^{-1} d^{-1}, respectively. LT ingestion rate values of *Coxliella* sp. were faster than ingestion rate values found during the ST grazing experiment. This is particularly evident when comparing ambient pCO_2 *Rhodomonas* sp. ingestion rates from ST and LT experiments.

Microzooplankton Growth Rate

Elevated pCO_2 resulted in a higher growth rates for all microzooplankton species during LT experiments (Table 11, Figure 23). *O. marina* had significantly higher growth rates in high pCO_2 (0.84 ± 0.05 d^{-1}) than ambient (0.56 ± 0.08 d^{-1}) (Table 12). *G. dominans* had higher growth rates in moderate (0.66 ± 0.06 d^{-1}) and high pCO_2 (0.65 ± 0.02 d^{-1}) than

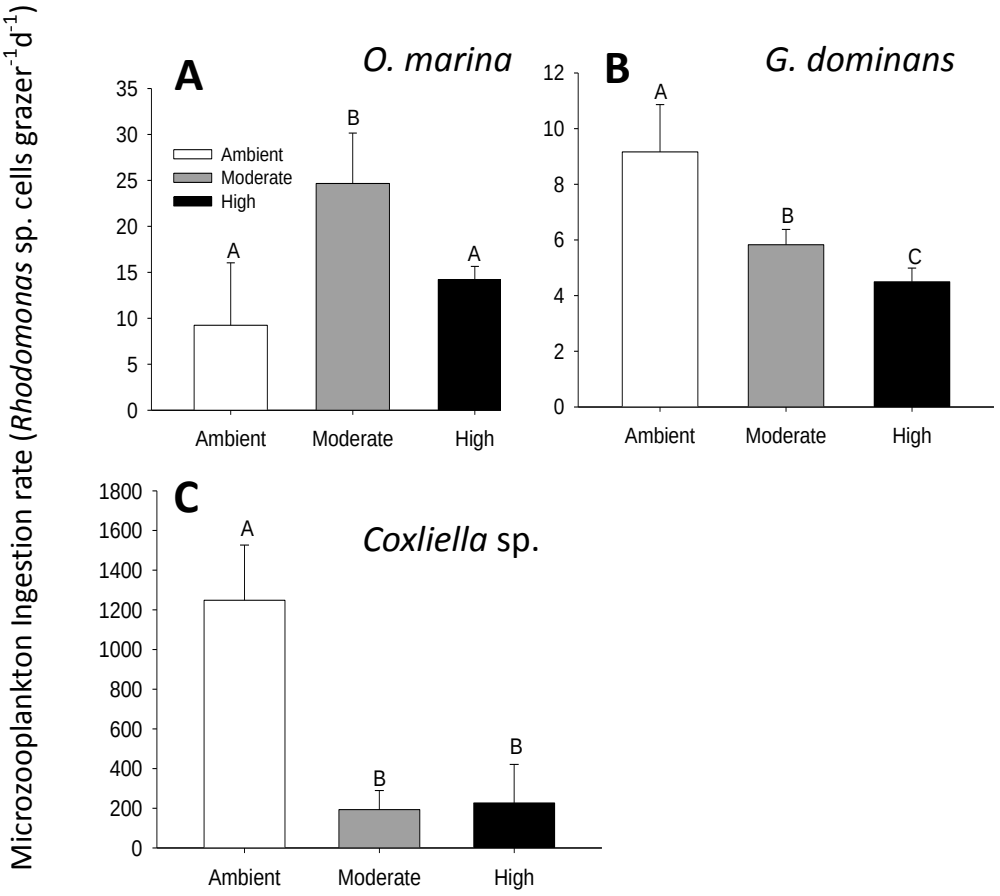


Figure 19. Long term ingestion rate of A) *Oxyrrhis marina* B) *G. dominans* and C) *Coxliella* sp. feeding on *Rhodomonas* sp. (average ± SD, n=5) cultured semi-continuously in pCO_2 treatments ambient, moderate, and high over 24 h, after a 5 day acclimation period. Ingestion rates are calculated using equations by Heinbokel (1978).The letters A and B on the graph indicate Tukey's post hoc significance ($p<0.05$). Refer to tables 11 and 12 for data.

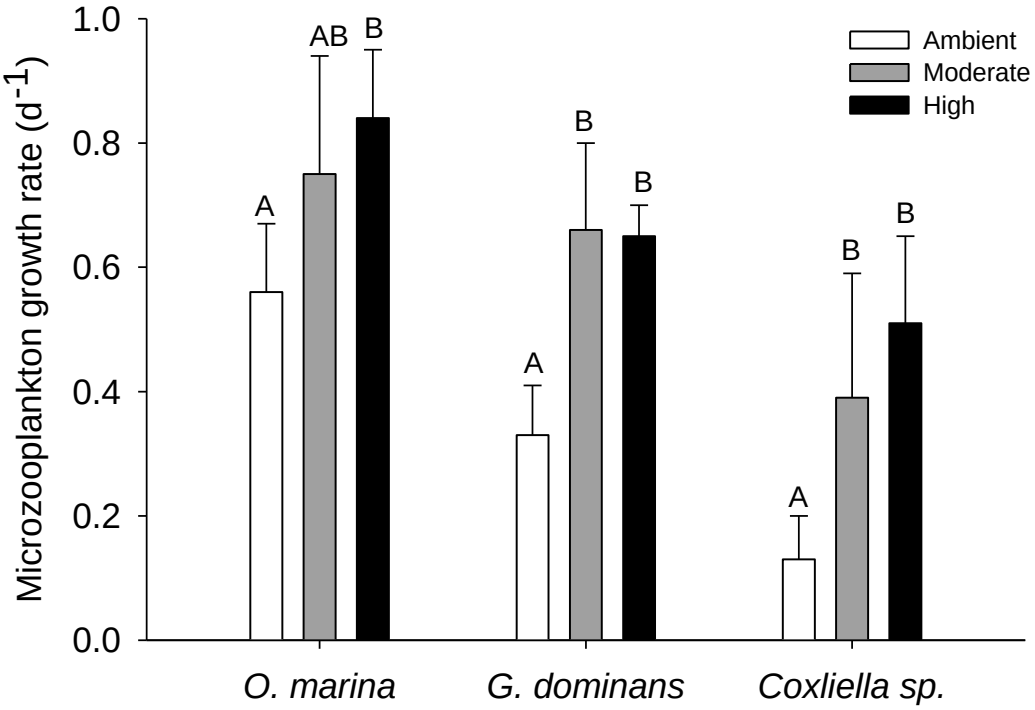


Figure 20. Microzooplankton growth rates (average ± SD, n=5) for the microzooplankton *Oxyrrhis marina, Gyrodinium dominans*, and *Coxliella* sp. The letters A and B on the graph indicate Tukey's post hoc significance ($p<0.05$). Refer to tables 11 and 12 for data.

ambient (0.33 ± 0.03 d^{-1}) (Table 12). Finally, growth rates of *Coxliella* sp. in high pCO_2 (0.59 ± 0.07 d^{-1}) were significantly higher than ambient (0.13 ± 0.04 d^{-1}) (Table 12).

Microzooplankton Gross Growth Efficiency

Microzooplankton ingestion rates from both ST and LT grazing experiments were used to calculate *Rhodomonas* sp. C ingested in order to calculate microzooplankton GGE values. When using ST ingestion rate values to determine prey carbon consumed GGE increased in elevated pCO_2 for *O. marina* and *G. dominans* (Table 11; Figure 24A). GGE values for *O. marina* were 10.9 ± 2.2, 12.2 ± 3.1 and 13.7 ± 1.8 % for ambient, moderate and high pCO_2, respectively. This trend suggests *O. marina* GGE is higher in moderate and high pCO_2 in comparison to ambient. However, results of a one way ANOVA reveal this trend to be not statistically significant. GGE values for *G. dominans* were 3.6 ± 0.9, 5.9 ± 1.3 and 4.4 ± 0.4 % for ambient, moderate and high, respectively. These results suggest *G. dominans* GGE is higher in moderate conditions than ambient and high (Figure 24A). A non-parametric Kruskal-Wallis (K) test confirms there is a significant difference in growth efficiency between pCO_2 treatments (Table 11; p=0.004). GGE values for *Coxliella* sp. were 35.6 ± 14.8, 66.9 ± 32.5 and 85.8 ± 25.5% for ambient, moderate and high, respectively. GGE values for *Coxliella* sp. were significantly higher when feeding on high pCO_2 cultured *Rhodomonas* sp. than ambient (Table 12).

When using LT ingestion rate values to estimate prey of carbon consumed, GGE values for *O. marina* were 36.8 ± 30.1, 23.4 ± 9.7 and 43.3 ± 7.1 % for ambient, moderate and high pCO_2, respectively (Table 11; Figure 24B). These values suggest a slight increase in GGE for *O. marina* under high pCO_2 in comparison to ambient and moderate. However, as a

result of high variability this trend is not significant. For *G. dominans,* GGE values calculated using LT ingestion rates were 33.0 ± 11.3, 84.6 ± 12.7 and 95.4 ± 9.3 % for ambient, moderate and high pCO_2, respectively. GGE values for *G. dominans* are significantly higher when feeding on *Rhodomonas* sp. cultured in moderate and high pCO_2 conditions in comparison to ambient (Table 12). *Coxliella* sp. GGE from LT ingestion rate values were 2.5 ± 1.1, 33.2 ± 18.0 and 56.7 ± 48.6% for ambient, moderate and high, respectively. GGE values for *Coxliella* sp. were significantly higher when feeding on moderate and high pCO_2 cultured *Rhodomonas* sp. than ambient (Table12).

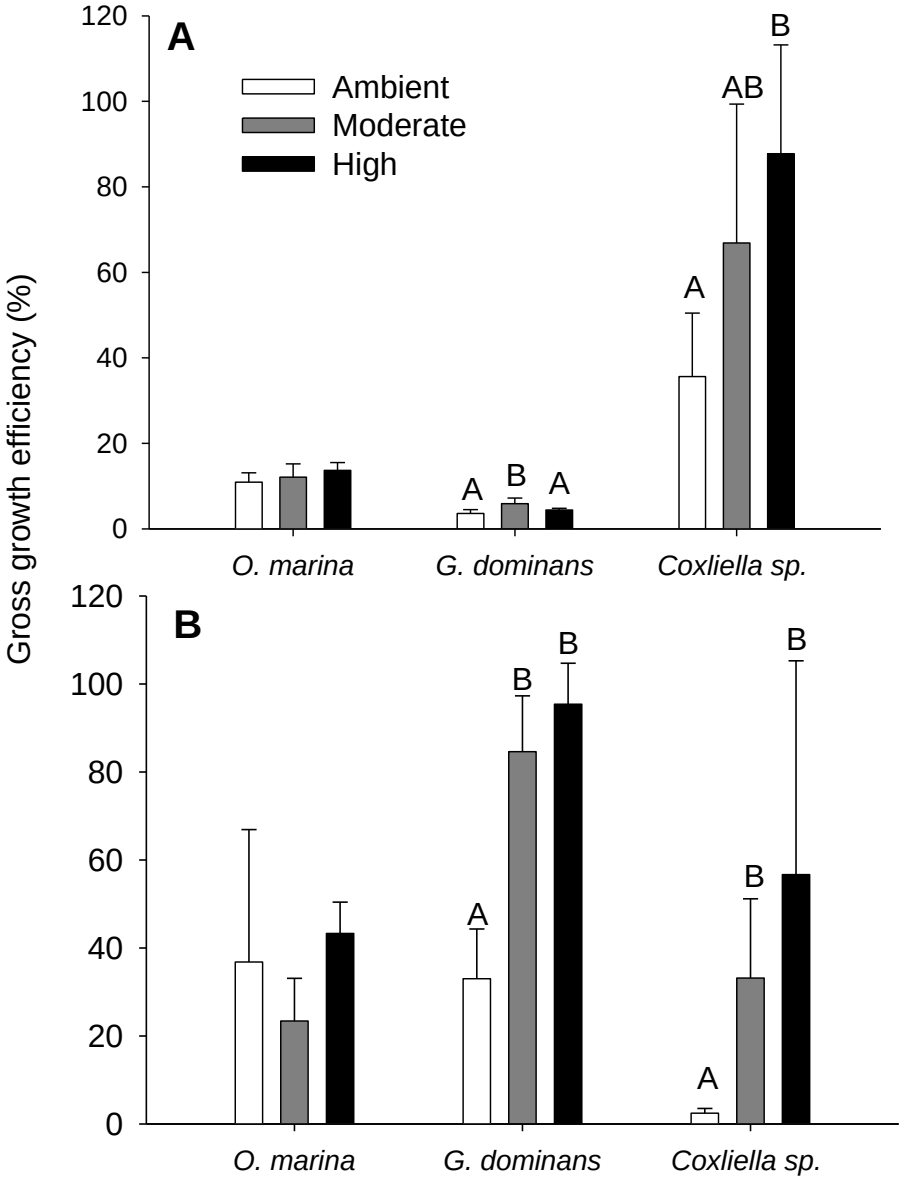


Figure 21. Gross growth efficiency (%) (average ± SD, n=5) of microzooplankton feeding on *Rhodomonas* sp. cultured semi-continuously in ambient moderate and high pCO_2. A) Short term and B) long term ingestion rate values were used for determining *Rhodomonas* sp. pg C ingested by microzooplankton. The letters A and B on the graph indicate Tukey's post hoc significance ($p<0.05$). Refer to tables 11 and 12 for data.

DISCUSSION

The purpose of this study was to determine how OA can indirectly affect microzooplankton ingestion, population growth and GGE. I accomplished this by first characterizing direct effects of OA on *Rhodomonas* sp. morphology, physiology and biochemistry. Subsequently pCO_2, acclimated *Rhodomonas* sp. were fed to microzooplankton, and microzooplankton ingestion and growth rates were measured, and from this, GGE was calculated. I found that OA directly affected *Rhodomonas* sp. morphology and biochemistry. Further, I found that microzooplankton grazing and growth was affected by OA-induced changes in *Rhodomonas* sp. In order for me to clearly express how the OA-induced changes that I observed in *Rhodomonas* sp. affected microzooplankton grazing and growth, I begin with a review of what is known about the mechanisms that govern microzooplankton-phytoplankton predator-prey dynamics.

Microzooplankton Selection of Phytoplankton Prey

Microzooplankton are acutely sensitive to the size and condition of their prey and can be very selective of the phytoplankton they consume (e.g. Andersson et al. 1986, Verity 1988, Flynn et al. 1996, John and Davidson 2001, Tillmann 2004, Meunier et al. 2011). Prey cell size and morphology are recognized as 1st order determinants that influence microzooplankton-phytoplankton feeding relationships, including microzooplankton ingestion rates (e.g. Jonsson 1986, Hansen 1992, 1994, Kamiyama and Arima 2001, Tillmann 2004). This, in part, results from microzooplankton being physically constrained to ingestion of prey within a defined size range. The width of this size window is dependent

upon the microzooplankton feeding mechanism and apparatus (Goldman et al. 1989, Hansen 1992, Hansen 1994, Jakobsen and Hansen 1997). Ciliates like *F. ehrenbergii* and *Coxliella* sp. suspension feed. The size of the ciliate's oral diameter determines the phytoplankton size range it can ingest (Fenchel 1980, Capriulo 1982, Jonsson 1986). In contrast to ciliates, some athecate dinoflagellates like *O. marina* and *G. dominans* feed by direct engulfment of their prey (Jeong et al. 2010). These dinoflagellates are capable of ingesting prey at predator: prey size ratios between 0.15:1 and 5.2:1 (Tillman 2004). The optimal linear size ratio for heterotrophic dinoflagellate and their prey across dinoflagellate taxa approximates 1:1 (Hansen 1994).

While prey cell size is important, it does not exclusively explain observed differences in microzooplankton grazing behavior (Shannon et al. 2007, Montagnes et al. 2011). Size selection by microzooplankton could be considered a passive mechanism that depends on contact probabilities and morphological limitations of the feeding structures. In comparison, microzooplankton have shown to actively select for phytoplankton based on qualities other than size selection such as the secondary constituents like: food quality (e.g. Verity 1991, John and Davidson 2001, Chen et al. 2010, Roberts et al. 2011), surface chemical signatures (Verity 1988, Strom et al. 2003, Barofsky et al. 2010,) and prey physiological state (Verity 1988, Christaki et al. 1998, Landry et al. 1991, Gruber et al. 2009).

The optimal foraging theory predicts grazers actively select for food with high nutritional value (MacArthur and Pianka 1966). Research has been done to elucidate the mechanism behind biochemical recognition, and it is believed that microzooplankton ingestion and capture of prey may be facilitated by receptor mediated recognition of

particles, but these findings are still in their infancy (Wootton et al. 2007, Hartz et al. 2008, Roberts et al. 2011). Ultimately, determining the optimal food for microzooplankton can be assessed by studying the growth efficiency of the grazer on various food resources. Prey cellular essential fatty acids, carbohydrates, proteins and C:N stoichiometry all have been shown to contribute to prey food quality and can influence microzooplankton ingestion, growth and GGE (Flynn et al. 1996, John and Davidson 2001, Kattner et al. 2007, Suida and Dam 2010, Meunier et al. 2011, Leu et al. 2013).

Given what is known about the microzooplankton-phytoplankton feeding relationships described above, I now discuss the observed pCO_2 induced changes in *Rhodomonas* sp. that could explain the differences in microzooplankton grazing and growth rates found in this study, and discuss the implications my findings may have on higher trophic levels and nutrient and other biogeochemical cycles in an acidifying ocean.

Direct OA Effects on *Rhodomonas* sp.

The most pronounced effect of elevated pCO_2 on *Rhodomonas* sp. was a ~60 and 100% increase in *Rhodomonas* sp. cell bio-volume when cultured in moderate and high pCO_2, respectively, relative to cells cultured under ambient pCO_2. The increase in cell bio-volume was stepwise, whereby cell bio-volume was significantly greater in each succeeding pCO_2 treatment concentration (Tables 6 and 8). This response was also observed in experiments by Kendall (2015). Similarly, an increase in cell bio-volume was observed for a different phytoplankton species, *Emiliania huxleyi,* cultured under the same pCO_2 conditions used in this study (Wuori 2012). In other phytoplankton species, observed increase in cell size under elevated pCO_2 generally is correlated with increased rates of production of

organic carbon, resulting in higher organic carbon per cell (Zondervan et al. 2002, Iglesias-Rodriguez et al. 2008). While *Rhodomonas* sp. cultured in high pCO_2 had, on average, more POC $cell^{-1}$ than cells cultured under ambient and moderate pCO_2, this trend was not significant and did not scale with the increase in *Rhodomomas* sp. cell bio-volume (Tables 6 & 8). Kendall (2015) and Wuroi (2012) also found no correlation between the increase in cell size and POC $cell^{-1}$. Wuroi (2012) suggested that the increase in *E. huxleyi* cell bio-volume may be due to water entering the cell as a response to intracellular concentration of osmolytes and internal pH.

In this study, the observed increase in *Rhodomonas* sp. total lipids $cell^{-1}$ in moderate and high pCO_2-cultured cells could also be contributing to the increased bio-volume of *Rhodomonas* sp. The increase in *Rhodomonas* sp. lipids was found in experiments 3 and 4, the only experiments when lipids were measured. *Rhodomonas* sp. cultured in moderate and high pCO_2 had, on average, ~1.4 and 1.5 times more total lipids $cell^{-1}$, respectively, than *Rhodomonas* sp. cultured in ambient pCO_2 (Table 10). Increases in cellular lipids have been observed in other phytoplankton species under elevated pCO_2 (Carvalho and Malcata 2005, Hoshida et al. 2005, Malzahn et al. 2007, Leu et al. 2013). For example, Malzahn et al. (2007) also found that the cryptophyte *Rhodomonas salina* increased their cellular concentrations of highly unsaturated fatty acids when grown under elevated pCO_2. While these studies have shown cellular total lipids of phytoplankton to be affected by elevated pCO_2, very little is known about the effect OA may have on total lipid density. For my study, when *Rhodomonas* sp. cellular total lipids were normalized to bio-volume, there was no longer a significant increase in total lipids μm^{-3}. This was because the percent change in

lipids (36% and 50% for moderate and high pCO_2 *Rhodomonas* sp. cells, respectively) was smaller than the percent change I found in *Rhodomonas* sp. bio-volume (~ 60% and 100% increase in moderate and high pCO_2 *Rhodomonas* sp. cells, respectively). Therefore, as suggested by Wuroi (2012), there are likely additional variables that are contributing to the observed increase in *Rhodomonas* sp. bio-volume.

