Summer 8-31-2020

The effect of phytoplankton properties on the ingestion of marine snow by Calanus pacificus

Grace Cawley
University of San Diego

UNIVERSITY OF SAN DIEGO

San Diego

The effect of phytoplankton properties on the ingestion of marine snow by *Calanus pacificus*

A thesis submitted in partial satisfaction of the requirements for the degree of

Master of Science in Environmental and Ocean Sciences

by
Grace Frances Cawley

Thesis Committee

Jennifer C. Prairie, Ph.D., Chair

Moira Décima, Ph.D.

Nathalie Reyns, Ph.D.

2020

The thesis of Grace Frances Cawley is approved by:

_____

Jennifer C. Prairie, Ph.D., Chair
University of San Diego

_____

Moira Décima, Ph.D.
University of California San Diego,
Scripps Institution of Oceanography

_____

Nathalie Reyns, Ph.D.
University of San Diego

University of San Diego

San Diego

DEDICATION

To my parents this one's for you!

To the copepods for making this possible.

ACKNOWLEDGMENTS

Thank you to my super women team that is my thesis committee. Thank you Jenny for dealing with my antics these last five years (and forever… sorry) and showing me the way of the research world. I am grateful for all of your guidance, and the hours spent sorting copepods into delirium. Thank you for everything, I'm a better scientist and person for working with you. Moira, thank you for arriving and solving our problems. Our questions grew weirder and your answers never ceased to make our ideas well rounded. I am grateful for our collaboration and for your help all around the world. Nathalie, thank you for agreeing to be on my committee, even after dealing with undergrad Grace and guiding me into remembering the bigger picture when I was too focused on copepods and what they ate.

I would like to thank the Environmental and Ocean Sciences Department for the support and resources given to me throughout my time at USD, especially for Russ for driving the boat! And of course for making a BA/MS program so I could keep talking about copepods. I would like to acknowledge NSF CAREER OCE-1654276 for also supplying the funding needed to pull this thesis off.

Thank you to my friends who have dealt with my crazy throughout this whole process. A special thanks to Riley for doing it all

with me – I'm grateful for you and wouldn't have wanted to bother Jenny with anyone else. Thank you to everyone in the Prairie lab, past and present, for teaching me the ways of being a lab mate, and also helping me in the field collecting copepods.

Thank you to my graduate cohorts. I love all of you and am grateful for your input and encouragement during this whole process.

To Rory, thanks for being a good older brother who is successful and makes me want to at least measure up. And of course thank you to the people who made it all possible, my parents. Mom and Dad, you know what you have done for me, and this is all possible because of you. Thank you for always picking up the phone, and bucking me up!

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The aggregation of phytoplankton into marine snow provides a mechanism by which smaller particles can coagulate to form larger particles, which can be consumed at various depths or readily transported to the deep ocean and sequestered from the atmosphere on time scales of a thousand years or more. Zooplankton interacting with these large carbon-rich aggregates can obtain nutrition in environments where the phytoplankton size spectrum is small and not directly available, enhancing the possibility of obtaining adequate nutrition in environments dominated by small cells. In addition, interactions between zooplankton and marine snow can result in fragmentation, thus affecting the particle sinking rate and changing the export of carbon. Unfortunately, these interactions are understudied and poorly understood. This study focuses on how two factors – phytoplankton growth phase and species – affect copepod feeding on marine snow, providing insight into the role of this food source in planktonic trophic dynamics and export of carbon to depth.

We conducted a series of grazing experiments using gut pigment and stable isotope methods to quantify the ingestion rate of the copepod, *Calanus pacificus*, on marine snow aggregates in comparison to their ingestion rate on individual phytoplankton. We also examined how the ingestion of copepods on marine snow was affected by the phytoplankton species and phytoplankton growth phase from which the aggregates were formed. Results demonstrate that marine snow represents a substantial food source for copepods, with ingestion rates comparable to those on individual phytoplankton as measured with both gut

pigment and stable isotope analysis. We found that phytoplankton growth phase can significantly affect the ingestion of marine snow. Finally, ingestion of marine snow was affected by phytoplankton species, and while aggregates formed from *Thalassiosira weissflogii* resulted in consistent patterns of ingestion rate between experiments and methodologies, the same was not the case for aggregates formed from *Skeletonema marinoi*. These findings suggest that marine snow is likely an important source of nutrition for copepods, but that its role in planktonic food webs may differ depending on the phytoplankton community composition and the stage of phytoplankton blooms.

CHAPTER 1: INTRODUCTION

1.1 Plankton Ecology

Though plankton are among the smallest inhabitants of the ocean, their impact on large-scale trophic dynamics and global carbon cycling makes these organisms and their interactions with the environment crucial to understand. Phytoplankton utilize photosynthesis to make energy using a combination of carbon dioxide and light along with a variety of nutrients. With this ability they form the base of the food chain as primary producers. Phytoplankton come in many different shapes and sizes, with temperature, nutrient availability, and light penetration all controlling the composition of local phytoplankton communities (Gower et al. 1980, Dai et al. 2016). Since these factors vary throughout the ocean, phytoplankton communities demonstrate strong spatial heterogeneity. Phytoplankton are the main food source for many types of zooplankton, and so these trophic interactions between predator and prey – along with planktonic trophic dynamics more broadly – also vary regionally (Messié and Chavez 2017).