With the significant increase in *Rhodomonas* sp. total lipids $cell^{-1}$ that I observed, I expected to see a concurrent increase in POC $cell^{-1}$ with elevated pCO_2, as was observed in *R. salina* by Malzahn et al. (2007). As stated above, POC $cell^{-1}$ was, on average, greater in cells cultured under high pCO_2, but this difference was not statistically significant, which as described above was also observed in Wuori (2012) for *Emiliania huxleyi* strain CCMP 2668. The likely explanation is that the variability in POC $cell^{-1}$ replicate measurements for each treatment was too high to identify a statistically significant treatment effect (Table 6 and 8).

Despite the significant effect of OA on *Rhodomonas* sp. cell bio-volume and cellular total lipids, many of the additional phytoplankton characteristics measured in this study (i.e. growth rate, Chl *a,* cellular PON and C:N) did not change significantly, or changes were variable between days and experiments under elevated pCO_2. Burkhardt et al. (1999) and Rost et al. (2003) suggest that because phytoplankton species possess different carbon acquisition mechanisms, some phytoplankton species may be more sensitive to changes to OA. Phytoplankton species that evolved efficient CCM's will be less sensitive to increasing levels of pCO_2 than those that haven't. Elzenga et al. (2000) suggests that *Rhodomonas* sp. acquires inorganic carbon for photosynthesis exclusively in the form of CO_2. In contrast, however, Camiro-Vargas et al. (2005) showed that *Rhodomonas* sp. was able to continue to

grow in the absence of CO_2 at pH levels of 10.1, suggesting *Rhodomonas* sp. is capable of direct HCO_3^- uptake. Cryptomonads have evolved to have a Rubisco structure that has high specificity for CO_2 over O_2 (Graham et al. 2009). Therefore, if *Rhodomonas* sp. has an effective CCM, this could explain why I observed little to no changes in most *Rhodomonas* sp. physiological variables between treatments with the exception of total lipids. Mechanisms for which elevated pCO_2 affects phytoplankton fatty acids are unclear, but it is possible, and this study suggests, *Rhodomonas* sp. is capable of allocating more fixed carbon towards fatty acid synthesis and storage under elevated pCO_2 (Bermúdez et al. 2015).

Microzooplankton Ingestion Rate

Short Term Response

My data show that microzooplankton grazing was significantly affected by OA-induced changes to their prey. ST microzooplankton ingestion rates were higher when grazers fed on moderate and high pCO_2 acclimated *Rhodomonas* sp. compared to the ambient treatment for three of the four grazers tested. I also observed *O. marina* to have a significant non-linear grazing response. *O. marina* ingested moderate pCO_2 *Rhodomonas* sp. significantly faster than ambient pCO_2 *Rhodomonas* sp. Despite the differences in size, feeding strategies and metabolism between ciliates and dinoflagellates, the increased feeding rates were found in both microzooplankton groups.

In the short term grazing experiments I observed species-specific ingestion rate responses for the two tintinnids ciliates, *F. ehrenbergii* and *Coxliella* sp. *F. ehrenbergii* grazed *Rhodomonas* sp. that was cultured under elevated pCO_2 ~1-1.2 times faster than *Rhodomonas* sp. cultured under ambient pCO_2. In comparison, I observed no difference in

ingestion rates of *Coxliella* sp. across all *Rhodomonas* sp. treatments. Tintinnids like *F. ehrenbergii* and *Coxliella* sp. have very narrow windows of optimal prey size because of their restrictive lorica (Jonsson 1986, Jeong et al. 2010). The maximum food particle size for tintinnids is limited by the oral lorica diameter (Heinbokel 1978), and maximum clearance rates are a linear function of food particle size until a maximum size is reached (Fenchel 1980). Jonsson (1986) found tintinnids feed most efficiently on particle sizes ranging between 10 and 40% of their lorica diameter size. In general, the optimal predator: prey cell size ratio for tintinnids is 8:1 (with the relevant predator size being the lorica opening diameter) (Hansen 1994). *Coxliella* sp. has a lorica opening diameter of 45 µm (Verity 1985) compared to *F. ehrenbergii* with a lorica opening diameter of 104 µm (Jörgensen 1924). Therefore, the increase in prey cell size under OA conditions affected predator:prey size ratios differently for the two tintinnids and their difference in oral lorica diameter could help explain the species specific grazing responses observed in the ST grazing experiments for these ciliates. To illustrate, *F. ehrenbergii* feeds most effectively on prey with an equivalent spherical diameter > 10µm (Stoecker et al. 1995, Kamiyama and Arima 2001). Predator: prey size ratios for *F. ehrenbergii* and *Rhodomonas* sp. were 16.5, 15.4 and 14.2 in ambient, moderate and high pCO_2 treatments, respectively. Therefore, the increase in cell size under OA resulted in the prey being closer to the optimal predator:prey size ratio for *F. ehrenbergii* (8:1) (Table 15). The predator:prey size ratio of *Coxliella* sp. and ambient pCO_2 cultured *Rhodomonas* sp. was 7.21, deviating only 0.79 from the reported optimal predator:prey size ratio for tintinnids (Hansen 1994) (Table 15). For moderate and high pCO_2 treatments the deviation in predator:prey size ratio from the optimal size ratio was

even greater. Predator: prey size ratios for moderate and high pCO_2 treatments were 5.82 and 5.44, respectively, deviating 2.18 and 2.56 from the optimal predator:prey size ratio (Hansen 1994) (Table 15). Because the increase in *Rhodomonas* sp. size resulted in the predator: prey size ratio decreasing below the optimal value, I would have expected to maybe see a reduction in feeding rates by *Coxliella* sp. on moderate and high pCO_2 *Rhodomonas* sp. While *Coxliella* sp. did ingest, on average, slightly more ambient pCO_2 *Rhodomonas* sp. than moderate and high pCO_2 *Rhodomonas* sp., I found no significant difference in *Coxliella* sp. ingestion rate between pCO_2 treatments (Figure 20). It's possible that the difference in observed prey size in this study was not great enough for *Coxliella* sp. to alter its grazing response, or perhaps *Coxliella* sp. may be more acutely sensitive to other changes in prey such as food quality.

The heterotrophic dinoflagellate, *O. marina,* fed on *Rhodomonas* sp. that was cultured under in moderate pCO_2 ~1.2 times faster than *Rhodomonas* sp. cultured under ambient pCO_2. I did not observe a significant stepwise increase in *O. marina* ingestion of *Rhodomonas* sp. with increasing pCO_2 acclimated prey. Instead, I observed a non-linear response, with *O. marina* ingesting high pCO_2 acclimated prey slower than moderate acclimated prey (Table 9). *O. marina* (18 µm ESD, Jeong et al. 2010) has been reported to ingest prey within a size spectrum ranging from 2 µm to 20 µm (Montagnes et al. 2011, Roberts et al. 2011, Guo et al. 2013). When offered prey of varying size, *O. marina* can feed and achieve high growth rates on flagellates >4 µm but will actively select prey larger in size (>7 µm) (Goldman et al. 1989, Hansen et al. 1996, Jeong et al. 2003). A review by Jeong et al. (2010) reported that for *O. marina*, the prey *Heterosigma akashiwo* (11.5 µm ESD), with

a predator: prey ratio of 1.2, supported maximum growth, ingestion and clearance rates. Predator: prey size ratios for *O. marina* and *Rhodomonas* sp. in this study were 2.82, 2.48 and 2.31 in ambient, moderate and high pCO_2 treatments, respectively. Therefore, the increase in cell size under OA resulted in the predator:prey size ratio trending towards a more optimal ratio for *O. marina* (1.2) (Table 15).

The dinoflagellate, *G. dominans* (20µm ESD, Naustvoll 2000), fed on *Rhodomonas* sp. cultured under moderate and high pCO_2 ~ 1.1 and 1.25 times faster than *Rhodomonas* sp. cultured under ambient pCO_2 (Table 9). *G. dominans* is capable of ingesting prey cells ranging in size from 6 to 43 µm ESD (Naustvoll 2000). Nakamura et al. (1995) found that *G. dominans* clearance rates on *Heterocapsa triquetra* (15.3µm ESD) were 2 times faster than when feeding on the smaller *Nephroselmis* aff. *rotunda* (4.5µm ESD). In agreement, when *G. dominans* was offered twelve prey of varying sizes and types, including *Rhodomonas baltica* (6.4 µm ESD), *H. triquetra* (10.9 µm ESD) supported the highest growth rate (~0.5 d^{-1}), and had a predator: prey size ratio of 2.4 (Naustvoll 2000). Therefore, *G. dominans* appears to feed most efficiently on larger prey. In this study the increase in prey cell size under moderate and high pCO_2 resulted in *G. dominans* predator: prey size ratios of 2.96 and 2.72, respectively. These ratios are closer to the optimal 2.4 ratio compared to the ambient treatment (3.18) (Table 15).

While there are other possible mechanisms that could explain the increase in microzooplankton ingestion rates observed in this study under elevated pCO_2, multiple

Table 15. Predator:prey size ratios of *O. marina, G. dominans, F. ehrenbergii* and *Coxliella* sp. feeding on *Rhodomonas* sp. cultured under ambient, moderate and high pCO_2. Equivalent spherical diameter (ESD) of *Rhodomonas* sp. and of the dinoflagellates *O. marina* and *G. dominans* were used to calculate predator:prey size ratios. ESD of *Rhodomonas* sp. was calculated using: $(\text{biovolume}/0.523)^{0.33}$ (Hansen 1994).For the ciliates, *F. ehrenbergii* and *Coxliella* sp., predator:prey size ratios were obtained using the oral lorica diameter and. *Rhodomonas* sp. ESD. The optimal predator:prey size ratios listed in the table are from Jeong et al. (2010) (*O. marina*), Naustvoll (2000) (*G. dominans)* and Hansen et al. (1994) (*F. ehrenbergii* and *Coxliella* sp.).

Species	**Ambient *Rhodomonas* sp.**	**Moderate *Rhodomonas* sp.**	**High *Rhodomonas* sp.**	**Optimal predator:prey**
O. marina (18µm ESD)	2.82	2.48	2.31	1.2
G. dominans (20 µm ESD)	3.18	2.96	2.72	2.4
Favella ehrenbergii (104 µm oral diameter)	16.5	15.4	14.2	8
Coxliella sp. (45 µm oral diameter)	7.21	5.82	5.44	8

linear regression models showed that the stepwise increase in *Rhodomonas* sp. cell bio-volume across ambient, moderate and high pCO_2 treatments explained 43, 82, and 88% of the variability in *O. marina, G. dominans* and *F. ehrenbergii* short term ingestion rates, respectively (Table 10). The non-linear short term grazing response observed for *O. marina* suggests that in addition to cell size, other OA-induced prey characteristics were influencing the microzooplankton's grazing response.

Long Term Response

In this study I observed a statistically significant OA effect on microzooplankton LT ingestion of pCO_2 acclimated *Rhodomonas* sp., where cells grown in ambient pCO_2 were consistently grazed at faster rates by the dinoflagellate *G. dominans* and the ciliate, *Coxliella* sp. This finding is in contrast, for these two grazers, to what I found in ST ingestion rate experiments (Table 9). In LT grazing experiments *G. dominans* ingested ambient pCO_2 treatment *Rhodomonas* sp. ~ 2 and 1.6 times faster than moderate and high *Rhodomonas* sp., respectively, whereas the ciliate *Coxliella* sp. ingested ambient pCO_2 treatment *Rhodomonas* sp. ~ 6 times faster than moderate and high treatments, respectively. When compared to the literature findings, *Coxliella* sp. average LT ingestion rate of ambient pCO_2 cultured *Rhodomonas* sp. was higher than I expected (Verity 1985). Average *Coxliella* sp. LT ingestion rates of ambient, moderate and high pCO_2 *Rhodomonas* sp. were: 1248.4 ± 278.3, 193.1 ± 96.0 and 226.3 ± 194.8 cells grazer^{-1} d^{-1}, respectively. However, under similar temperature and saturating prey carbon concentration conditions to this study, Verity (1985) observed that *Tintinnopsis vasculum* (80µm length, 45 µm oral lorica diameter) ingested 600-800 pg C h^{-1} (equivalent to ~288-384 *Rhodomonas* sp. cells day^{-1}). Therefore,

there is a possibility that the high *Coxliella* sp. grazing rates in ambient pCO_2 treatments are an experimental artifact. The high ambient *Coxliella* sp. ingestion rate calculated using Heinbokel (1978) equations is being driven by the unexpectedly low *Rhodomonas* sp. growth rates values that were found in the microzooplankton treatment bottles. Because I was unable to dilute *Rhodomonas* sp. cell densities prior to the 24 h ingestion rate experiment, I believe the low *Rhodomonas* sp. growth rate values in the microzooplankton treatment bottles are from prey reaching capacity in the ambient pCO_2 treatments (due to high prey cell densities). However, despite *Coxliella* sp. ingestion rates of ambient pCO_2 *Rhodomonas* sp. being greater than published values, I did observe a significant trend towards lower microzooplankton ingestion rates on moderate and high pCO_2 cultured *Rhodomonas* sp. compared to ambient pCO_2 cultured *Rhodomonas* sp. for both the ciliate *Coxliella* sp. and the dinoflagellate *G. dominans.*

A fundamental question for *Coxliella* sp. and *G. dominans* is, why were the observed trends in microzooplankton grazing different between short- and long-term grazing experiments? It has been recognized that when working with species that show compensatory feeding, i.e. the ability to adjust feeding rates and behavior as a consequence of changes in prey food quality (Meunier et al. 2011), that it is important to conduct long incubations and allow the organism to precondition to the prey for several days to allow for stabilization of feeding rates (Calbet et al. 2013). The longer term grazing experiments conducted here allowed the microzooplankton species to acclimate and adjust their grazing response to any secondary pCO_2 changes in *Rhodomonas* sp., such as food quality. Therefore the grazing response by microzooplankton in long term experiments may have

been different from ST grazing rates because microzooplankton were altering their grazing in response to both primary (prey cell size) and secondary (biochemical constituents) OA induced changes in *Rhodomonas* sp. biology.

A secondary constituent that may explain the observed change in trend in microzooplankton grazing response between short- and long-term experiments is the 1.4 and 1.5 times more total fatty acid content in cells of *Rhodomonas* sp. cultured in moderate and high pCO_2, respectively, than under ambient pCO_2. On a per cell basis microzooplankton gain more nutritional value from ingesting the lipid rich high pCO_2 acclimated *Rhodomonas* sp. cell than an ambient pCO_2 acclimated *Rhodomonas* sp. cell. An additional benefit to ingesting lipid rich cells at slower rates is that *G. dominans* and *Coxliella* sp. reduce energy expenditure towards prey searching and capturing. The grazing response I observed is consistent with John and Davidson (2001) and Chen et al. (2010). Chen et al. (2010) showed that the oligotrichous ciliate, *Strobilidium* sp., consumed the phytoplankton species, *Nannochloropsis* sp., at lower rates than *Isochrysis galbana,* presumably because *Nannochloropsis* sp. contained more polyunsaturated fatty acids than *I. galbana.* In another study, the microflagellate, *Paraphysomonas vestita*, also demonstrated a similar feeding behavior when presented two prey of similar size but with differing fatty acid composition (John and Davidson 2001). In that study, *P. vestita* ingested the prey containing more total fatty acids, *Pavlova lutheri*, at a lower rate than the fatty acid poor *I. galbana*. These findings suggest that my observations of *G. dominans* and *Coxliella* sp. ingesting moderate and high pCO_2 *Rhodomonas* sp. cells at a slower rate than ambient pCO_2 cultured

Rodomonas sp. may result from grazers potentially achieving the same nutritional benefit because of the difference in prey total fatty acids.

The lower ingestion rates of moderate and high cultured pCO_2 *Rhodomonas* sp. for *G. dominans* and *Coxliella* sp. in LT grazing experiments was a pattern not observed in the dinoflagellate *O. marina.* Instead, *O. marina* ingested moderate pCO_2 *Rhodomonas* sp. ~2.7 and 1.7 times faster than ambient and high pCO_2 *Rhodomonas* sp., respectively. A similar non-linear grazing trend by *O. marina* was also observed in the short term experiments (*O. marina* ingested moderate pCO_2 *Rhodomonas* sp. ~1.2 faster than ambient pCO_2 *Rhodomonas* sp. and high pCO_2 *Rhodomonas* sp. at equal rates to the moderate and ambient pCO_2 treatment) (Table 9).

It is unclear to me why no change in grazing response was observed between short and long term ingestion rate experiments for *O. marina.* In addition, the question remains, why did *O. marin*a ingest moderate pCO_2 cultured *Rhodomonas* sp. significantly faster than ambient and high in both short and long term grazing experiments? A similar inverted U-shaped ingestion rate response to elevated pCO_2 was also observed in the copepod species, *Pseudocalanus acuspes*, isolated from Svalbard (Thor and Oliva 2015). The authors proposed that this population of copepod species had adapted to feeding most efficiently in a mid-range pH because it resided in estuarine waters that can commonly experience changes in pH due to discharges of low-pH water from rivers and glaciers. Microzooplankton have demonstrated to have a high tolerance to direct effects of pCO_2/pH (Suffrian et al. 2008, Aberle et al. 2013), and the population of *O. marina* used in this study

has been maintained in a laboratory setting for many years. As such, pH variability wasn't a likely explanation for the observed grazing responses observed here.

Based on the OA induced increase in *Rhodomonas* sp. cell size and cellular total lipid content with increasing pCO_2, I expected to see a linear increase in *O. marina* ingestion rate during short term grazing experiments and linear decrease in *O. marina* long term ingestion rates as demonstrated by the other dinoflagellate, *G. dominans*. But because I observed the same grazing response pattern to occur for *O. marina* in both ST and LT experiments there is strong evidence to suggest the non-linear grazing response is true for this microzooplankton species when feeding on pCO_2 acclimated prey and not coincidental. *O. marina* grazing response appears to be more complex and may be dependent upon the interplay of prey characteristics, thus making it difficult to identify the mechanism behind the ingestion rate responses.

While, *O. marina* has been shown to discriminate prey based on size and biochemical composition (Wootton et al. 2007, Roberts et al. 2011, Meunier et al. 2011), the grazer's response may be affected by additional prey qualities (e.g. prey protein content, motility, chemical exudates etc.). It is possible a characteristic of *Rhodomonas* sp., which was not characterized in this study, may have been affected by pCO_2 in a non-linear fashion. For example, Wuori (2012) observed that under elevated pCO_2, a non-calcifying strain of *E. huxleyi* showed a non-linear response in particulate dimethylsulfoniopropionate ($DMSP_p$), a chemical known to retard some microzooplankton grazing response (Strom et al. 2003). While *Rhodomonas* sp. does not produce DMSP (Keller et al. 1989), another

secondary biochemical constituent of *Rhodomonas* sp. that was uncharacterized in this study may have behaved in a non -linear fashion.

Microzooplankton Population Growth

My data show that microzooplankton specific growth rates and GGE were significantly affected by the influence of OA on their prey. Higher growth rates were observed for all microzooplankton species tested when feeding on *Rhodomonas* sp cultured under moderate and high pCO_2 (Table 9). The dinoflagellates, *O. marina* and *G. dominans*, grew at rates ~ 1.5 and 2 times faster when feeding on moderate and high pCO_2 cultured *Rhodomnonas* sp., respectively, in comparison to ambient pCO_2 cultured *Rhodomonas* sp. The ciliate, *Coxliella* sp. grew at rates ~ 3 and 4 times faster when feeding on moderate and high pCO_2 cultured *Rhodomonas* sp., respectively, compared to ambient cultured *Rhodomonas* sp. The fact that microzooplankton grew at higher rates on *Rhodomonas* sp. cultured under high pCO_2, yet grazed these same cells at the lowest observed rates, supports my conclusion that OA-induced changes to *Rhodomonas* sp. food quality is affecting microzooplankton grazing. This is further supported by the higher observed GGEs for *G. dominans* and *Coxliella* sp. when feeding on high pCO_2 cultured *Rhodomonas* sp.

The results of this study show that when feeding on high pCO_2 *Rhodomonas* sp. the grazers *O. marina, G. dominans* and *Coxliella* sp. all showed higher GGE, calculated from using both short- and long-term ingestion rates (Table 9). This trend was only statistically significant for *G. dominans* and *Coxliella* sp. (Table 9). In the LT grazing experiments, *G. dominans* GGE increased by ~50 and 62% when feeding on moderate and high pCO_2 cultured *Rhodomonas* sp. compared to ambient grown cells. Similarly in LT grazing

experiments with *Coxliella* sp., GGE by ~30 and 54% when feeding on moderate and pCO_2 cultured *Rhodomonas* sp. compared to ambient grown cells.

Microzooplankton GGE values can range from 0 to 80%, but in general fall within the accepted 20-30% average range (Straile 1997). Microzooplankton GGEs calculated here fall below, within and above the generally accepted 20-30% average range. Overall, for all microzooplankton tested, I found GGEs from ST ingestion rate experiments likely underestimated the true growth efficiency because microzooplankton feeding rates may not have been at steady state. A possible explanation for this is that starved microzooplankton may feed at faster rates compared to rates averaged over longer time periods, with resulting lower assimilation efficiencies than microzooplankton maintained on a constant prey concentration. For example, Schmoker et al. (2011) observed lower GGE values (6, 17 and 30%) for *G. dominans* when given a single pulse of food in comparison to a constant concentration over time. I also found that *G. dominans* GGE values calculated from LT ingestion rates on moderate (GGE 84.6%) and high (GGE 95.4%) pCO_2 *Rhodomonas* sp. are likely overestimates since they fall above the 0 to 80% range stated above (Table 9). Some additional error in the GGE calculations may exist because I used reported microzooplankton pg C values from literature rather than empirically deriving this value by measuring each microzooplankton's bio-volume.

The significant increase in total fatty acids found in high pCO_2 *Rhodomonas* sp. could be contributing to the higher microzooplankton specific growth rates and GGE under elevated pCO_2. Both dinoflagellates and ciliates are observed to grow faster and have higher GGE when feeding on prey with increased fatty acids (John and Davidson 2001, Chen

et al, 2010). Certain essential fatty acids are a critical factor of food quality for microzooplankton and other consumers to ensure maximum population growth (Kattner et al. 2007, Leu et al. 2013). In addition, I found no negative OA effects on *Rhodomonas* sp. food quality such as elevated cellular C:N stoichiometry or low concentrations of cellular carbohydrates. Because I only measured total cellular lipids of *Rhodomonas* sp. and not specific lipid groups, I can only speculate on the degree to which increased total lipids added to the nutritional value of the prey. Protein content of *Rhodomonas* sp. was also not measured in this study, so it is unclear whether protein content may be a contributing food quality variable. But because I didn't observe a significant difference in *Rhodomonas* sp. cellular PON content with elevated pCO_2 it is probably unlikely there would be a significant difference in protein content.

Ecological Significance

Microzooplankton are instrumental players in the trophic interactions and biogeochemical processes at the base of the pelagic marine food web (Calbet & Landry 2004, Sherr and Sherr 2007). Despite studies suggesting microzooplankton have a high tolerance to direct effects of increasing pCO_2 concentrations (Suffrian et al. 2008, Aberle et al. 2013), this study provides evidence that there are direct OA effects on phytoplankton that can act as mechanisms for changes in microzooplankton grazing, population growth and GGE. With studies showing the direct response of phytoplankton morphology, physiology and biochemistry to OA to be species specific it is uncertain how widespread and prevalent the observed microzooplankton grazing and growth responses in this study will be in nature in future acidifying oceans . But if these microzooplankton responses are

prevalent, the question is: how could such changes in microzooplankton grazing and growth impact the marine food web in an acidifying ocean?

Elevated pCO_2 directly affected aspects of *Rhodomonas* sp. that are important to microzooplankton selection and ingestion of prey. The increase in cell size resulted in the prey being more optimal in size for some of the microzooplankton species studied. Similarly, the increase in total fatty acids possibly resulted in the microzooplankton species ingesting more lipids $cell^{-1}$ under elevated pcO_2 conditions. Depending on how OA affects the biology of the wide array of phytoplankton species, there will likely be an effect on microzooplankton prey selectivity. Microzooplankton may also be more selective of some phytoplankton species, like *Rhodomonas* sp., that are larger in size and more lipid rich than phytoplankton that are lipid poor, e.g. *Thalassiosira pseudonona* (Rossoll et al. 2012), under OA conditions. This active selection by microzooplankton could lead to changes in the overall phytoplankton community composition and structure in future acidified oceans. The direct bottom-up influence of OA on phytoplankton will also put selective pressure on which microzooplankton species are capable of thriving. For example, prey availability for microzooplankton may be impacted under OA conditions if there is a shift towards larger prey cell sizes, as suggested in this current study. However, it is important to recognize phytoplankton community composition may be more heavily influenced by other changing environmental variables, like increasing temperatures under future climate conditions rather than OA. Increasing surface ocean temperature could lead to a more stratified water column and limiting nutrients concentrations (Lewandowska et al. 2014). Consequently, this may favor smaller phytoplankton cells that are more competitive at taking up nutrients.

Therefore, it will be important to consider interactive effects of all changing environmental variables in future surface oceans when predicting phytoplankton community structure.

The microzooplankton-mediated changes resulting from OA may propagate through the food web and have potential effects on the fitness and recruitment of higher trophic levels (Malzahn et al. 2010, Schoo et al. 2012, Rossoll et al. 2012). Because microzooplankton GGE increased, the efficiency of the marine pelagic food web potentially may increase, possibly translating into more biomass in higher trophic levels, including commercially valuable fish populations.

While increased microzooplankton GGE may lead to stronger coupling with higher trophic levels, lower microzooplankton grazing and increased assimilation efficiency (as suggested by elevated GGE) could result in reduced POM/DOM production. Decreased egestion of organic matter and increased assimilation rates would dampen the role microzooplankton play in remineralizing nutrients in future acidified oceans. As a result this would have cascading effects on nutrient regeneration in the euphotic zone.

This is the first study to determine the indirect effects of OA on microzooplankton ingestion, growth and GGE. My findings show several pathways by which microbial food webs may be affected in a future ocean, and provide the foundation for future studies exploring this topic. Further research can explore whether other microzooplankton may have a species-specific response like I observed to OA induced changes in prey biology. This study was limited to only a few microzooplankton species and one phytoplankton prey species. In order improve the ability to extrapolate laboratory-based experiments on

individual species to natural communities more research is needed on a variety of microzooplankton and prey species.

APPENDIX A

CONTINUATION OF PHYTOPLANKTON CHARACTERIZATION

Table A1. Physiological parameters (average ± SD) of *Rhodomonas* sp. measured over time for semi-continuous experiments 1, 2, 3 and 4 in pCO_2 treatments ambient (400ppmv), moderate (750ppmv) and high (1000ppmv).

Parameter	Expt #, (days)	Ambient	Moderate	High
Growth rate (d^{-1})	1 (2-10)	0.59 ± 0.09	0.57 ± 0.10	0.56 ± 0.10
	2 (2-17)	0.55 ± 0.07	0.57 ± 0.05	0.57 ± 0.08
	3 (2-16)	0.58 ± 0.12	0.61 ± 0.09	0.65 ± 0.04
	4 (2-16)	0.56 ± 0.07	0.56 ± 0.08	0.55 ± 0.06
Cell bio-volume (μm^3)	1 (1-10)	145.2 ± 13.0	176.7 ± 15.3	186.9 ± 25.6
	2 (8-12, 16-17)	154.3 ± 19.2	216.4 ± 32.9	283.6 ± 47.0
	3 (8-16)	151.0 ± 18.1	247.6 ± 25.1	289.5 ± 25.7
	4 (8-16)	155.6 ± 18.3	241.5 ± 29.6	296.0 ± 32.8

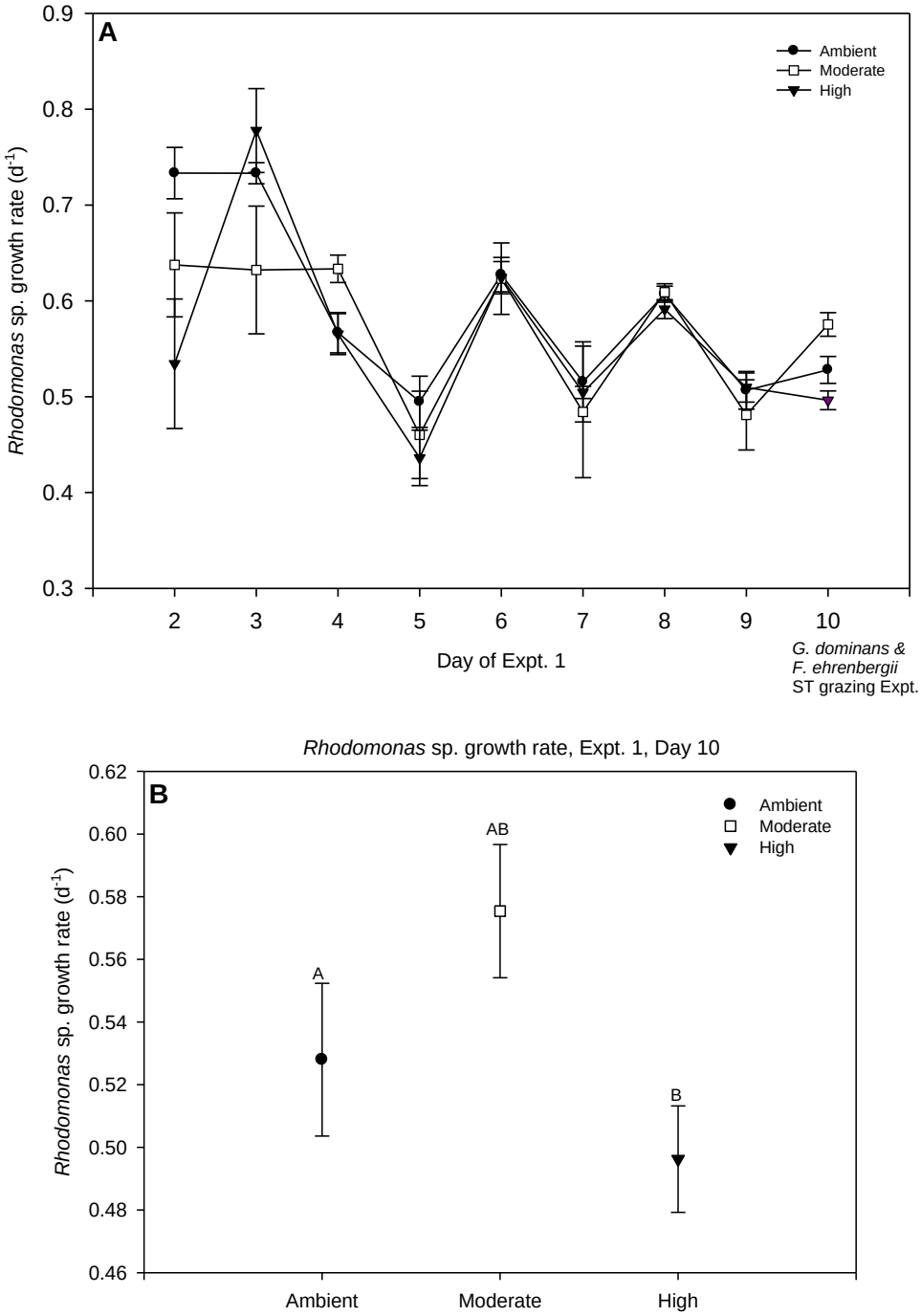


Figure A1. Growth rate (d^{-1}) (average ± SD, n=3) of *Rhodomonas* sp. cultured semi-continuously A) from day 1 to day 10 B) day 9 to 10 in pCO_2 treatments ambient, moderate and high for experiment 1. Experiment 1 corresponds to *Rhodomonas* sp. prey used in short term grazing experiments with *G. dominans* and *F. ehrenbergii* on day 10

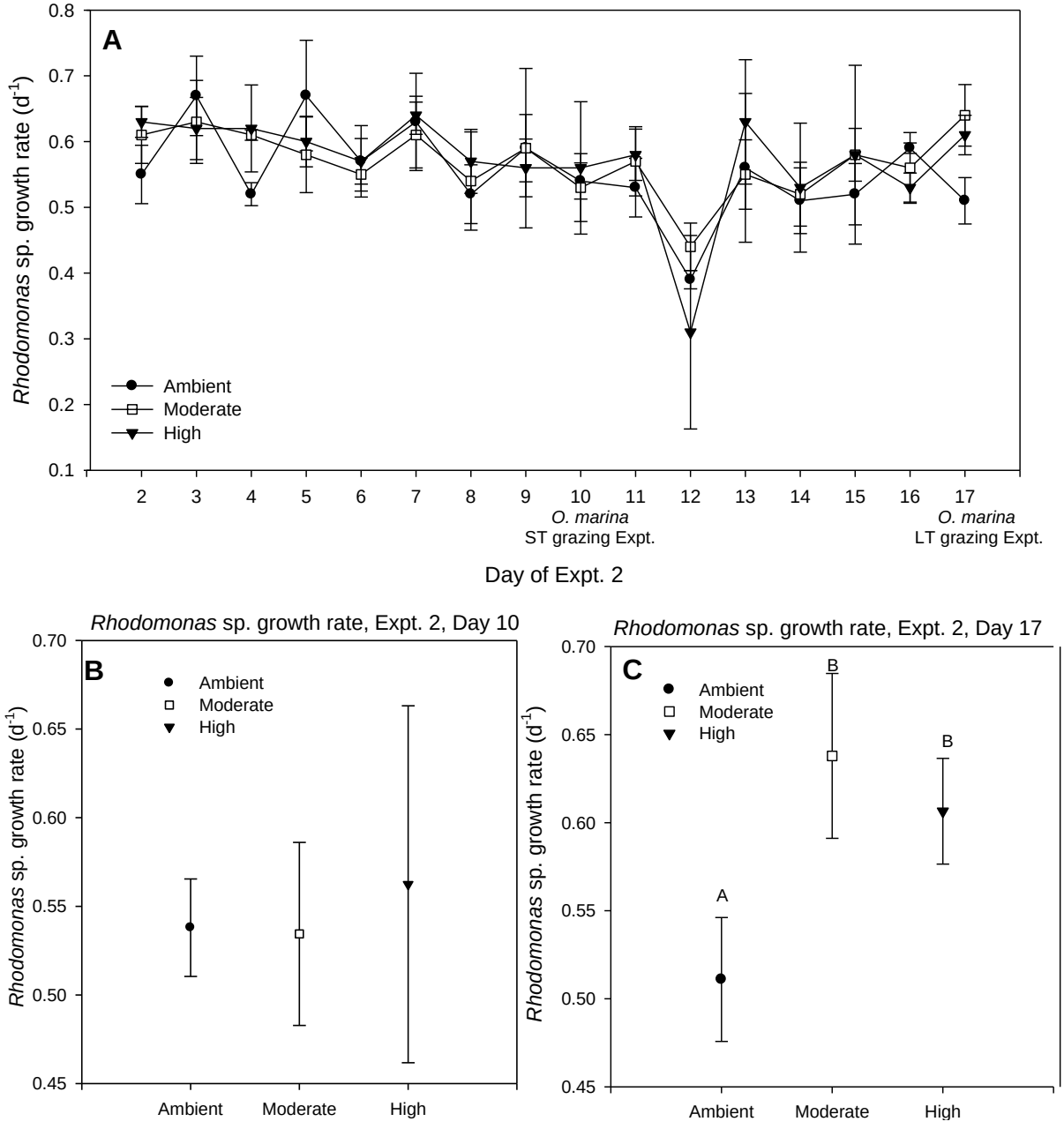


Figure A2. Growth rate (d^{-1}) (average ± SD, n=3) of *Rhodomonas* sp. cultured semi-continuously A) from day 1 to day 10, B) day 9 to 10 and C) day 16 to 17 in pCO_2 treatments ambient, moderate and high for experiment 2. Experiment 2 corresponds to *Rhodomonas* sp. prey used in short term and long term grazing experiments with *O. marina* on days 10 and 17, respectively.

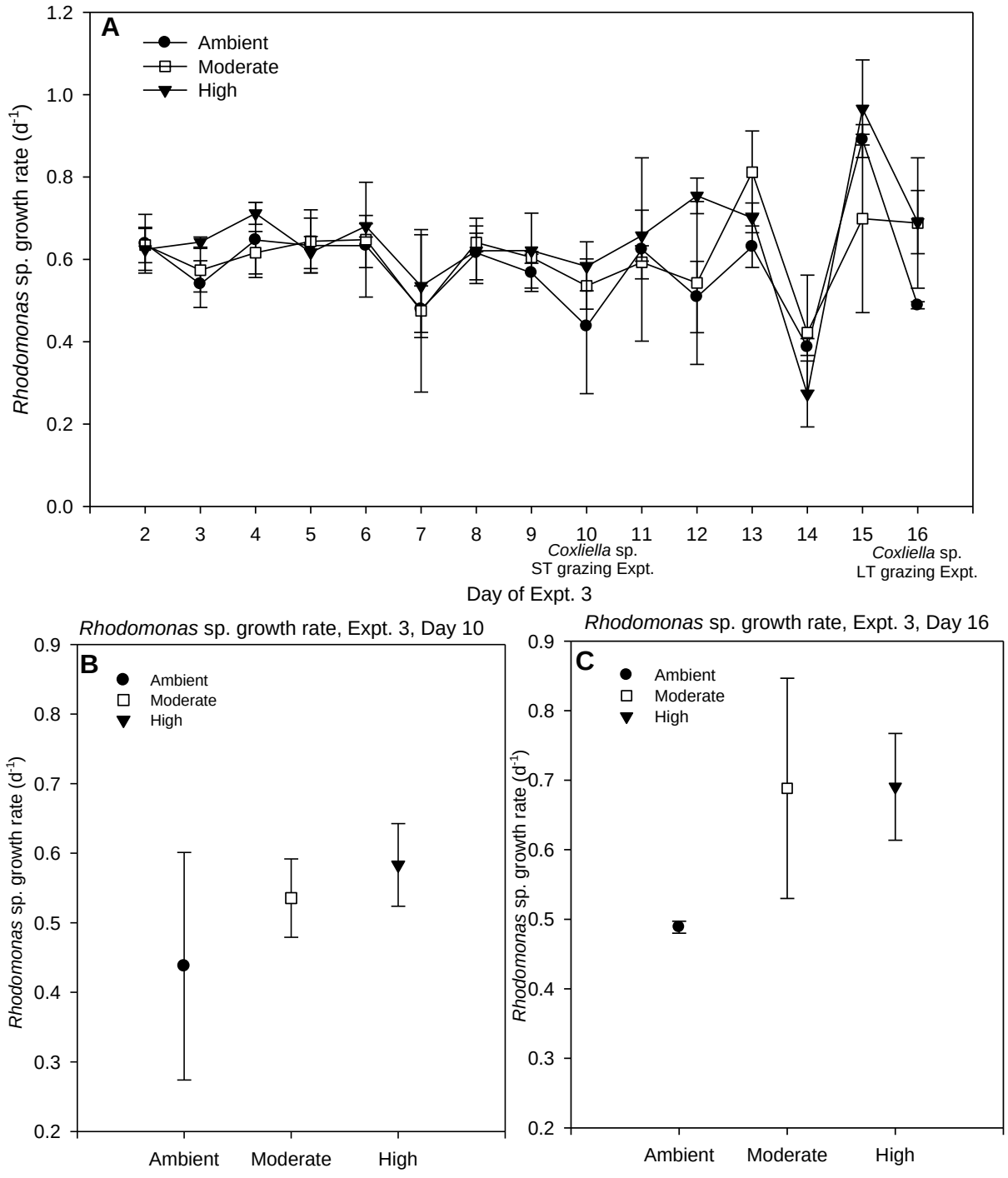
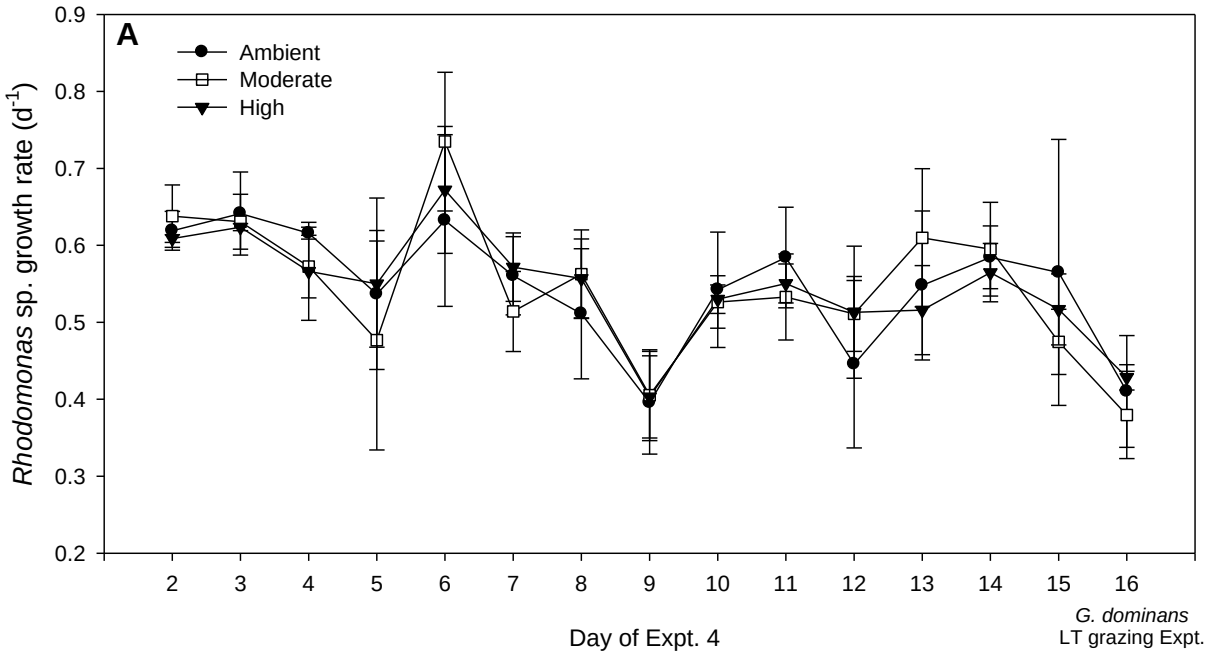