Zooplankton include organisms that remain as plankton throughout their whole life history (holoplankton) and organisms which only are planktonic during their larval stage (meroplankton). Copepods are small crustaceans that are a group of holoplanktonic zooplankton found in a vast range of aquatic habitats, including the benthos, the inside of shark's mouths, and even in the damp leaf litter of the redwood forests (Oldewage and Smale 1993, Camann et al. 2001). There are over 10,000 species of copepods with calanoids, harpacticoids, and cyclopoids being

the major groups (Brun et al. 2017). Due to the extensive abundance and diversity of copepods and their wide range of life history strategies (Turner 2004), understanding these organisms and their interactions with the marine environment is critical.

Copepods have a diversity of feeding modes (Kiørboe 2011) and play an important role in pelagic food webs, including as primary grazers. Copepods have the ability to chemically sense their environment, which allows them to not only find food, but also in some cases sense the pheromones of potential mates (Paffenhöfer and Lewis 1990, Yen et al. 1998). Some have suggested that the bacterial activity on marine aggregates, which provide a home for diverse microbial communities, create chemical plumes that make it easier for copepods to find this potential food source (Lombard et al. 2013). *Calanus pacificus*, the study organism of this thesis, is a species of calanoid copepod commonly found off the coast of southern California. *C. pacificus* is known to be a size selective feeder, making size and concentration of prey a factor in foraging (Frost 1972). It is suggested that foraging upon phytoplankton happens when present, but carnivory can also be an option (Landry 1981).

1.2 The Biological Pump

The biological pump is the export and sequestration of biologically-derived particulate carbon into the deep ocean. The deep ocean is the largest sink of actively cycled carbon on Earth. When carbon is sequestered into the deep ocean, more carbon can be absorbed by the surface ocean from the atmosphere

(Turner 2015). Thus, the biological pump plays an important role in mediating climate change. Understanding the complex factors affecting the efficiency of the biological pump is important to understand, not only for ocean dynamics, but also for Earth's global carbon cycle.

The biological pump is initiated by phytoplankton taking up carbon dioxide in the surface ocean and transforming it into particulate organic carbon (POC) through photosynthesis. Although some of this carbon will be returned to the surface ocean in dissolved form, some of this carbon will be transported to the deep ocean through various ocean processes, such as sedimentation, deep ocean circulation, and sinking of organisms and their molts (Ducklow et al. 2001, Buesseler and Boyd 2009, Boyd et al. 2019).

Aggregations of phytoplankton called marine snow (a term coined based on what these aggregates look like as they fall through the ocean) serve as one of the major vehicles that bring carbon to the deep ocean. Some species of phytoplankton and many marine bacteria produce a sticky substance referred to as transparent exopolymer particles (TEP) (Passow and Alldredge 1995). The stickiness of TEP along with physical encounters of phytoplankton lead to the formation of marine snow (Kiørboe 2001. Marine snow aggregates are primarily composed of organic matter such as phytoplankton and mucus houses used in filter feeding of some zooplankton, but these particles can also contain sediment and other inorganic matter.

Due to the larger size of marine snow particles, these aggregates sink faster than individual phytoplankton (Shanks and Trent 1980). Marine snow thus

provides one of the main transport mechanisms for carbon into the deep ocean. In addition, these marine snow particles serve as a source of nutrition to many organisms in the ocean including bacteria and zooplankton (Azam and Long 2001, Kiørboe et al. 2003).

1.3 Zooplankton Grazing on Marine Snow

The role of marine snow as a food source for copepods has important implications for both the biological pump and plankton trophic dynamics. Field studies have imaged zooplankton associated with and interacting with marine snow particles (Shanks and Walter 1997, Möller et al. 2012), and some studies additionally collected zooplankton and inferred ingestion through gut pigment analysis (Bochdansky and Herndl 1992) and fecal pellet production (Lampitt et al. 1993).

A laboratory study by Dilling et al. (1998) was the first to use fecal pellet production to confirm the consumption of marine snow by *Euphasia pacifica* and *Calanus pacificus*; this study also explained the difficulty of using gut pigment analysis for quantifying ingestion of marine snow due to the uncertainty of cell concentration within aggregates (Dilling et al. 1998). Dilling and Brzezinski (2004) investigated the preference of zooplankton for marine snow and dispersed phytoplankton. Using two varieties of silicon isotopes, they found that marine snow served as a food source even in the presence of individual phytoplankton. Though ingestion of marine snow by zooplankton is now understood to occur, many questions still remain. In particular, it is unclear how different factors, such

as the composition of aggregates, may affect the ingestion of marine snow by zooplankton.

1.4 Objective and Hypotheses

The objective of this study was to determine if properties of aggregates, specifically growth phase and species of phytoplankton from which the aggregates are formed, impact the grazing of zooplankton on marine snow. Towards this goal, I addressed the following questions:

1) What is the ingestion rate of *Calanus pacificus* on marine snow, and how is this ingestion rate comparable to the ingestion rate on dispersed phytoplankton?
2) Does the ingestion rate of *Calanus pacificus* on marine snow differ for aggregates formed from different growth phases of phytoplankton?
3) Is there a difference in the ingestion rate of *Calanus pacificus* on marine snow formed from different species of phytoplankton?