Figure A3. Growth rate (d^{-1}) (average ± SD, n=3) of *Rhodomonas* sp. cultured semi-continuously A) from day 1 to day 16, B) day 9 to 10 and C) day 15 to 16 in pCO_2 treatments ambient, moderate and high for experiment 3. Experiment 3 corresponds to *Rhodomonas* sp. prey used in short term and long term grazing experiments with *Coxliella* sp. on days 10 and 16, respectively.



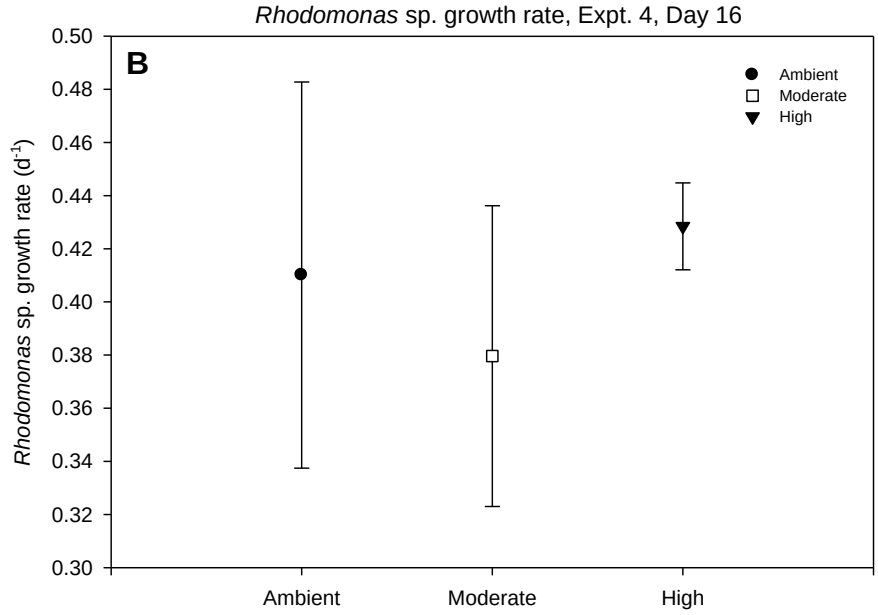


Figure A4. Growth rate (d^{-1}) (average ± SD, n=3) of *Rhodomonas* sp. cultured semi-continuously A) from day 1 to day 16 and B) day 15 to 16 in pCO_2 treatments ambient, moderate and high for experiment 4. Experiment 4 corresponds to *Rhodomonas* sp. prey used in long term grazing experiments with *Gyrodinium dominans* on day 16.

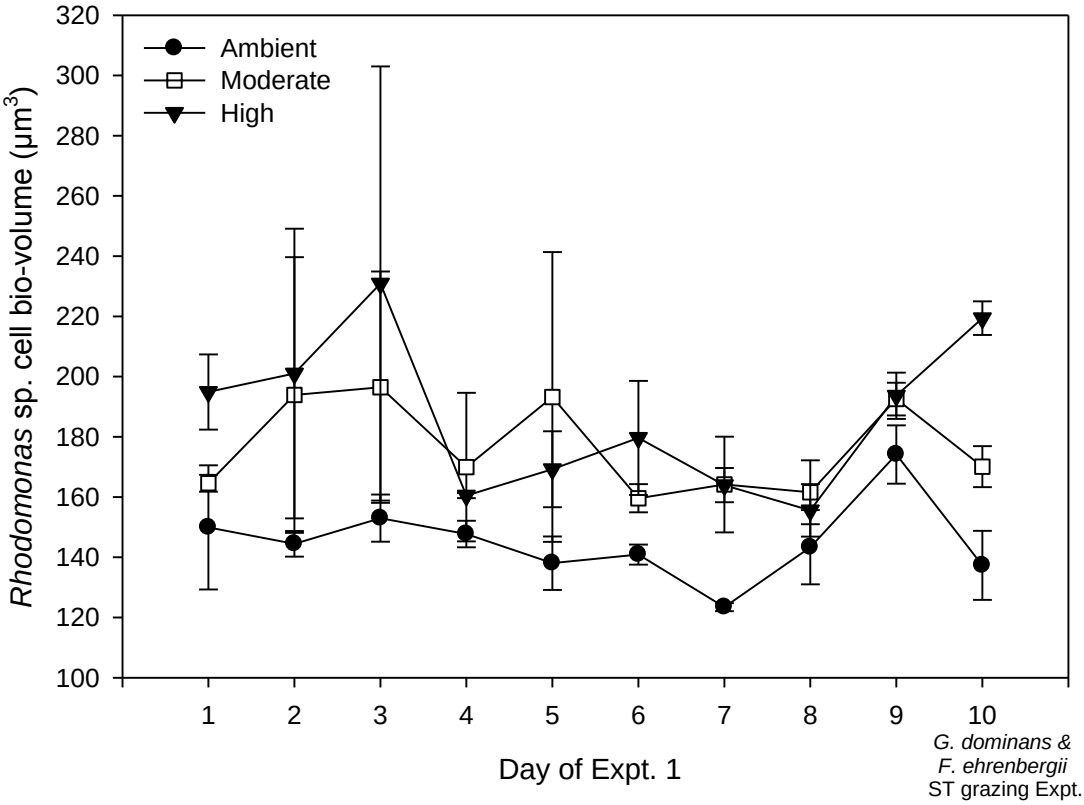


Figure A5. *Rhodomonas* sp. cell bio-volume (µm^3) (average ± SD, n=3) cultured semi-continuously on days 1 to 10 in pCO_2 treatments ambient, moderate and high for experiment 1. Experiment 1 corresponds to *Rhodomonas* sp. prey used in short term grazing experiments with *G. dominans* and *F. ehrenbergii* on day 10.

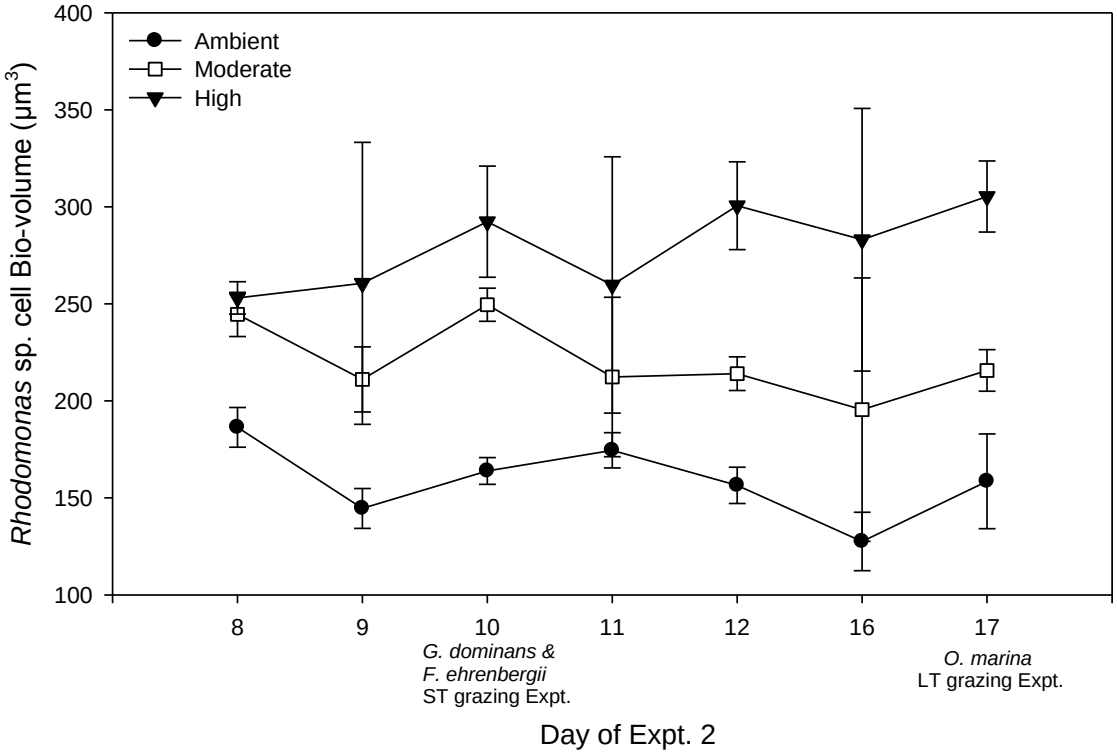


Figure A6. *Rhodomonas* sp. cell bio-volume (µm^3) (average ± SD, n=3) cultured semi-continuously on days 8-12; 16-17 in pCO_2 treatments ambient, moderate and high for experiment 2. Experiment 2 corresponds to *Rhodomonas* sp. prey used in short and long term grazing experiments with *O. marina* on day 9 and 17, respectively.

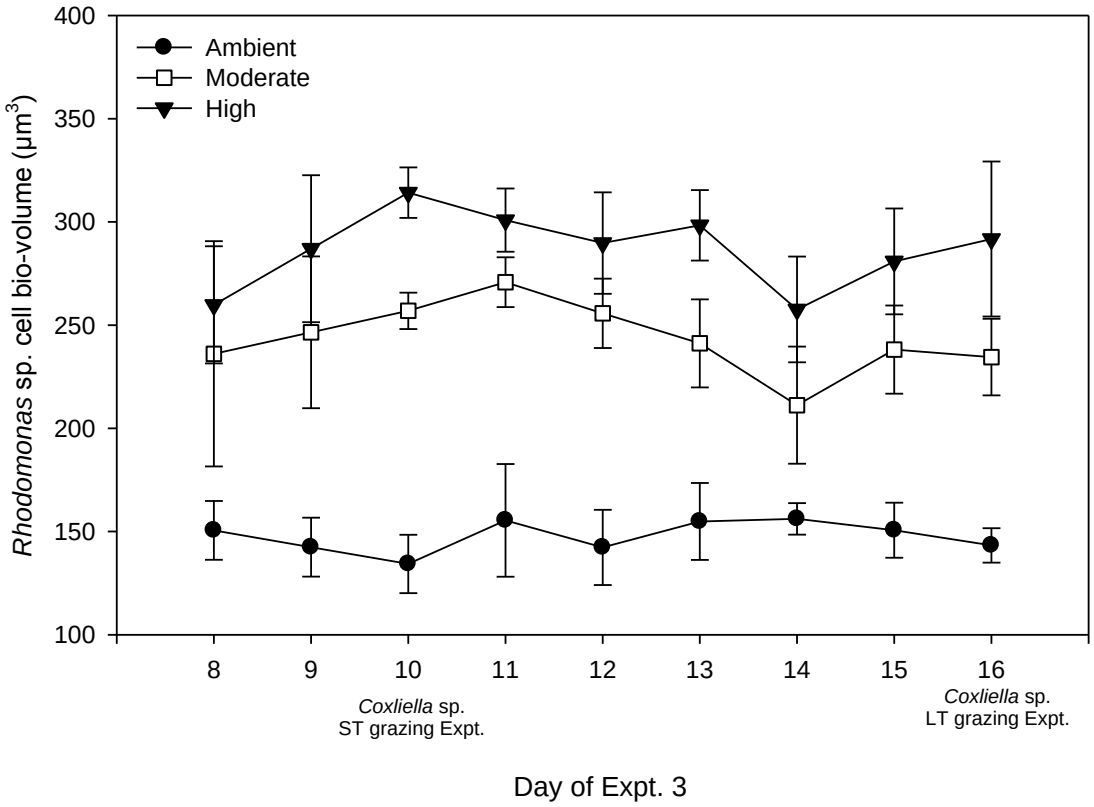


Figure A7. *Rhodomonas* sp. cell bio-volume (μm^3) (average ± SD, n=3) cultured semi-continuously on days 8 to 16 in pCO_2 treatments ambient, moderate and high for experiment 3. Experiment 3 corresponds to *Rhodomonas* sp. prey used in short and long term grazing experiments with *Coxliella* sp. on day 10 and 16, respectively.

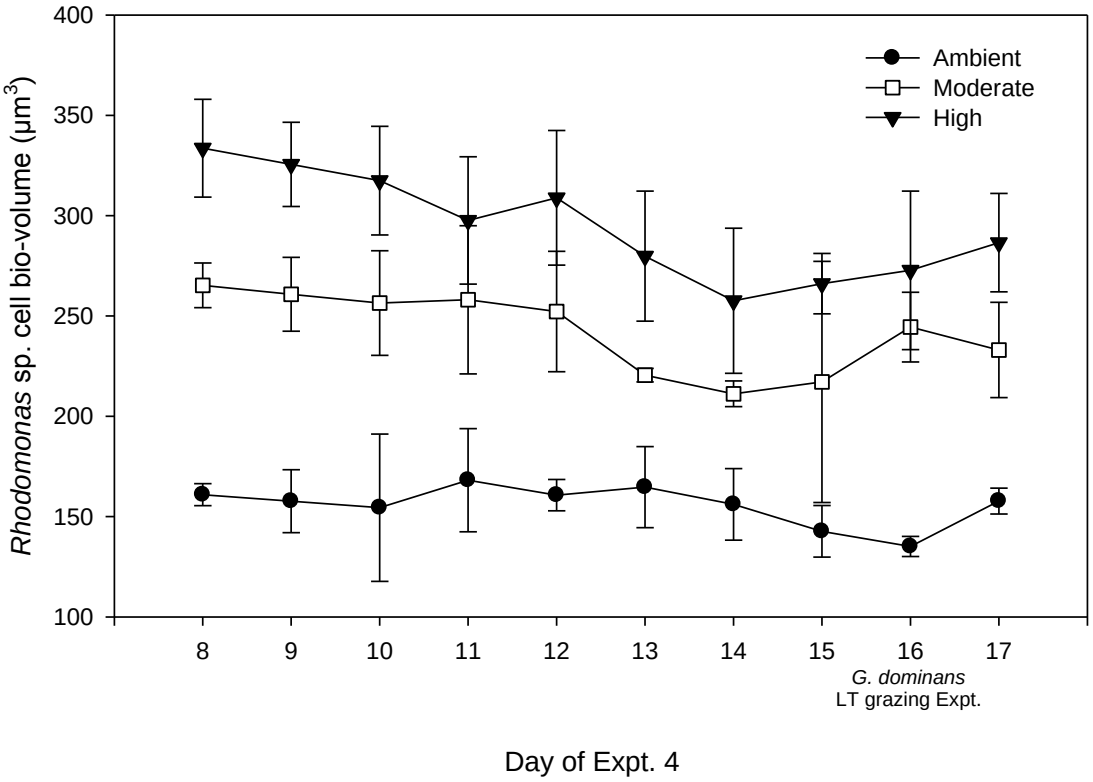


Figure A8. *Rhodomonas* sp. cell bio-volume (μm^3) (average ± SD, n=3) cultured semi-continuously on days 8 to 17 in pCO_2 treatments ambient, moderate and high for experiment 4. Experiment 4 corresponds to *Rhodomonas* sp. prey used in long term grazing experiments with *Gyrodinium dominans* on day 16.

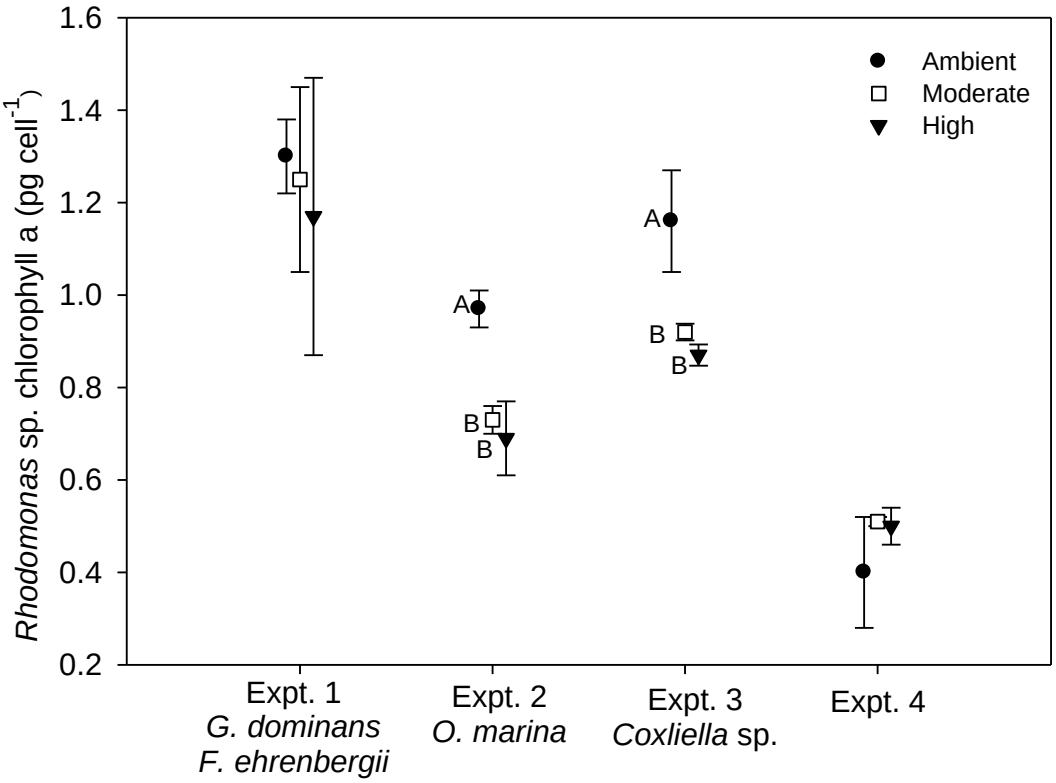


Figure A9. *Rhodomonas* sp. cellular chlorophyll a (pg cell^{-1}) (average ± SD, n=3) for pCO_2 treatments ambient, moderate and high on day 10 of semi-continuous culturing during ST grazing experiments with *G. dominans* and *F. ehrenbergii* (Expt. 1), *O. marina* (Expt. 2), *Coxliella* sp. (Expt. 3). No short term grazing experiment was done during experiment 4.