REFERENCES

Azam, Farooq, and Richard A. Long. "Sea snow microcosms." *Nature* 414, no. 6863 (2001): 495-498.

Bochdansky, Alexander B., and Gerhard J. Herndl. "Ecology of amorphous aggregations (marine snow) in the Northern Adriatic Sea. 111. Zooplankton interactions with marine snow." *Mar Ecol Prog Ser* 87 (1992): 135-146.

Boyd, Philip W., Hervé Claustre, Marina Levy, David A. Siegel, and Thomas Weber. "Multi-faceted particle pumps drive carbon sequestration in the ocean." Nature 568, no. 7752 (2019): 327-335.

Brun, Philipp Georg, Mark Payne, and Thomas Kiørboe. "A trait database for marine copepods." Earth System Science Data 9, no. 1 (2017): 99-113.

Buesseler, Ken O., and Philip W. Boyd. "Shedding light on processes that control particle export and flux attenuation in the twilight zone of the open ocean." Limnology and Oceanography 54, no. 4 (2009): 1210-1232.

Camann, Michael A., Karen L. Lamoncha, and Clinton B. Jones. "Old-growth redwood forest canopy arthropod prey base for arboreal wandering salamanders: a report prepared for the Save-the-Redwoods League." (2001).

Dai, Chuanjun, Min Zhao, and Hengguo Yu. "Dynamics induced by delay in a nutrient–phytoplankton model with diffusion." *Ecological Complexity* 26 (2016): 29-36.

Dilling, Lisa, Jacqueline Wilson, Deborah Steinberg, and Alice Alldredge. "Feeding by the euphausiid Euphausia pacifica and the copepod Calanus pacificus on marine snow." *Marine Ecology Progress Series* 170 (1998): 189-201.

Dilling, Lisa, and Mark A. Brzezinski. "Quantifying marine snow as a food choice for zooplankton using stable silicon isotope tracers." *Journal of plankton research* 26, no. 9 (2004): 1105-1114.

Ducklow, Hugh W., Deborah K. Steinberg, and Ken O. Buesseler. "Upper ocean carbon export and the biological pump." *OCEANOGRAPHY-WASHINGTON DC-OCEANOGRAPHY SOCIETY-* 14, no. 4 (2001): 50-58.

Gower, J. F. R., K. L. Denman, and R. J. Holyer. "Phytoplankton patchiness indicates the fluctuation spectrum of mesoscale oceanic structure." *Nature* 288, no. 5787 (1980): 157-159.

Jackson, George A., and David M. Checkley Jr. "Particle size distributions in the upper 100 m water column and their implications for animal feeding in the plankton." *Deep Sea Research Part I: Oceanographic Research Papers* 58, no. 3 (2011): 283-297.

Kiørboe, Thomas. "Formation and fate of marine snow: small-scale processes with large-scale implications." *Scientia marina*65, no. S2 (2001): 57-71.

Kiørboe, Thomas, Kam Tang, Hans-Peter Grossart, and Helle Ploug. "Dynamics of microbial communities on marine snow aggregates: colonization, growth, detachment, and grazing mortality of attached bacteria." *Applied and Environmental Microbiology* 69, no. 6 (2003): 3036-3047.

Kiørboe, Thomas. "How zooplankton feed: mechanisms, traits and trade-offs." Biological reviews 86, no. 2 (2011): 311-339.

Lampitt, R. S., W. R. Hillier, and P. G. Challenor. "Seasonal and diel variation in the open ocean concentration of marine snow aggregates." *Nature* 362, no. 6422 (1993): 737-739.

Lombard, Fabien, Marja Koski, and Thomas Kiørboe. "Copepods use chemical trails to find sinking marine snow aggregates." *Limnology and oceanography* 58, no. 1 (2013): 185-192.

Messié, Monique, and Francisco P. Chavez. "Nutrient supply, surface currents, and plankton dynamics predict zooplankton hotspots in coastal upwelling systems." Geophysical Research Letters 44, no. 17 (2017): 8979-8986.

Möller, Klas O., Michael St John, Axel Temming, Jens Floeter, Anne F. Sell, Jens-Peter Herrmann, and Christian Möllmann. "Marine snow, zooplankton and thin layers: indications of a trophic link from small-scale sampling with the Video Plankton Recorder." *Marine Ecology Progress Series* 468 (2012): 57-69.

Oldewage, W. H., and M. J. Smale. "Occurrence of piscine parasitic copepods (Crustacea) on sharks taken mainly off Cape Recife, South Africa." *South African Journal of Marine Science* 13, no. 1 (1993): 309-312.

Paffenhöfer, Gustav-Adolf, and Kellie D. Lewis. "Perceptive performance and feeding behavior of calanoid copepods." *Journal of Plankton Research* 12, no. 5 (1990): 933-946.

Passow, Uta, and Alice L. Alldredge. "Aggregation of a diatom bloom in a mesocosm: The role of transparent exopolymer particles (TEP)." *Deep Sea Research Part II: Topical Studies in Oceanography* 42, no. 1 (1995): 99-109.

Shanks, Alan L., and Jonathan D. Trent. "Marine snow: sinking rates and potential role in vertical flux." *Deep Sea Research Part A. Oceanographic Research Papers* 27, no. 2 (1980): 137-143.

Shanks, Alan L., and Keith Walters. "Holoplankton, meroplankton, and meiofauna associated with marine snow." *Marine Ecology Progress Series* 156 (1997): 75-86.

Turner, Jefferson T. "Zooplankton fecal pellets, marine snow, phytodetritus and the ocean's biological pump." *Progress in Oceanography* 130 (2015): 205-248.

Turner, Jefferson T. "The importance of small planktonic copepods and their roles in pelagic marine food webs." *Zoological studies* 43, no. 2 (2004): 255-266.

Yen, Jeannette, Marc J. Weissburg, and Michael H. Doall. "The fluid physics of signal perception by mate-tracking copepods." *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 353, no. 1369 (1998): 787-804.

CHAPTER 2: The effect of phytoplankton properties on the ingestion of marine snow by *Calanus pacificus*

2.1 Introduction

In plankton ecology, determining the factors that drive changes in foraging behavior on small temporal and spatial scales is important for building a larger understanding of trophic dynamics and carbon cycling in the ocean. Zooplankton are the primary consumers of phytoplankton, making up a crucial part of the pelagic food web (Parsons et al. 1967, Bautista and Harris 1992, Turner and Tester 1997). Copepods make up the largest biomass of metazoans in the ocean (Turner 2004) and are often the dominant grazers of plankton communities in pelagic ecosystems (Landry 1977, Bautista and Harris 1992). As grazers, copepods impose top down control in pelagic ecosystems, which is important in regulating phytoplankton blooms and other aspects of microzooplankton and phytoplankton dynamics (Christaki and Wambeke 1995, Armengol et al. 2017). In addition, copepod foraging behavior can impact carbon cycling and export, particularly as they interact with sinking particles like marine snow.

Marine snow are aggregates that form in the ocean from phytoplankton, fecal pellets, and other organic and inorganic matter (Alldredge and Silver 1988). These aggregates play an important role in the carbon cycle since they sink significantly faster than individual phytoplankton (Iverson and Ploug 2010, McDonnell et al. 2010). Many field observations of zooplankton associated with marine snow suggest that aggregates may be a substantial food source in addition

to phytoplankton (Steinberg et al. 1994, Möller et al. 2012). For example, using SCUBA to collect marine snow, Green and Dagg (1997) observed a variety of zooplankton, including various copepod species like *Oncaea spp*. and *Microsetella norvegica,* in association with these aggregates. Shanks and Walters (1997) also observed copepods associated with aggregates in the field, and in laboratory experiments used a vertical flume to further investigate interactions between copepods and marine snow aggregates. Gut content analysis has been used to confirm the consumption of marine snow by many different types of zooplankton (Dagg 1993, Uttal and Buck 1996, Wilson and Steinberg 2010), including multiple species of copepods (Shanks and Edmonson 1990).

Our somewhat limited understanding of interactions between zooplankton and marine snow can be attributed to the fragile nature of marine snow, which cannot be sampled by traditional field methods. Observations of these interactions in situ have been bolstered by the advancement of imaging systems (Möller et al. 2012). Further challenges with working with marine snow in the lab have provided barriers to experimentally quantifying ingestion of marine snow by zooplankton. Dilling et al. (1998) noted issues with many classical methods for quantifying ingestion when applied to marine snow, but was able to measure consumption of field-collected marine snow by *Calanus pacificus* using fecal pellet production. In one set of experiments, they were able to quantify the ingestion rate of *Euphasia pacifica* on marine snow through changes in particulate organic carbon concentration, but acknowledged that high abundances of marine snow needed to be used because of the sensitivity in taking these measurements.

Dilling and Brzezinski (2004) carried out grazing experiments with *Euphausia pacifica* and *Calanus pacificus* in tanks with both individual phytoplankton and marine snow, which were labeled with different isotopic tracers. This study showed that marine snow is a viable food choice even when other alternatives were present; however, absolute ingestion rates were not quantified. These previous studies highlight the complications that come in measuring marine snow ingestion in the lab, making it difficult to address certain questions regarding zooplankton-aggregate interactions.

Interactions between zooplankton and marine snow can have an impact on the pelagic food web by providing an alternative food source for copepods. Aggregation of phytoplankton cells may transform particles into a larger, more manageable size for ingestion by size-selective grazers (Frost 1972, Hansen et al. 1994). In this way, the formation of marine snow can allow phytoplankton that are too small to be eaten by some zooplankton to become newly available as a food source, creating a sort of trophic shortcut. Thus, marine snow has the potential to increase the food availability for zooplankton in oligotrophic regions, where smaller phytoplankton tend to thrive. Along with affecting trophic dynamics, zooplankton ingestion of marine snow aggregates also impacts the biological pump in multiple ways. Even if the zooplankton are not directly feeding on the aggregates, fragmentation of the particles can occur when zooplankton interact with them (Dilling and Alldredge 2000, Kiørboe 2001, Goldthwait et al. 2004, Kiko et al. 2017). This fragmentation will result in changes in the size and density of the marine snow particles, which will then alter

their sinking rate (Gibbs 1985, Prairie et al. 2019), thus changing the efficiency of the biological pump. In addition, zooplankton foraging on marine snow repackages marine snow aggregates into dense fecal pellets (Turner and Ferrante 1979, Shanks and Edmonson 1990), which generally sink at faster rates then marine snow (Bruland and Silver 1981).