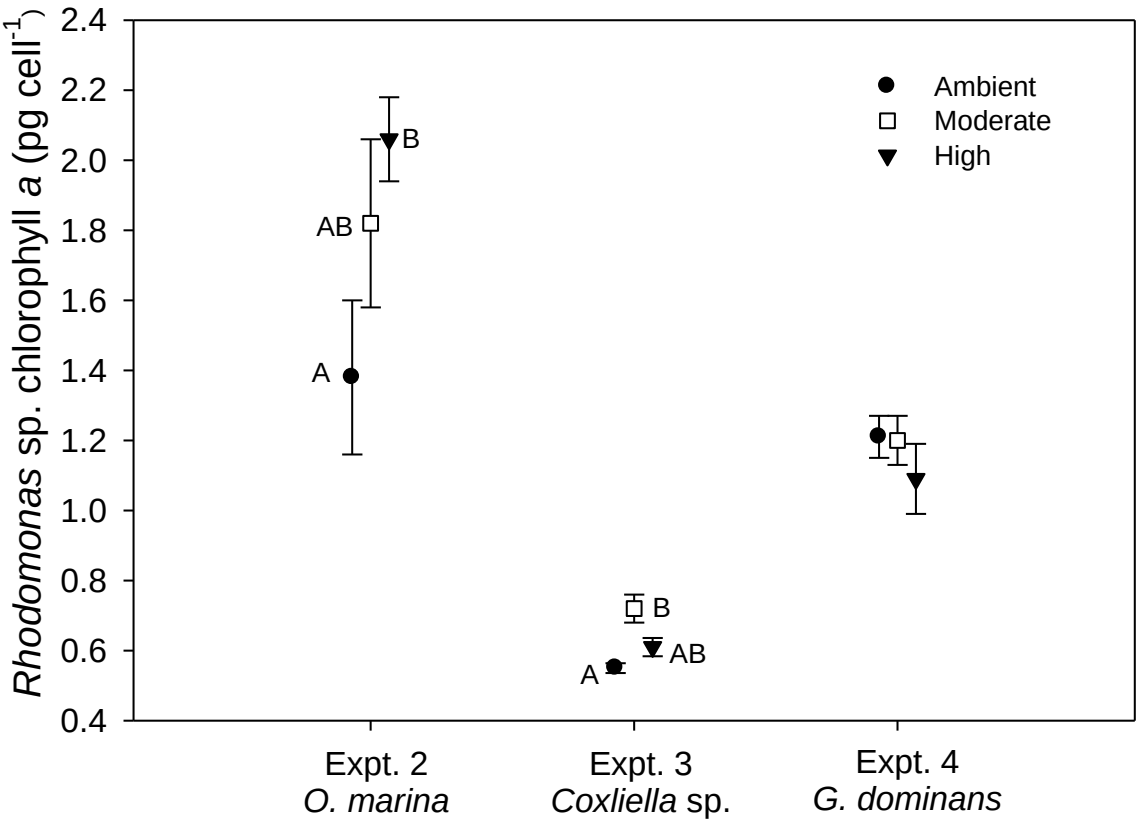


Figure A10. *Rhodomonas* sp. cellular chlorophyll *a* (pg cell^{-1}) average ± SD, n=3) for pCO_2 treatments ambient, moderate and high on the last day of semi-continuous culturing during LT grazing experiments with *O. marina* (Expt. 2), *Coxliella* sp. (Expt. 3) and *G. dominans* (Expt. 4).

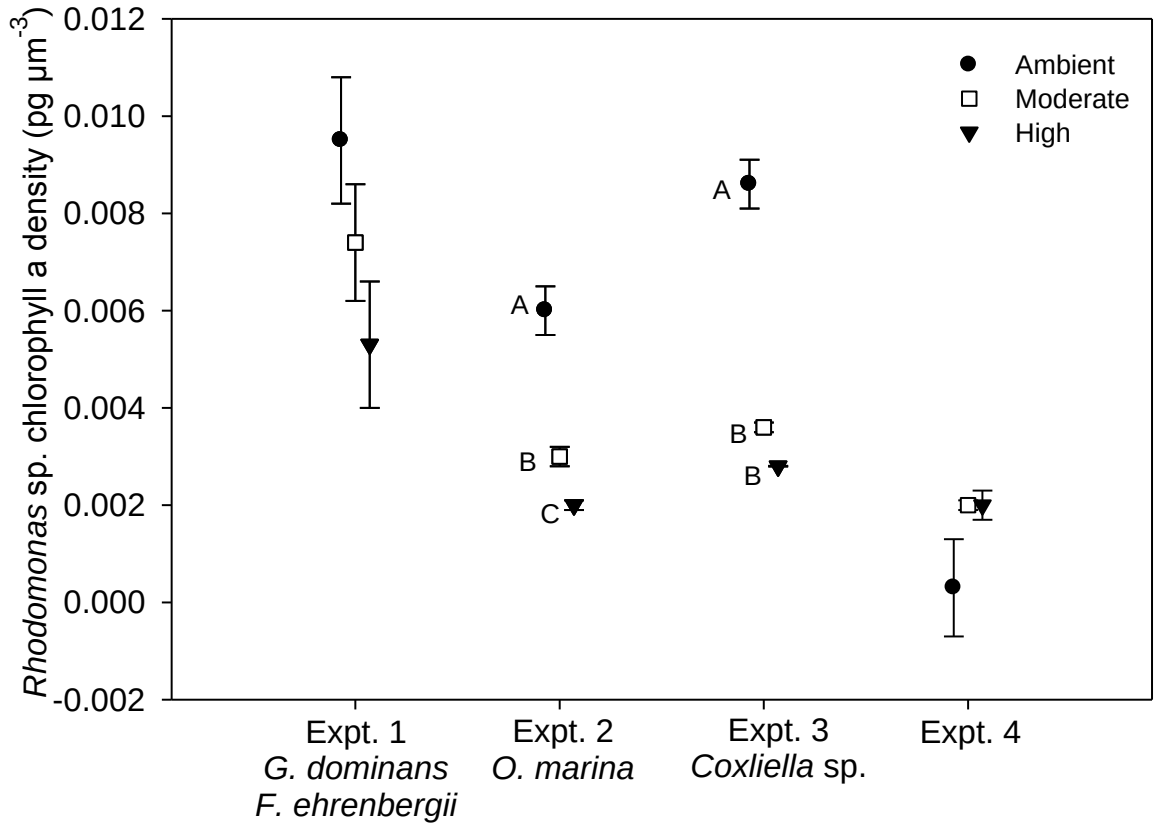


Figure A11. *Rhodomonas* sp. cellular chlorophyll *a* density (pg µm^3) (average ± SD, n=3) for pCO_2 treatments ambient, moderate and high on day 10 of semi-continuous culturing during ST grazing experiments with *G. dominans* and *F. ehrenbergii* (Expt. 1), *O. marina* (Expt. 2), *Coxliella* sp. (Expt. 3).

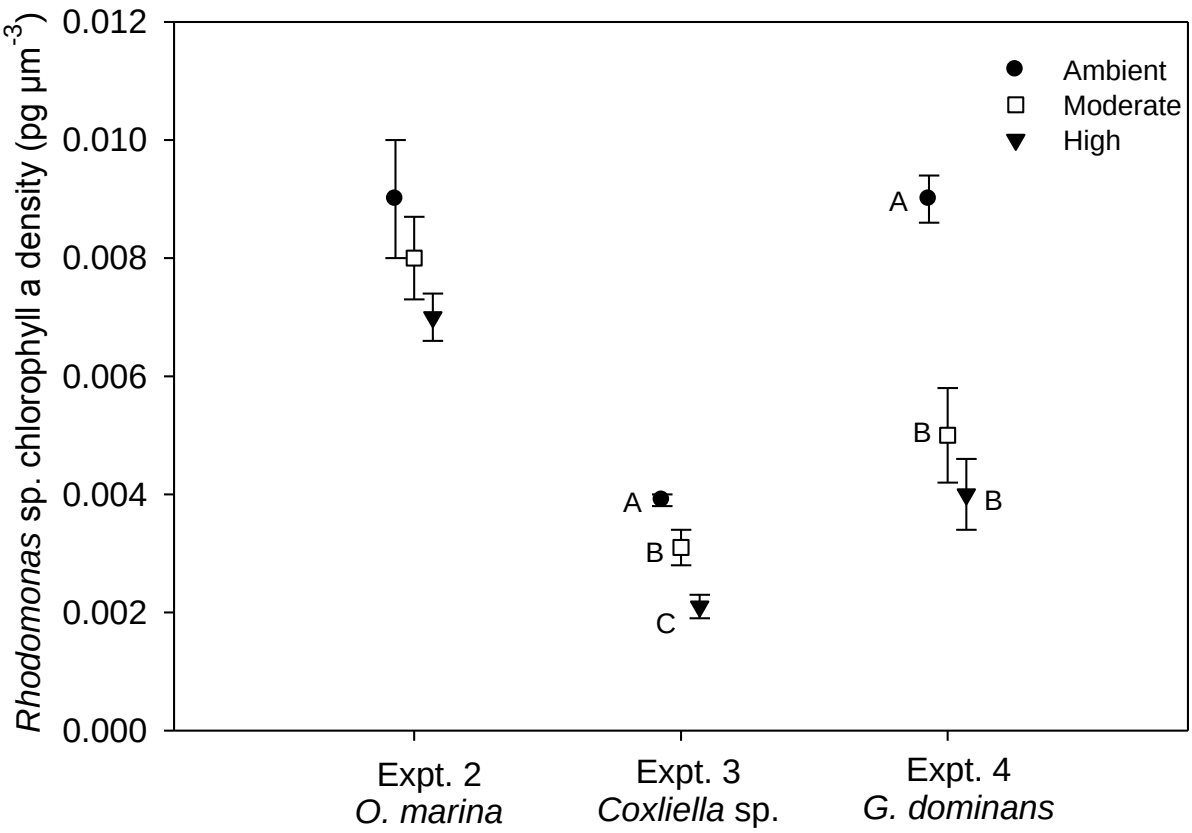


Figure A12. *Rhodomonas* sp. cellular chlorophyll *a* density (pg μm^3) average ± SD, n=3) for pCO_2 treatments ambient, moderate and high on the last day of semi-continuous culturing during LT grazing experiments with *O. marina* (Expt. 2), *Coxliella* sp. (Expt. 3) and *G. dominans* (Expt. 4).

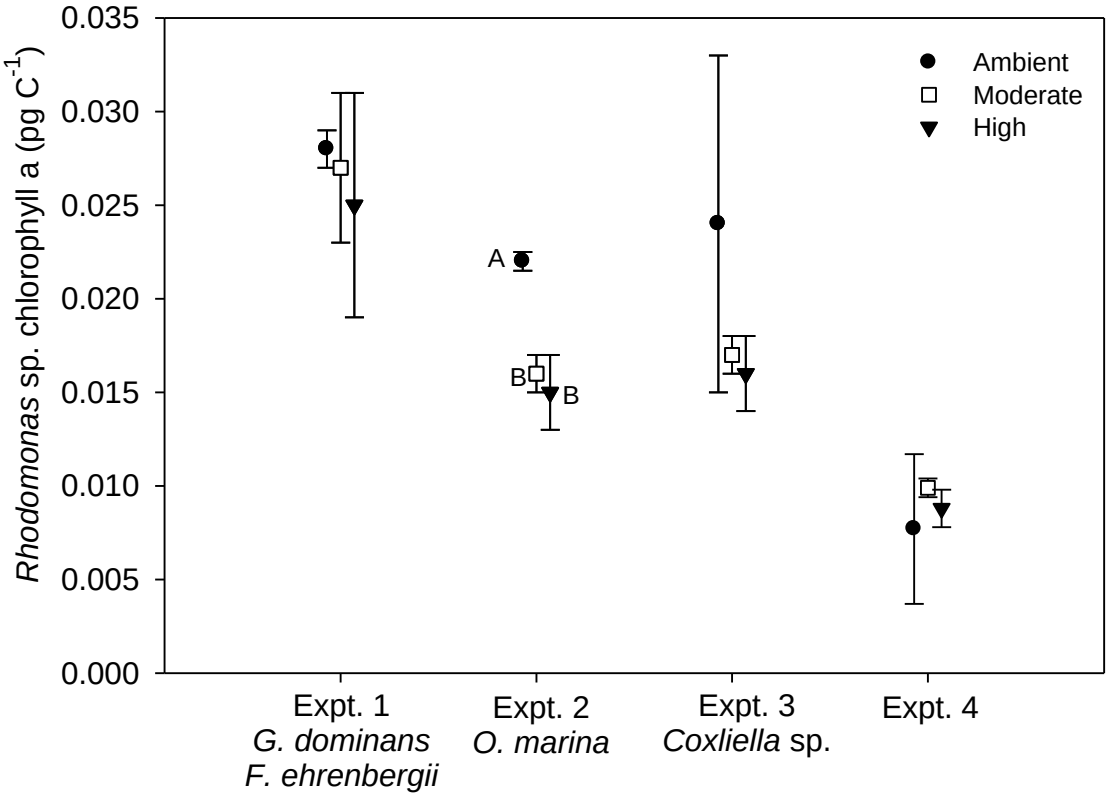


Figure A13. *Rhodomonas* sp. cellular chlorophyll *a* per carbon (pg C^{-1}) (average ± SD, n=3) for pCO_2 treatments ambient, moderate and high on day 10 of semi-continuous culturing during ST grazing experiments with *G. dominans* and *F. ehrenbergii* (Expt. 1), *O. marina* (Expt. 2), *Coxliella* sp. (Expt. 3).

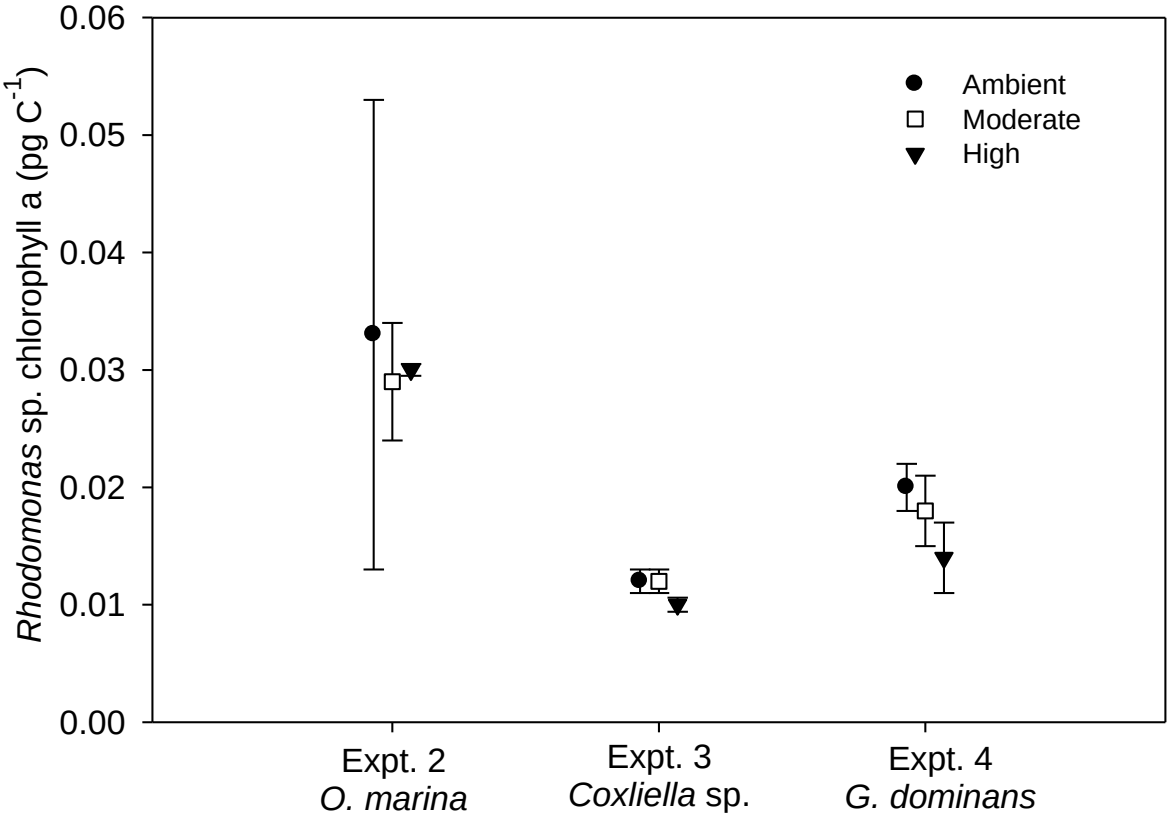


Figure A14. *Rhodomonas* sp. cellular chlorophyll *a* per pg carbon (pg chl a pg C^{-1}) (average ± SD, n=3) for pCO_2 treatments ambient, moderate and high on the last day of semi-continuous culturing during LT grazing experiments *O. marina* (Expt. 2), *Coxliella* sp. (Expt. 3) and *G. dominans* (Expt. 4).

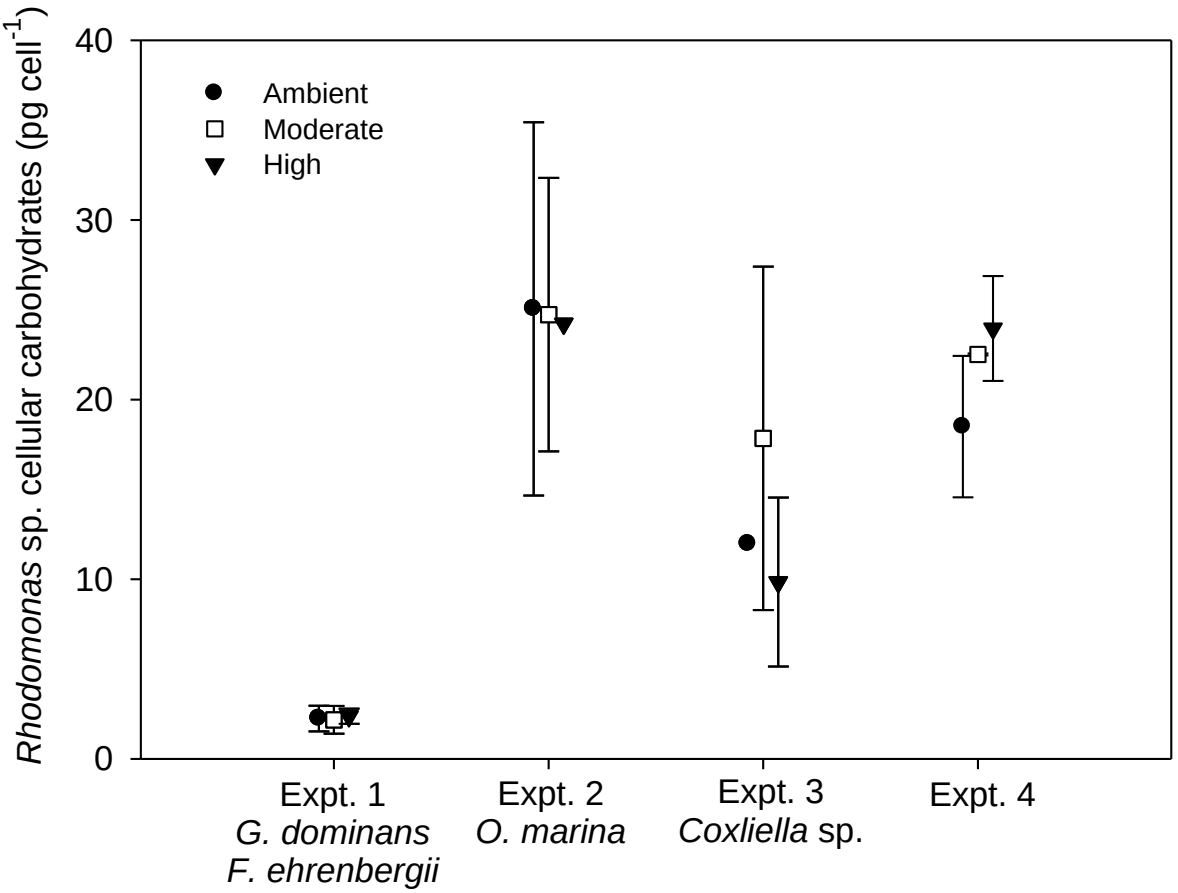


Figure A15. *Rhodomonas* cellular carbohydrates per cell (pg cell^{-1}) (average ± SD, n=3) for pCO_2 treatments ambient, moderate and high on day 10 of semi-continuous culturing during ST grazing experiments with *G. dominans* and *F. ehrenbergii* (Expt. 1), *O. marina* (Expt. 2), *Coxliella* sp. (Expt. 3).