Although many studies have examined zooplankton, including copepods, foraging on marine snow, there is not a lot known on how different properties of marine snow may impact consumption by zooplankton. Since copepods are known to select phytoplankton prey based on size and other factors (Kiørboe 2008), it is likely that the ingestion of marine snow by copepods may also depend on the physical and biological characteristics of the aggregates. For example, marine snow can vary in size and composition based on the different species and physiology of the phytoplankton present and other variables (Alldredge and Gotschalk 1988, Thorton and Thake 1998, Engel et al. 2007, Yamada et al. 2013). Properties of marine snow have also been shown to depend on the amount of TEP (transparent exopolymer particles) produced, which is the sticky matrix produced by phytoplankton and bacteria that acts like a glue helping with the aggregation process (Alldredge et al. 1998, Passow 2002). Phytoplankton, specifically diatoms, also develop different physiological characteristics with age (De Troch et al. 2012), and previous studies have shown that zooplankton can demonstrate a food preference based on phytoplankton growth phase (Long and Hay 2006, Barofsky 2009). Given this, ingestion of marine snow may also depend on the age of the phytoplankton from which they are formed, particularly since Prairie et al.

(2019) showed that phytoplankton growth phase affected the TEP production and density of marine snow. Despite this, no study has specifically looked at the effect of phytoplankton growth phase on the ingestion of marine snow aggregates by zooplankton, although Dilling et al. (1998) observed copepod foraging on aged marine snow from the ocean. Examining how factors like phytoplankton species and growth phase may affect copepod ingestion of marine snow is important to predicting how these interactions could impact carbon export temporally and spatially in the ocean.

In this study, we investigated how specific properties of phytoplankton impact copepod foraging on marine snow aggregates. With a series of lab experiments, the ingestion rate of the copepod, *Calanus pacificus,* on marine snow was quantified using both gut pigment analysis and stable isotope analysis. Ingestion of marine snow and dispersed phytoplankton were compared for different phytoplankton growth phases and for two different species of phytoplankton.

2.2 Methods

During the summer of 2018 and the fall of 2019, six experiments were conducted to investigate the effect of phytoplankton properties on the ingestion rate of *Calanus pacificus* (Table 1). All experiments included two or three growth phases, and phytoplankton as a food source presented in both dispersed (i.e. as individual cells) or aggregated form. Each growth phase included three treatments: 1) a control, in which copepods were placed in tanks with filtered

seawater and no food source, 2) a phytoplankton treatment, in which copepods were placed in tanks with dispersed phytoplankton as a food source, and 3) an aggregate treatment, in which copepods were placed in tanks with aggregates as a food source (Figure 1). Ingestion rate was quantified in these experiments using two methods: gut pigment analysis and stable isotope analysis.

2.2.1 Copepod collection

C. pacificus was collected using a small boat off the coast of La Jolla, CA (32° 51.720' N, 117° 16.816' W) 5-20 days before each experiment with a 333 μm mesh plankton net (0.5 m diameter mouth). Contents of each tow were diluted and chilled, and samples were sorted for individuals of the species *C. pacificus* (copepodite V and adult female stages). Copepods were maintained with regular water changes in an incubator at 18°C in the dark. When being kept before experiments, copepods were fed a mixed diet of phytoplankton as to not develop a preference: *Thalassiosira weissflogii* and haptophytes (*Tisochrysis sp. and Pavlova sp.*) for the 2018 experiments and *Thalassiosira weissflogii* and *Skeletonema marinoi* for the 2019 experiments. Copepods were acclimated to room temperature and starved 24 hours prior to the experiment to avoid residual food in their gut (Dam and Peterson 1988). Beakers were wrapped in aluminum foil during acclimation period to keep copepods in the dark.

2.2.2 Phytoplankton cultures and aggregate formation

Prior to each experiment, non-axenic phytoplankton cultures of the species *T. weissflogii* (Experiments 1, 2, 3, and 5) or *S. marinoi* (Experiments 4 and 6) were started in 2 L flasks (Table 1). All cultures were grown in f/2 media at room temperature under a 12:12 hour LED light:dark cycle. Experiments 1 and 2 were carried out for three different growth phases (corresponding to early exponential, late exponential, and late stationary stages of the phytoplankton growth curve), while Experiments 3 through 6 were carried out with just the first two growth phases. For each growth phase, two cultures were started: one to be used for the dispersed phytoplankton treatment and one to be used for the aggregate treatment. Cultures for the early exponential growth phase were grown for 5 days, cultures for the late exponential growth phase were grown for 11 days (Exp. 1 and 2) or 12 days (Exp. 3-6), and cultures for the late stationary growth phase were grown for 17 days (Table 1). In all cases, cultures for the aggregate treatment were started three days earlier then the cultures for the phytoplankton treatment to account for the three-day period used for rolling the culture to form marine snow (see description below). The cell concentration of each phytoplankton culture was measured every day on a particle counter (Multisizer 3, Beckman Coulter Counter) to track phytoplankton growth over time (Figure 2).