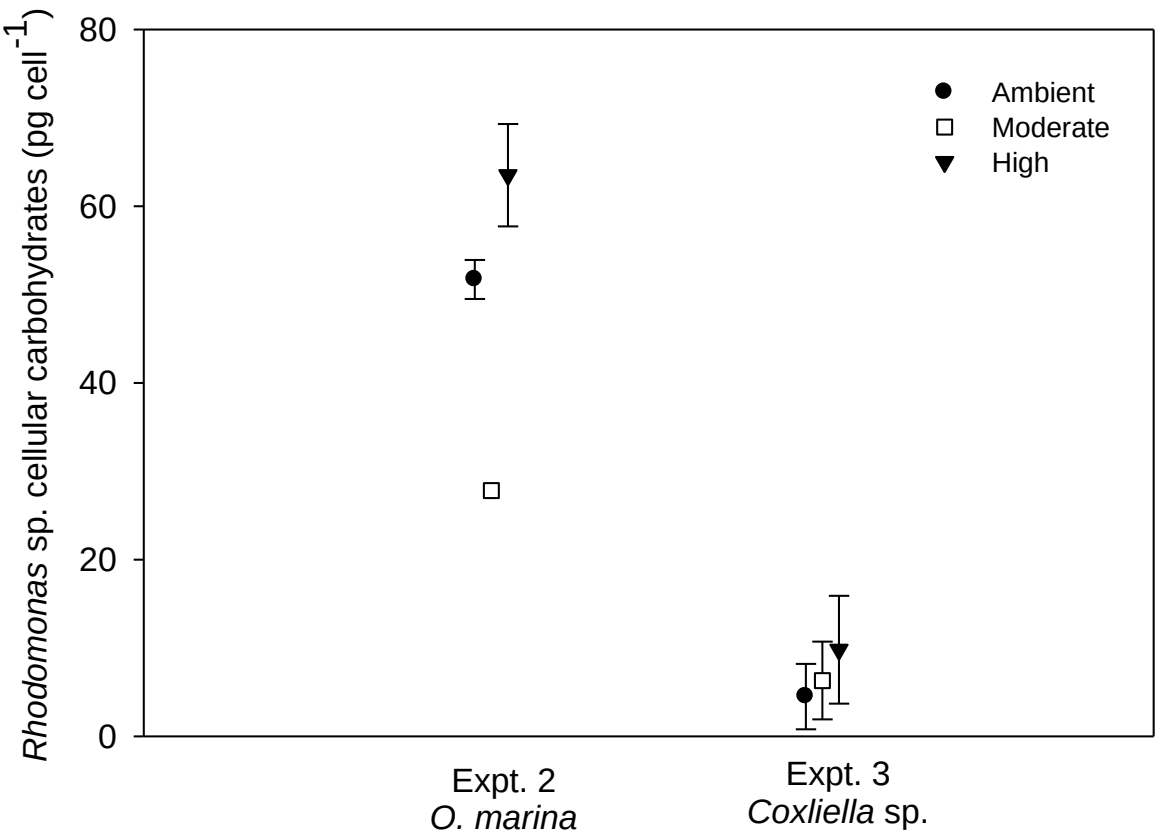


Figure A16. *Rhodomonas* cellular carbohydrates per cell (pg cell^{-1}) (average ± SD, n=3) for pCO_2 treatments ambient, moderate and high on the last day of semi-continuous culturing during LT grazing experiments with *O. marina* (Expt. 2) and *Coxliella* sp. (Expt. 3).

APPENDIX B

CONTINUATION OF MICROZOOPLANKTON GRAZING

Microzooplankton Short Term Ingestion Rate

Total Prey Cells Ingested

Microzooplankton, with the exception of *Coxliella* sp., showed increased feeding on high and moderate pCO_2 *Rhodomonas* sp. cells compared to ambient at the first time point (Figures B1, B2, B3 and B4). Though there was a strong treatment effect on total prey cells ingested by microzooplankton for the first time point, the size of this effect varied over time for each microzooplankton. *O. marina* showed the greatest difference between treatments in number of *Rhodomonas* sp. cells ingested at the second sample time point, 60 minutes (Figure B1). At the final sample time point, 120 minutes, the number of cells ingested by *O. marina* in the different treatments no longer increased linearly with time (Figure B1). For *G. dominans* and *F. ehrenbergii* the number of cells ingested in each treatment continued to increase in a linear fashion over the time of the ST grazing experiment (Figure B2 and B3). For *G. dominans,* more moderate and high pCO_2 cells are ingested at time point sampled than ambient pCO_2 *Rhodomonas* sp. (Figure B2). For *F. ehrenbergii,* a stepwise increase in the number of cells of *Rhodomonas* sp. occurs for ambient, moderate and high pCO_2 (Figure B3). The number of cells of *Rhodomonas* sp. ingested by *Coxliella* sp. over time does not significantly vary between pCO_2 treatments (Figure B4).

Microzooplankton Ingestion Rate: For Each Sample Time Point

Although the overall microzooplankton ingestion rates are the primary results of this study, looking in detail at rates calculated for individual time points can provide additional

Table B1. Replicate Linear fit short term ingestion rates (cells grazer^{-1} min^{-1}) of *Favella ehrenbergii, Gyrodinium dominans,* and *O. marina* when feeding on ambient, moderate and high pCO_2 *Rhodomonas* sp. The linear regression model equation and R^2 is listed for each treatment replicate (A, B and C).

Microzooplankton	pCO_2 treatment	Treatment replicate	Linear regression model equation	R^2	Average slope = ingestion rate
Favella ehrenbergii **(0-45min)**	Ambient	A	$y = 0.3333x + 0.4434$	0.9879	0.35 ± 0.015
		B	$y = 0.3608x + 0.4082$	0.9955	
		C	$y = 0.3513x + 0.5946$	0.9597	
	Moderate	A	$y = 0.3864x + 0.9122$	0.9707	0.39 ± 0.030
		B	$y = 0.3636x + 1.2741$	0.9620	
		C	$y = 0.4177x + 0.9579$	0.9727	
	High	A	$y = 0.465x + 1.2788$	0.9795	0.47 ± 0.015
		B	$y = 0.4633x + 1.2362$	0.9808	
		C	$y = 0.4923x + 0.9844$	0.9817	
Gyrodinium dominans **(0-90min)**	Ambient	A	$y = 0.0569x + 0.7374$	0.9798	0.053 ± 0.003
		B	$y = 0.0506x + 0.7524$	0.9677	
		C	$y = 0.0524x + 0.7457$	0.9706	
	Moderate	A	$y = 0.0586x + 0.9962$	0.9458	0.058 ± 0.003
		B	$y = 0.0581x + 1.0633$	0.9358	
		C	$y = 0.0583x + 0.9893$	0.9479	
	High	A	$y = 0.0677x + 1.0888$	0.9470	0.067 ± 0.001
		B	$y = 0.0659x + 1.2648$	0.9189	
		C	$y = 0.0677x + 1.1126$	0.9447	
Oxyrrhis marina **(0-60min)**	Ambient	A	$y = 0.0256x + 0.26003$	0.9816	0.026 ± 0.004
		B	$y = 0.0258x + 0.4173$	0.9715	
		C	$y = 0.0264x + 0.4853$	0.9541	
	Moderate	A	$y = 0.0306x + 0.4963$	0.9607	0.031 ± 0.001
		B	$y = 0.0315x + 0.5473$	0.9479	
		C	$y = 0.0322x + 0.5383$	0.9439	
	High	A	$y = 0.0322x + 0.5373$	0.9350	0.029 ± 0.003
		B	$y = 0.0265x + 0.7413$	0.8110	
		C	$y = 0.0278x + 0.6472$	0.8697	

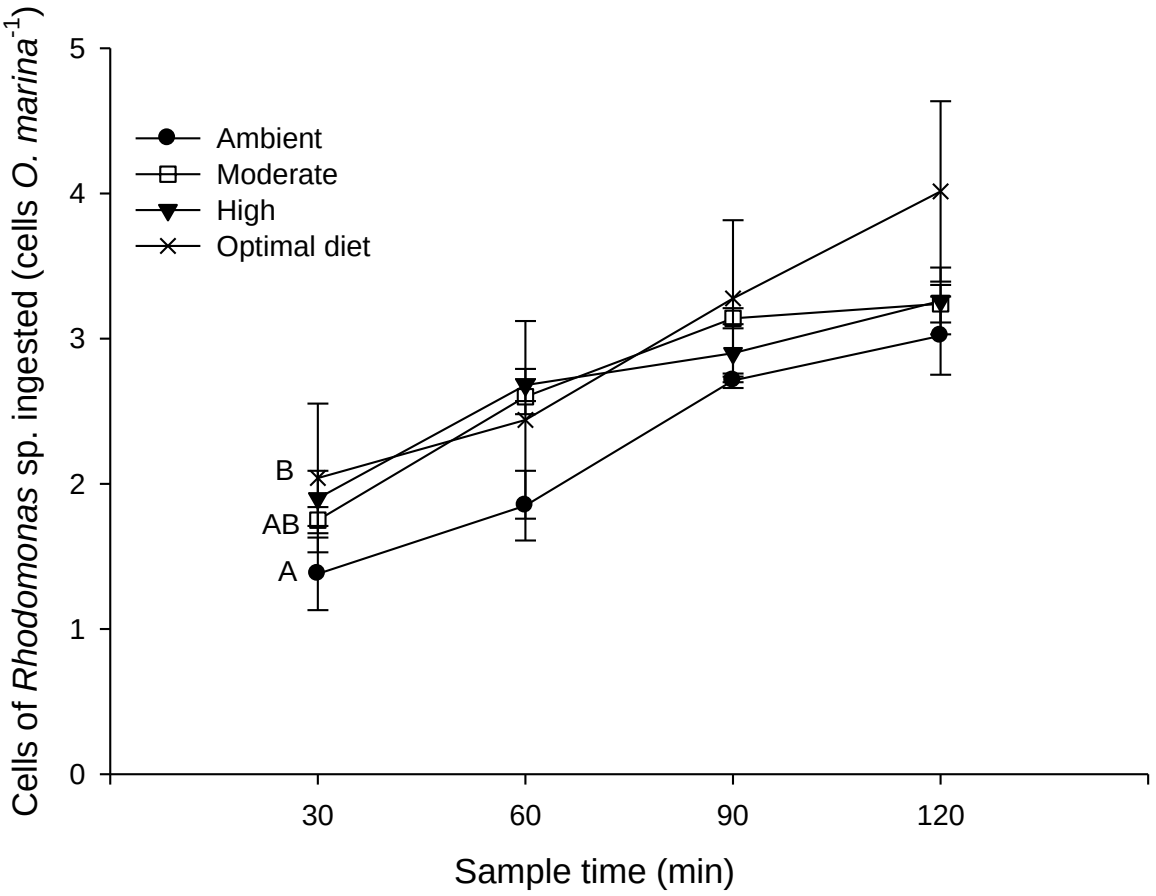


Figure B1. Total cells of *Rhodomonas* sp. (average ± SD, n=3) ingested by *Oxyrrhis marina* in pCO_2 treatments ambient, moderate, high at time points 30, 60, 90 and 120 minutes during a short term ingestion rate experiment. *Isochrysis galbana* was used as prey in the optimal diet treatment.

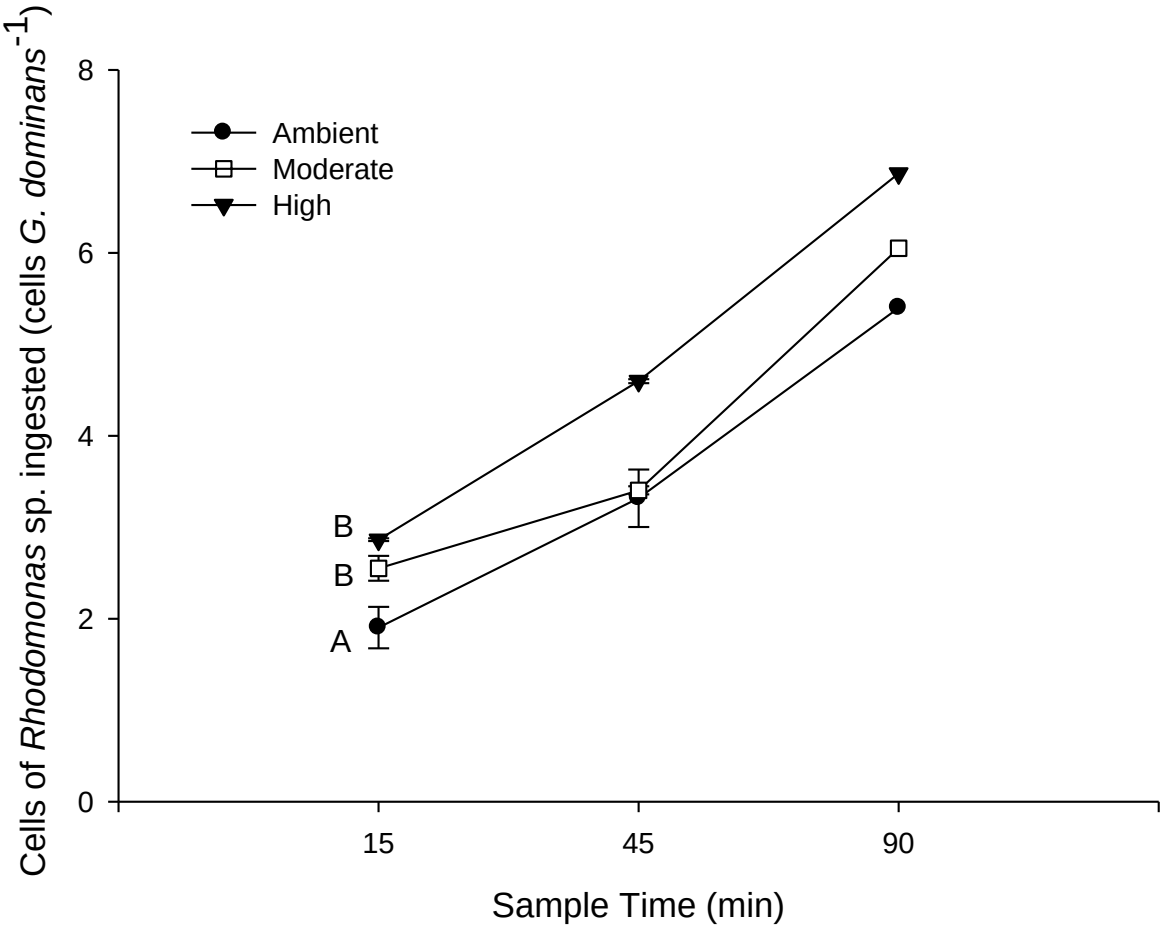


Figure B2. Total cells of *Rhodomonas* sp. (average ± SD, n=3) ingested by *G. dominans* in pCO_2 treatments ambient, moderate and high at time points 15, 45 and 90 minutes during a short term ingestion rate experiment.

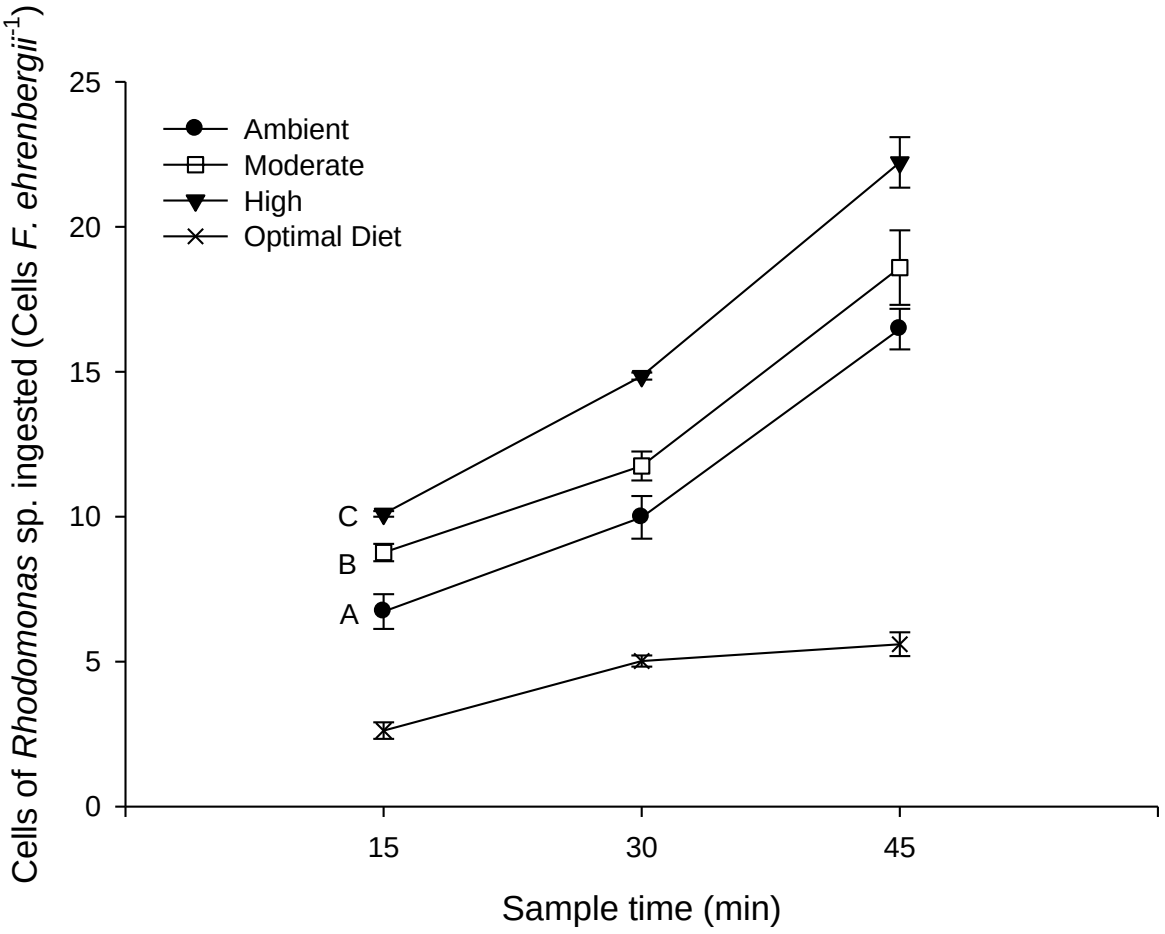


Figure B3. Total cells of *Rhodomonas* sp. (average ± SD, n=3)ingested by *F. ehrenbergii* in pCO_2 treatments ambient, moderate and high at time points 15, 30 and 45 minutes during a short term ingestion rate experiment. *Heterocapsa triquetra* was used as prey in the optimal diet treatment.