To carry out ingestion rate measurements using stable isotope analysis, 1.7 mL of an ^{15}N nitrate solution (7.5 g/L ^{15}N-potassium nitrate salt in DI water) was added to each phytoplankton culture 3 days before the culture was to be stopped (except for Experiment 1 where 12.75 mL of ^{15}N nitrate solution was

added 2 days before each culture was stopped, and Experiment 2 where 1.7 mL of ^{15}N nitrate solution was added 5 days before each culture was stopped). Right before adding ^{15}N nitrate solution to each culture, 25 mL of the culture was filtered onto GF/F filters in triplicate, to be used as initial measurements of naturally occurring ^{15}N concentrations in the phytoplankton cultures. Filters were frozen in a −20 °C freezer until all isotope samples were ready to be packed for analysis.

After the cultures for the aggregate treatments grew for their allotted time (Table 1), they were diluted and added to two cylindrical acrylic tanks, each with a volume of 550 mL. Due to the size difference between *T. weissflogii* (average diameter 10 μm) and *S. marinoi* (average diameter 8 μm), the cultures of the two species were diluted to 20,000 cells/mL and 39,000 cells/mL, respectively, to allow for a roughly equivalent concentration by cell volume between the two species. These tanks were incubated on a roller table, and allowed to rotate at a speed of 4.6 rpm for 3 days to form aggregates. This method has been widely used previously to form aggregates (Shanks and Edmondson 1989, Dilling and Brezinski 2004, Prairie et al. 2013). Tanks were incubated on the roller table in the dark to ensure that no further growth of the phytoplankton culture occurred.

2.2.3 Grazing experiments

Experiments for each growth phase were conducted using three treatments: one treatment with no food added (control), one treatment with individual/dispersed phytoplankton, and one treatment with aggregates (Figure 1).

For each treatment, two replicate cylindrical tanks were used, each with a volume of 2200 mL. For the aggregate treatment, aggregates formed in the 550 mL tanks were transferred to the experimental tank along with the seawater in which they were formed; the rest of the volume of the experimental tank (total 2200 mL) was filled with filtered seawater, thus resulting in an average cell concentration of 5000 cells/mL for *T. weissflogii* and 9750 cells/mL for *S. marinoi*. Before adding copepods, aggregates were photographed on a transparent mm-square grid sealed on the bottom of the cylindrical experimental tank to observe qualitative differences in the size and appearance of aggregates from different growth phases and experiments (photographs were not taken in Experiments 1 and 2). The phytoplankton treatment tanks were filled with individual phytoplankton from the culture grown for this treatment which was diluted to 5000 cells/mL for *T. weissflogii* and 9750 cells/mL for *S. marinoi*, such that the phytoplankton treatment tanks and the aggregate treatment tanks had equivalent cell concentrations. Control treatment tanks were filled with filtered seawater, and had a small amount of ^{15}N nitrate solution added (between 87 and 483 μL) such that the final concentration of ^{15}N in the control tanks was comparable to that in the phytoplankton and aggregate tanks for that experiment. For each replicate treatment tank, 30 copepods were added and allowed to forage for an hour while the tank rotated at ~1 rpm in the dark.

Once the one-hour incubation time had elapsed for each treatment tank, the tank was removed from the roller table and 40 mL of seltzer water was added to anesthetize the copepods and avoid regurgitation of gut contents. The copepods

were removed from the cylindrical experimental tank with gentle suctioning of water onto a mesh sieve. For gut pigment analysis, two copepods were placed in 6-10 amber vials (depending on the total number of copepods recovered), which contained 3 mL of 90% acetone. For stable isotope analysis, five sets of two copepods were transferred into tin cups for each tank of each treatment (only 3 to 4 sets of two copepods were collected for Experiments 1 and 2).

2.2.4 Gut pigment analysis

After copepods were transferred to amber vials, a sonicator was used at 40% amplitude for 5 seconds to break up the organisms and release their gut content into the acetone solution. In addition, the water from each experimental tank was evenly mixed, and three subsamples of 25 mL of tank water were filtered onto GF/F filters and placed into 5 mL of acetone. After a day in a −20 °C freezer, the copepod and tank water samples were analyzed using a Trilogy Laboratory Fluorometer (Turner Designs) to measure the concentration of total pigment (combined chlorophyll-*a* and pheophytin) in the acetone solution. For the copepod samples, this represents the gut pigment content per copepod from the experiment (G, in units of μg pigment/copepod) which was calculated using the equation (Dam and Peterson 1988):

$$G = \frac{K\left(\frac{r}{r-1}\right)(rR_a - R_a)\,E}{n} \quad (1)$$

where K is the fluorometer calibration constant, R_a represents the fluorescence reading after acidification, r is the acidification ratio, E is the volume in L of acetone used to extract chlorophyll, and n is the number of copepods per vial. To

account for differences in fluorescence between phytoplankton and aggregates (since the aggregates were formed in the dark for 3 days), the fluorescence per cell (F, in units of μg pigment/cell) was calculated from the fluorometer measurements of the tank water samples using the equation:

$$F = \frac{K\left(\frac{r}{r-1}\right)(rR_a - R_a)\,E}{S\,C} \quad (2)$$

where S is the sample volume in L (i.e. 0.025 L) and C is the phytoplankton concentration in the tank in cells/L (5,000,000 cells/L for *T. weissflogii* experiments and 9,750,000 cells/L for *S. marinoi* experiments).

To directly compare results between the two methods, we calculated ingestion rate (I, in units of μgC/copepod/hour) using :

$$I = \left(\frac{G}{F}\right) \times M \quad (3)$$

where M is the mass of carbon per cell as calculated by the relationship provided in Menden-Deuer and Lessard (2000). Note that F could not be directly calculated for the control treatment (since C is equal to 0 cells/L), and so ingestion rate for these samples was calculated using the F value for the phytoplankton treatment from the same experiment.

2.2.5 Stable isotope analysis

In addition to the copepods used in the grazing experiments, for each growth phase of each experiment five sets of two unfed copepods (which were starved alongside experimental copepods but not used in any treatment tank) were transferred into tin cups, and were used to measure the natural concentration of

^{15}N in copepods before being exposed to phytoplankton grown in ^{15}N nitrate solution. Right after grazing incubations were complete, subsamples of the remaining tank water for each treatment (three replicates of 250 mL) were each filtered onto GF/F filters and packed into a tin cup for ^{15}N measurements of the food that was fed to the copepods in each treatment. The GF/F filters taken prior to adding ^{15}N nitrate solution to the cultures were also packed into tin cups to be analyzed. All samples were processed by UC Davis Stable Isotope Facility (with the exception of Experiment 1, in which samples were processed on an Isotope Ratio Mass Spectrometer at Scripps Institution of Oceanography).

From the raw stable isotope data, isotopic fraction of each copepod sample (F_S) was calculated as (Verschoor 2005):

$$F_S = \frac{R_S}{(R_S+1)} \tag{4}$$

where R_S is the isotopic ratio of the sample calculated as:

$$R_S = \left(\left(\frac{\delta 15N}{1000}\right) + 1\right) * R_R \tag{5}$$

where R_R is the isotopic ratio of a reference standard (Sigman et al. 2009) and δ^{15}N is the measure of the ratio of ^{15}N to ^{14}N that is provided in the raw data. The mass of food eaten per copepod (M_{food}) was calculated as:

$$M_{Food} = \frac{M_{FedZoop}F_{FedZoop} - (F_{starved} * M_{Starved})}{F_{Food}} \tag{6}$$

where $M_{FedZoop}$ is the mass of carbon in the experimental copepod sample (as given in the stable isotope data) divided by the number of copepods, $F_{FedZoop}$ and $F_{Starved}$ are the isotopic fraction of the experimental copepods and the starved copepods, respectively (calculated using equation 4), and F_{Food} is the isotopic

fraction of the food source (calculated using equation 4 but from the measurements of the GF/F filters taken from the remaining water of the feeding experiments for the phytoplankton and aggregate treatment, and from the measurements of the GF/F filters taken from the cultures before ^{15}N was added for the control treatment). $M_{Starved}$ represents the mass of the experimental copepods before they were fed; however, because this measurement could not be obtained, $M_{FedZoop}$ was used in place of $M_{Starved}$ in equation 6 (which assumes that the difference in mass before and after the copepods were fed is negligible). In cases where equation 6 resulted in a negative value for M_{Food} (which occurred for some samples in the control treatment when $F_{Starved}$ was greater than $F_{FedZoop}$), a value of 0 was used for M_{Food} instead. Ingestion rate (I) was then calculated as:

$$I = \frac{M_{Food}}{t} \quad (7)$$

where t is time of incubation (which is 1 hour for these experiments).

2.2.6 Data analysis

A two-way mixed ANOVA was run for each experiment (and data from each methodology separately) with growth phase and treatment as fixed effects and tank number as a random effect. For Experiments 1 and 2 the ANOVA was only run on the first two growth phases so the results would be comparable to the other experiments. A Tukey-Kramer post-hoc test was used to determine differences pairwise between the three treatments. An F-Test was run on the ingestion rate data calculated from the gut pigment analysis to determine if there

was any difference in the variance of ingestion rates between the phytoplankton and aggregate treatments.

2.3 Results

Fluorescence values per cell for the phytoplankton treatment were greater than those for the aggregate treatment by a factor of 2 or more in some cases (Table 2), which resulted in substantially different patterns between the raw gut pigment data and the ingestion rate calculated from the gut pigment data after it was corrected for this difference (Figure 3 A, B). However, in other cases there was little difference between fluorescence per cell between the treatments, and so the patterns remained the same (Figure 3 C, D). *C. pacificus* appeared to consume both aggregates and dispersed phytoplankton as demonstrated by the higher mean gut pigment content in each of these treatments compared to the control in all experiments and growth phases (Figure 4, 5), although many of these differences were not significant (Table 3, 4). Similarly, mean ingestion rate as calculated from the stable isotope data was higher in both the aggregate and phytoplankton treatment compared to the control in all experiments and growth phases (Figures 4, 5), but again these differences were often not significant (Table 3, 5), and ingestion in the aggregate treatment was negligibly higher than the control in the early exponential growth phase of Experiment 5 (Figure 4H).