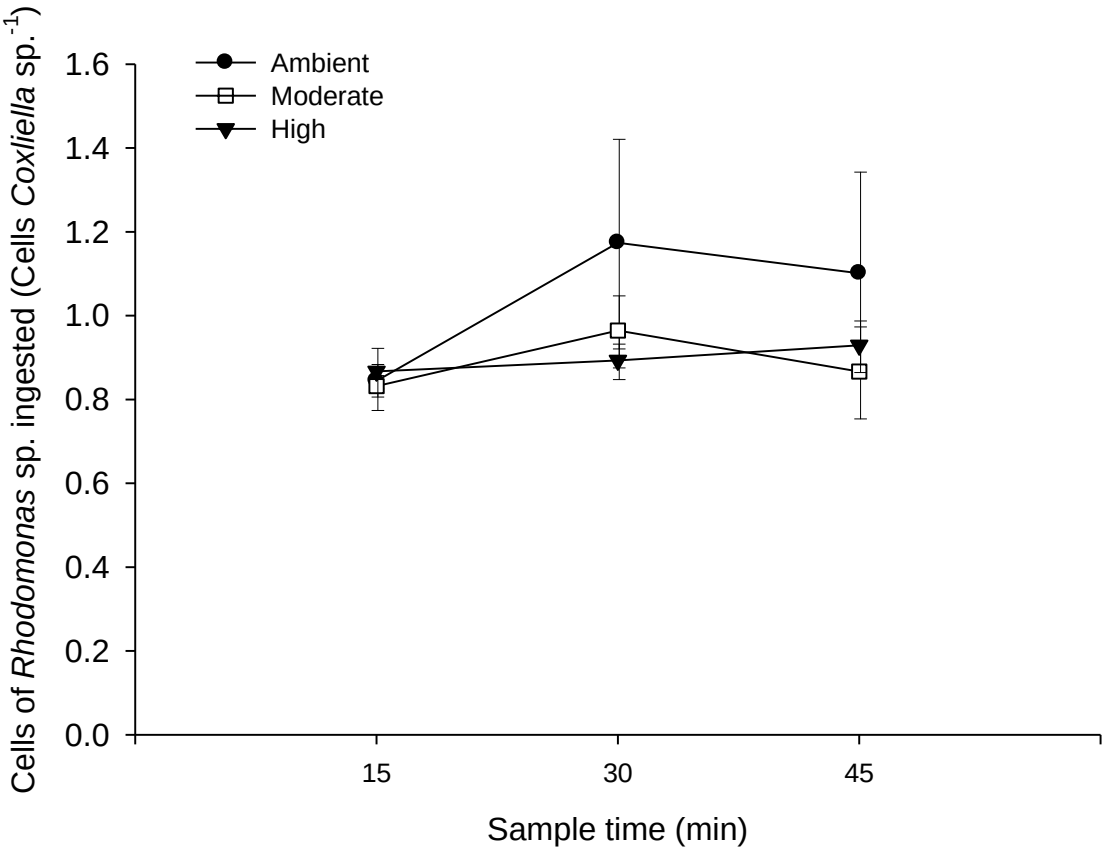


Figure B4. Total cells of *Rhodomonas* sp. (average ± SD, n=3) ingested by *Coxliella* sp. in pCO_2 treatments ambient, moderate and high at time points 15, 30 and 45 minutes during a short term ingestion rate experiment.

information in certain cases. Therefore, I also found individual microzooplankton ingestion rates between each sample time point by calculating the slope of the line relating cells ingested and time elapsed. The pCO_2 treatment effect on microzooplankton ingestion rates is particularly evident at the first time point for all grazers, with the exception of *Coxliella* sp. (Figures B5, B6, B7, and B8). The results from one way ANOVA revealed the ingestion rate for the first sample time point was significantly affected by pCO_2 for *G. dominans* ($F_{(2,6)}$=32.156, p=0.001), *F. ehrenbergii* ($F_{(2,6)}$=643.236, p<0.0001) and *O. marina* ($F_{(2,6)}$=6.048, p=0.036). G. dominans consumed moderate and high pCO_2 *Rhodomonas* sp. significantly faster than ambient pCO_2 *Rhodomonas* sp. after 15 minutes of feeding (Tukey's post hoc: ambient vs. moderate p=0.005, ambient vs. high p=0.001). A stepwise increase in F. *ehrenbergii* ingestion rate of ambient, moderate and high pCO_2 *Rhodomonas* sp. occurred after 15 minutes of feeding (Tukey's post hoc comparison: ambient vs. moderate p<0.0001, ambient vs. high p<0.0001 and moderate vs high p=0.001). Finally, *O. marina* fed on *Rhodomonas* cultured in high pCO_2 at a significantly faster rate than *Rhodomonas* sp. cultured in moderate and ambient pCO_2 (Tukey's post hoc comparison: ambient vs. moderate p=0.113, ambient vs. high p=0.035). No pCO_2 effect on *Coxliella* sp. ingestion of *Rhodomonas* sp. was found after 15 minutes of feeding (Figure B8, ANOVA, $F_{(2,6)}$=1.50, p=0.296).

Trends in the microzooplankton ingestion rate of *Rhodomonas* sp. after the first initial sample time differed between microzooplankton species (Figures B5, B6, B7 and B8). But, for all microzooplankton species, ingestion rates decreased after the first sample time point. For *O. marina* initial ingestion rates were 0.054 ± 0.006, 0.049 ± 0.003, and 0.037 ±

0.008 cells grazer^{-1} min^{-1} of high, moderate and ambient *Rhodomonas* sp., respectively. At 60 minutes, no significant pCO_2 treatment effect on *O. marina* ingestion rates was found and rates decreased to 0.026 ± 0.010, 0.0280 ± 0.004, and 0.016 ± 0.0008 cells grazer^{-1} min^{-1} for high, moderate and ambient pCO_2 *Rhodomonas* sp. (a 52, 43 and 57% decrease, respectively). After 60 minutes, at sample time 90 minutes *O. marina* ingested high pCO_2 *Rhodomonas* sp. significantly faster than ambient pCO_2 *Rhodomonas* sp. (ANOVA, $F_{(2,6)}$=17.244, p=0.003; Tukey's post hoc: ambient vs. high p=0.003). No pCO_2 treatment effect on *O. marina* ingestion of *Rhodomonas* sp. was found after 90 minutes of grazing. For *G. dominans,* initial ingestion rates were 0.17 ± 0.014, 0.15 ± 0.003 and 0.1 ± 0.009 cells grazer^{-1} min^{-1} of high, moderate and ambient pCO_2 *Rhodomonas* sp., respectively. Again, there was a decline in the rate of ingestion by *G. dominans* on *Rhodomonas* sp. at sampling point 45 minutes to 0.058 ± 0.007, 0.044 ± 0.004 and 0.047 ± 0.011 cells grazer^{-1} min^{-1} in high, moderate and ambient treatments (a 66, 71 and 56% decrease, respectively). No significant pCO_2 effect on the ingestion rate of *Rhodomonas* sp. existed at sample times after 15 minutes for *G. dominans*. A significant pCO_2 effect on the ingestion rate of *Rhodomonas* sp. prevailed at each sampling time point for *F*. *ehrenbergii* (Figure B7). After *F. ehrenbergii* fed on *Rhodomonas* sp. for 15 minutes ingestion rates were 0.5 ± 0.007, 0.47 ± 0.004 and 0.32 ± 0.008 in high, moderate and ambient pCO_2 treatments (ANOVA, $F_{(2,6)}$ 643.236, p<0.0001; Tukey's post hoc: ambient vs. moderate p<0.0001, ambient vs. high p<0.0001, moderate vs. high p=0.001). Between sampling points 15 and 30 minutes *F. ehrenbergii* ingestion rates declined to 0.22 ± 0.02, 0.16 ± 0.01 and 0.23 ± 0.03 in high,

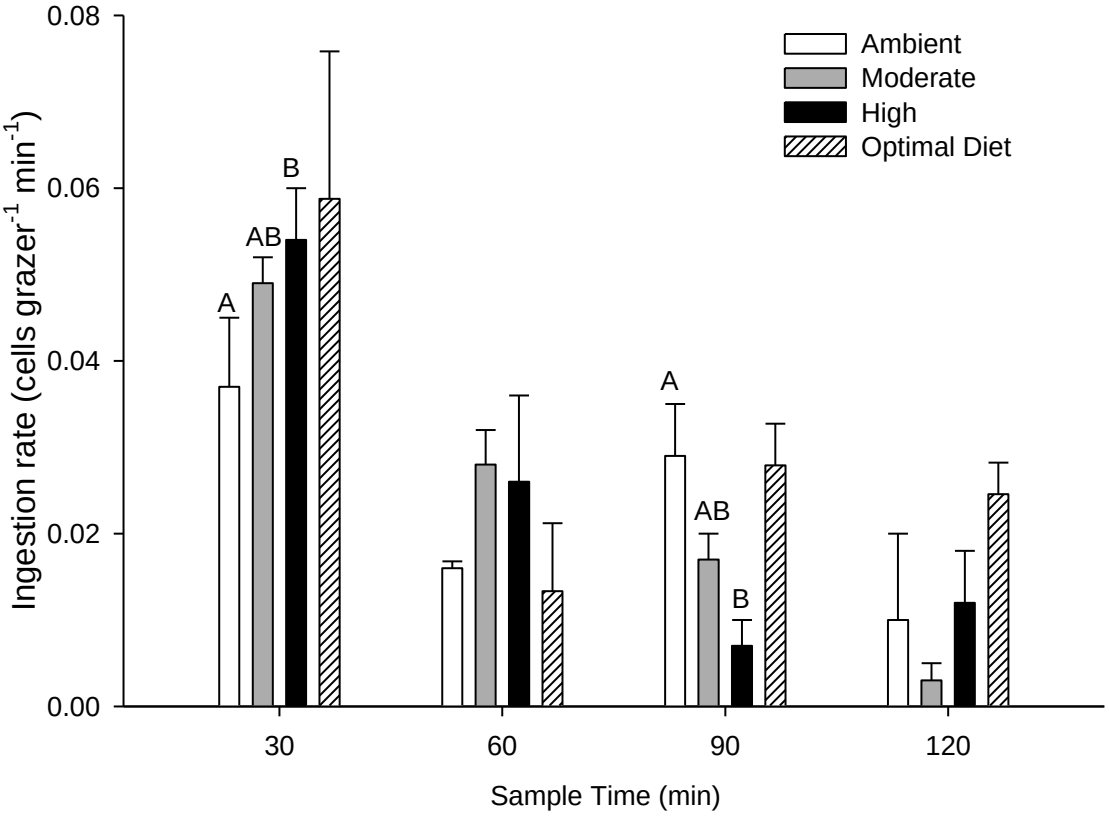


Figure B5. Short term ingestion rate of *Oxyrrhis marina* (average ± SD, n=3) (cells grazer^{-1} min^{-1}) feeding on *Rhodomonas* sp. cultured semi-continuously in pCO_2 treatments ambient moderate and high at time points 30, 60, 90 and 120 minutes. *Isochrysis galbana* was used as prey in the optimal diet treatment. The ingestion rate is the slope of the line relating cells ingested since last time point per minute. The letters A and B on the graph indicate Tukey's post hoc significance ($p<0.05$) at all sample time points.

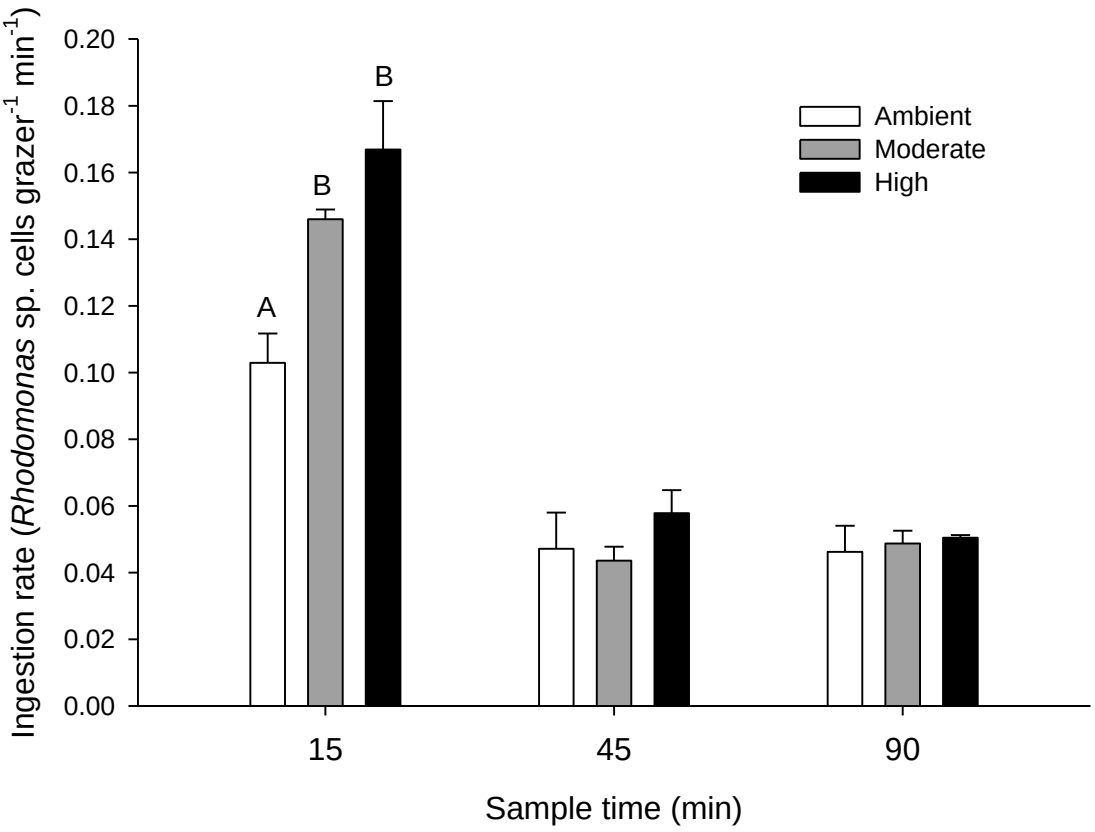


Figure B6. Short term ingestion rate of *Gyrodinium dominans* (average ± SD, n=3) feeding on *Rhodomonas* sp. cultured semi-continuously in pCO_2 treatments ambient moderate and high at time points 15, 45 and 90 minutes. See Fig. B5 for how the ingestion rate is found. The letters A, and B on the graph indicate Tukey's post hoc significance when applicable at all sample time points.

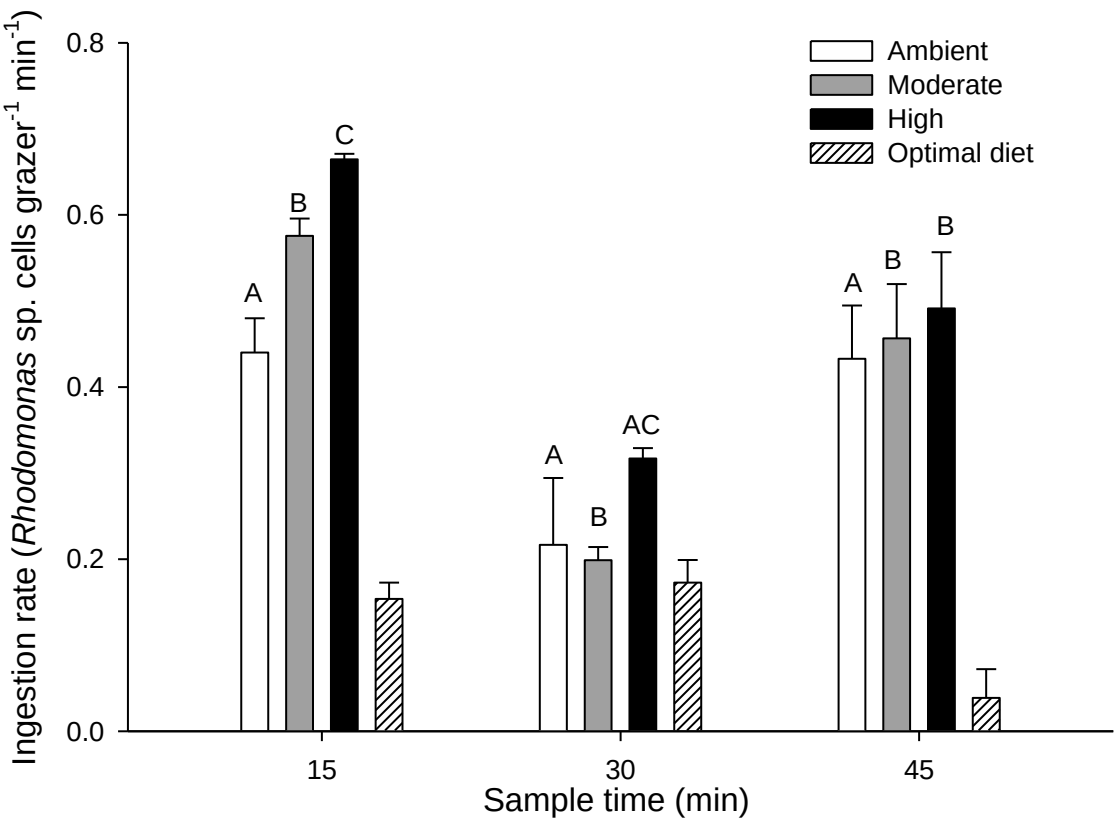


Figure B7. Short term ingestion rate of *Favella ehrenbergii* (average ± SD, n=3) (cells grazer^{-1} min^{-1}) feeding on *Rhodomonas* sp. cultured semi-continuously in pCO_2 treatments ambient moderate and high at time points 15, 30 and 45 minutes. *Heterocapsa triquetra* was used as prey in the optimal diet treatment. See Fig. B5 for how the ingestion rate is found. The letters A, B, and C on the graph indicate Tukey's post hoc significance ($p<0.05$) at all time points.

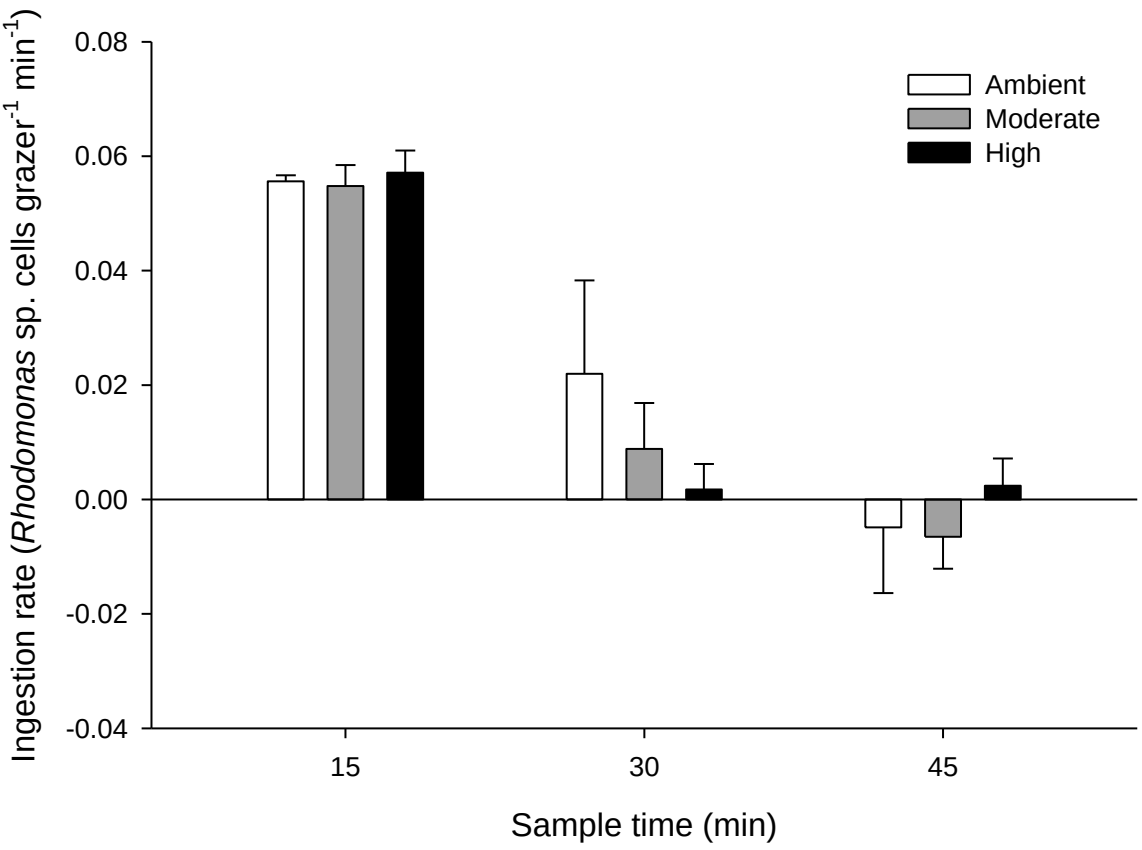


Figure B8. Short term ingestion rate of *Coxliella* sp. (average ± SD, n=3) feeding on *Rhodomonas* sp. cultured semi-continuously in pCO_2 treatments ambient moderate and high at time points 15, 30 and 45 minutes. See Fig. B5 for how the ingestion rate is found.

moderate and ambient pCO_2 treatments (a 56, 66 and 28% decrease, respectively). But *F. ehrenbergii* ingestion rates increased to 0.38 ± 0.01, 0.34 ± 0.01 and 0.29 ± 0.03 in high, moderate and ambient pCO_2 treatments after another 15 minutes passed at sampling point 45 minutes. *F. ehrenbergii* ingested *Rhodomonas* sp. cultured in moderate pCO_2 faster than ambient and high pCO_2 *Rhodomonas* sp. between the 15 and 30 minute sampling period (ANOVA, $F_{(2,6)}$=11.802, p=0.008; Tukey's post hoc: ambient vs. moderate p=0.010, moderate vs. high p=0.021). Between 30 and 45 sample times, *F. ehrenbergii* ingested moderate and high pCO_2 *Rhodomonas* sp. significantly faster than ambient pCO_2 *Rhodomonas* sp. (ANOVA, $F_{(2,6)}$=13.893, p=0.003; Tukey's post hoc: ambient vs. moderate p=0.029, ambient vs. high p=0.002).

Population of microzooplankton

Thus far, the data presented from ST ingestion rate experiments includes cells counts from all 100 microzooplankton food vacuoles encountered haphazardly. This includes any microzooplankton encountered with no ingested *Rhodomonas* sp. cells. In the case of empty microzooplankton food vacuoles, a zero was used and included in the calculation for average ingested cells by the microzooplankton at that time point. However, it is also valid to look at the data that only include microzooplankton that are actively feeding. Doing this provides additional information about the indirect effect elevated pCO_2 has on the ingestion of microzooplankton. When removing the microzooplankton not feeding from the data the overall patterns and trends of the results were the same.

Optimal Diet

On average *O. marina* ingested 2.04 ± 0.5, 2.44 ± 0.7, 3.28 ± 0.5 and 4.01 ± 0.6 cells of *I. galbana* at sample time points 30, 60, 90 and 120 minutes, respectively (Figure B1). *Rhodomonas* sp. cell biovolume (ambient: 144.6μm^3; moderate: 211.4 μm^3 and high: 260.6 μm^3) was larger than *I. galbana* (56 μm^3, Hansen et al. 1996). Therefore, even though it appears *O. marina* ingested a similar number of total prey cells in the optimal diet treatment for each sample time point, *O. marina* ingested less prey bio-volume in the optimal diet treatment. Average *O. marina* ingestion rates calculated from the slope of the lines relating *I. galbana* cells ingested and time elapsed were 0.059 ± 0.02, 0.013 ± 0.008, 0.028 ± 0.005 and 0.025 ± 0.004 cells $grazer^{-1}$ min^{-1} for the 0-30, 30-60, 60-90 and 90-120 minutes sample time intervals, respectively (Figure B5). To compare pCO_2 treatments to the optimal diet treatment prey ingested by *O. marina* was converted to biomass in pg C (Figure B13 and B14). On average, *O. marina* ingested less pg C of prey in the optimal diet treatment at each sample time point in comparison to ambient, moderate and high pCO_2 treatments (Figure B13) (30 minutes: ANOVA, $F_{(3,8)}$=121.891, p<0.0001; Tukey's post hoc hoc: optimal diet vs. ambient; moderate; high p<0.0001 ; 60 minutes: ANOVA, $F_{(3,8)}$=191.982, p<0.0001; Tukey's post hoc: optimal vs. ambient; moderate; high p<0.0001; 90 minutes: ANOVA, $F_{(3,8)}$=257.442, p<0.0001; Tukey's post hoc: optimal diet vs. ambient; moderate; high p<0.0001; 120 minutes: ANOVA, $F_{(3,8)}$=225.998, p<0.0001; Tukey's post hoc: optimal diet vs. ambient; moderate; high p<0.0001).

On average, F. *ehrenbergii* ingested 2.43 ± 0.29, 5.02 ± 0.19 and 5.60 ± 0.41 cells of *H. triquetra* after 15, 30 and 45 minutes of grazing, respectively (Figure B3). Cell bio-volume

of *H. triquetra* (~4179µm^3, Olenina et al. 2006) is much larger than *Rhodomonas* sp. (ambient: 137.3µm^3; moderate: 170.9 µm^3 and high: 219.4 µm^3). Therefore, although *F. ehrenbergii,* ingested fewer cells of *H. triquetra* at each sample time the microzooplankton actually ingested more total bio-volume of prey in the optimal diet treatment. Average *F. ehrenbergii* ingestion rates calculated from the slope of the lines relating *H. triquetra* cells ingested and time elapsed for each replicate were 0.15 ± 0.02, 0.17 ± 0.03 and 0.04 ± 0.03 cells grazer^{-1} min^{-1} for the 0-15, 15-30 and 30-45 minutes sample time intervals, respectively (Figure B7). To compare pCO_2 treatments to the optimal diet, treatment prey ingested by *F. ehrenbergii* was converted to biomass in pg C (Figure B15 and B16). On average, *F. ehrenbergii* ingested more pg C in the optimal diet treatment at each sample time point in comparison to ambient, moderate and high pCO_2 treatments (Figure B15) (15 minutes: ANOVA, $F_{(3,8)}$=237.85, p<0.0001; Tukey's post hoc hoc: optimal diet vs. ambient; moderate; high p<0.0001 ; 30 minutes: ANOVA, $F_{(3,8)}$=176.09, p<0.0001; Tukey's post hoc: optimal vs. ambient; moderate; high p<0.0001; 45 minutes: ANOVA, $F_{(3,8)}$=503.282, p<0.0001; Tukey's post hoc: optimal diet vs. ambient; moderate; high p<0.0001). Similarly, *F. ehernbergii* ingestion rate (pg C grazer^{-1} min^{-1}) was significantly higher in the optimal diet treatment in comparison to ambient, moderate and high pCO_2 treatments at sample times 0-15 and 15-30 minutes (Figure B16) (15 minutes: ANOVA, $F_{(3,8)}$=198.218, p<0.0001; Tukey's post hoc hoc: optimal diet vs. ambient; moderate; high p<0.0001 ; 30 minutes: ANOVA, $F_{(3,8)}$=62.858, p<0.0001; Tukey's post hoc: optimal vs. ambient; moderate; high p<0.0001). After 45 minutes of feeding, *F. ehrenbergii* ingestion rate (pg C grazer^{-1} min^{-1}) is not significantly different in any treatment.

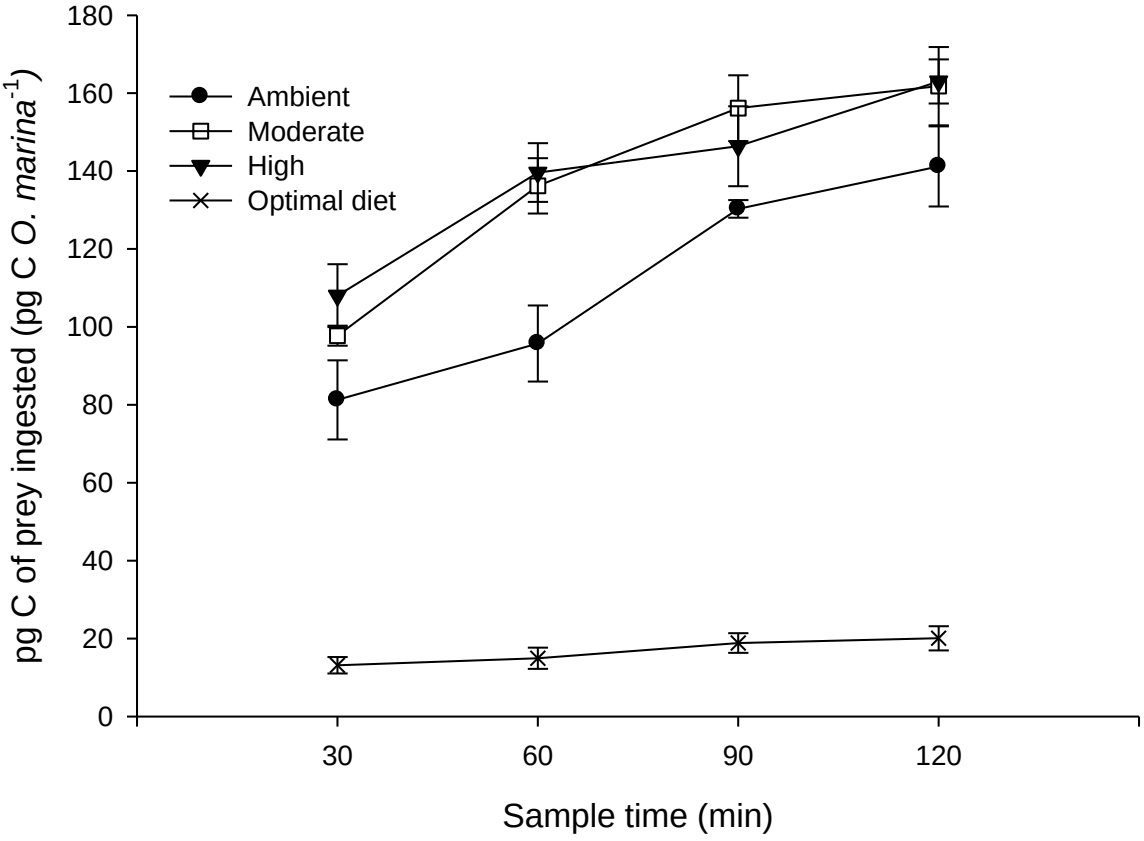


Figure B13. Total pg C of *Rhodomonas* sp. (average ± SD, n=3) ingested by *Oxyrrhis marina* in pCO_2 treatments ambient, moderate, high at time points 30, 60, 90 and 120 minutes during a short term ingestion rate experiment. *Isochrysis galbana* was used as prey in the optimal diet treatment

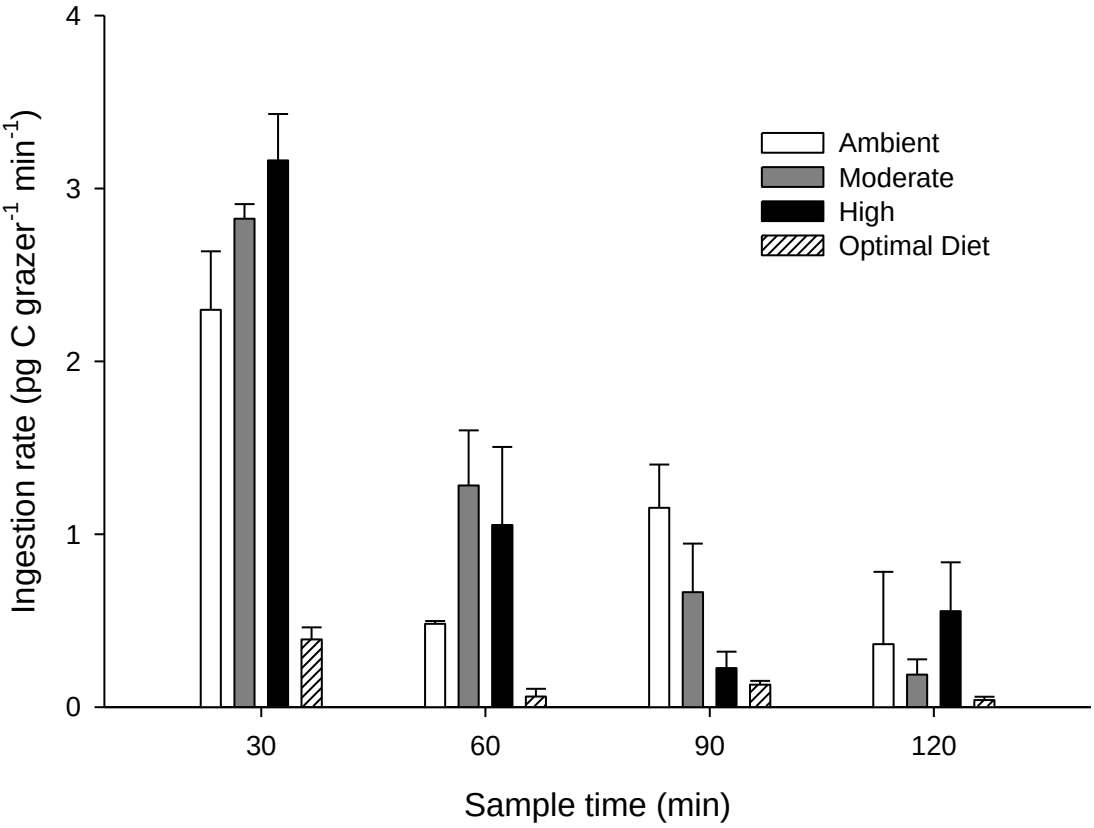


Figure B14. Short term ingestion rate of *Oxyrrhis marina* (average ± SD, n=3) (pg C grazer^{-1} min^{-1}) feeding on *Rhodomonas* sp. cultured semi-continuously in pCO_2 treatments ambient moderate and high at time points 30, 60, 90 and 120 minutes. *Isochrysis galbana* was used as prey in the optimal diet treatment. The ingestion rate is the slope of the line relating cells ingested and time between each sample time point.

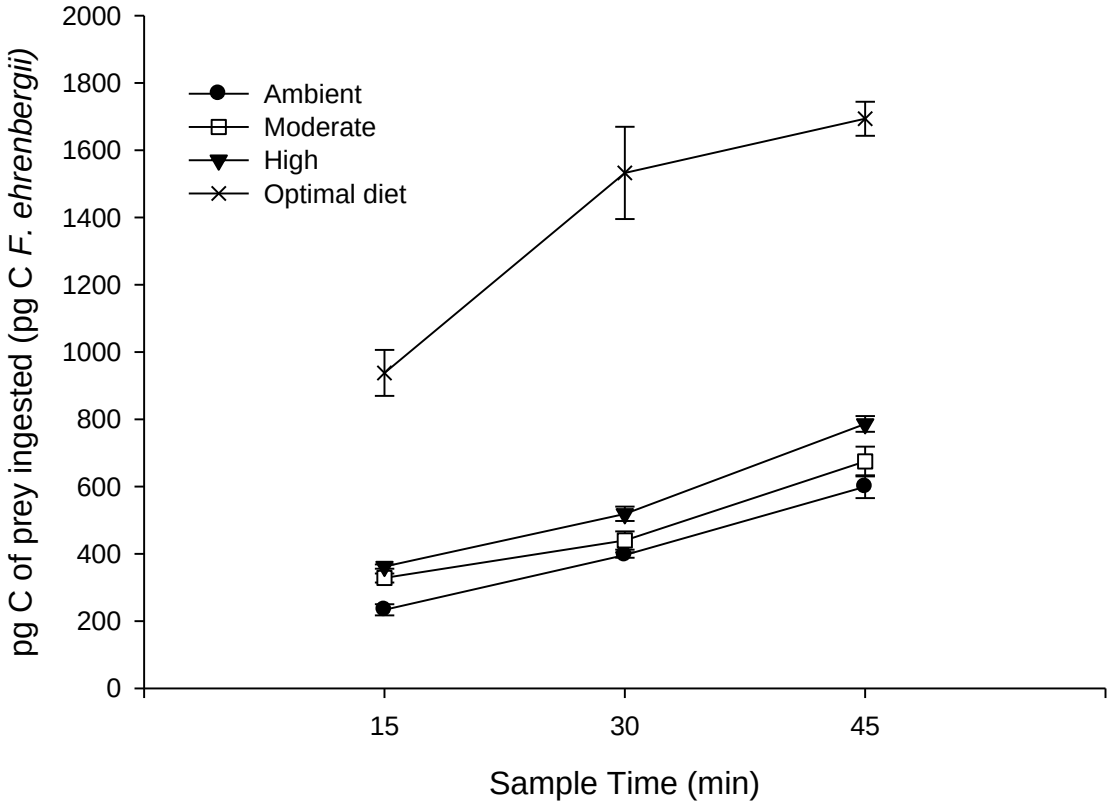


Figure B15. Total pg C of *Rhodomonas* sp. (average ± SD, n=3)ingested by *F. ehrenbergii* in pCO_2 treatments ambient, moderate and high at time points 15, 30 and 45 minutes during a short term ingestion rate experiment. *Heterocapsa triquetra* was used as prey in the optimal diet treatment.

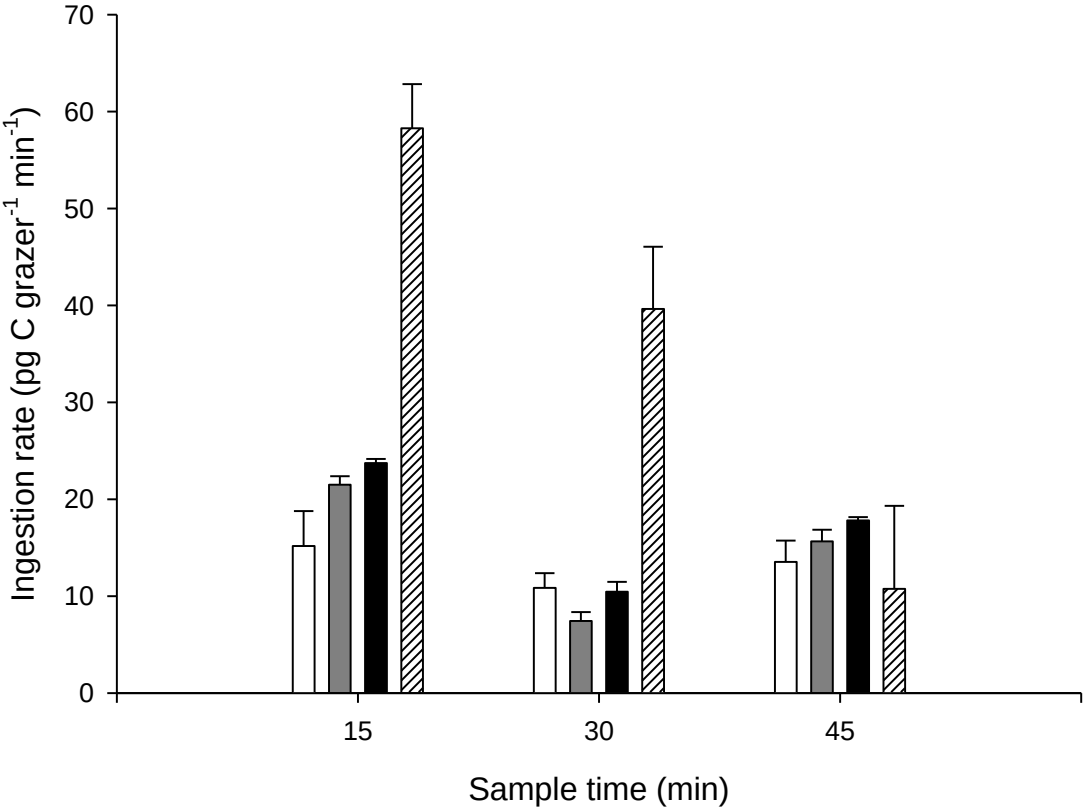


Figure B16. Short term ingestion rate of *Favella ehrenbergii* (average ± SD, n=3) (pg C grazer^{-1} min^{-1}) feeding on *Rhodomonas* sp. cultured semi-continuously in pCO_2 treatments ambient moderate and high at time points 15, 30 and 45 minutes. *Heterocapsa triquetra* was used as prey in the optimal diet treatment.

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