Differences in copepod ingestion rate of aggregates versus dispersed phytoplankton were present in some cases, but the magnitude and direction of these differences depended on both phytoplankton growth phase and the species

of phytoplankton. Our ANOVA results for ingestion rate as calculated from both methods showed there was a significant effect of treatment for all 6 experiments (Table 3). There was a significant effect of growth phase for only one of the six experiments with the ingestion rate data from gut pigment analysis (Experiment 6), but two of the six experiments had a significant effect of growth phase when using the stable isotope data (Experiments 1 and 5). A significant interaction effect was present in two experiments for ingestion calculated from gut pigment data (Experiments 1 and 3) and in one experiment for ingestion using stable isotope analysis (Experiment 6). No consistent differences were observed in variances of ingestion rate as calculated from the gut pigment data between the phytoplankton and the aggregate treatment; in early exponential growth phase, variance was significantly higher in the aggregate treatment for two out of the six experiments (Experiments 3 and 4), whereas in the late exponential growth phase variance was significantly higher in the phytoplankton treatments for two of the six experiments (Experiments 2 and 3) (Table 6).

Although most experiments did not show significant effects of growth phase, consistent patterns in ingestion of these two food sources were observed in experiments using *T. weissflogii*. Ingestion rate (as calculated from both gut pigment data and stable isotope data) of aggregates in early exponential growth phase was equal to or higher than that of phytoplankton in all experiments except for Experiment 5 (Figure 4). By contrast, higher ingestion of phytoplankton was observed in the late exponential growth phase (both when calculated from gut pigment data and stable isotope data), with the exception of the late exponential

growth phase in experiment 1 from the stable isotope data (Figure 4B). Ingestion rate on phytoplankton increased from the early exponential growth phase to the late exponential growth phase for all experiments with the exception of Experiment 1 when using the stable isotope data (Figure 4B). The opposite trend was observed for aggregates, with reduced consumption in the late exponential growth phase compared to the early exponential growth phase for all experiments except in Experiment 5 using the stable isotope data (Figure 4H). For the two experiments (Experiments 1 and 2) in which grazing experiments were also done for a third growth phase, a decrease in ingestion rate on both phytoplankton and aggregates was observed in relation to the previous growth phase (Figure 4 A, B, D). The ingestion rates calculated from both gut pigment data and stable isotope data were very similar in magnitude and trends (relating to treatment and growth phase) for each *T. weissflogii* experiment.

In experiments using the phytoplankton species *S. maranoi*, patterns in ingestion rate of aggregates versus phytoplankton were less clear (Figure 5). Based on the ingestion rates calculated from the gut pigment data, copepods ingested aggregates at a higher or equal rate compared to phytoplankton in both growth phases in both experiments (Figure 5 A, C). However, ingestion rates calculated from stable isotope data showed more varied results, with ingestion of phytoplankton being higher for both growth phases in Experiment 4 (Figure 5B), and ingestion of aggregates being higher in the early exponential growth phase while ingestion of phytoplankton being higher in the late exponential growth phase in Experiment 6 (Figure 5D). Ingestion rate was higher in the

phytoplankton treatment in the late exponential growth phase compared to the early exponential growth phase except for in Experiment 4 from the stable isotope data (Figure 5B). Ingestion of aggregates was lower in the late exponential growth phase than the early exponential growth phase in all experiments except for Experiment 6 from gut pigment analysis (Figure 5C).

Photographs of aggregates taken before each grazing experiment were examined for qualitative differences that might explain the variation in ingestion rate patterns between experiments and growth phases for *S. marinoi*. Aggregates in the early exponential growth phases for both experiments appeared less compact (as indicated by their lighter color) than their later growth phase counterparts (Figure 6). Between experiments there is a clear difference in size of aggregates, with larger aggregates observed in Experiment 6 compared to Experiment 4. By contrast, *T. weissflogii* did not show substantial differences in the color or compactness of aggregates between growth phases or experiments (Appendix B).

2.4 Discussion

Our study design including two methodologies, multiple sets of experiments, and different growth phases and phytoplankton species confirm previous experiments suggesting active consumption of *C. pacificus* on phytoplankton aggregates (Dilling et al. 1998). Our approach allowed us to additionally quantify ingestion rates, and show that rates of both food types (marine snow or dispersed phytoplankton) were similar, yet depended on

phytoplankton growth phase (Figures 4, 5). Utilization of two different species of phytoplankton, *T. weissflogii* and *S. maranoi*, further illustrated the diversity of factors that may affect consumption of marine snow in the field, as grazing experiments with the smaller diatom *S. maranoi* did not show consistent patterns between experiments or methodology (Figure 5, 6), unlike the reproducible patterns observed with *T. weissflogii* (Figure 4).

The goal of the present work was to investigate patterns of marine snow and phytoplankton consumption, using two different methods, because quantifying ingestion rates of marine snow are notoriously difficult (Dilling et al. 1998). We have structured our discussion below to address the strengths and limitations of our two methodological approaches, summarize our new understanding of aggregate consumption by copepods, and address the implications of these results for plankton ecology.

2.4.1 Comparison of gut pigment and stable isotope analyses for measuring ingestion of aggregates

Although many studies have demonstrated that copepods and other types of zooplankton consume marine snow (e.g., Steinberg et al. 1994, Dilling et al. 1998), quantifying the ingestion rate of marine snow by different organisms has been lacking, since many methods used to measure ingestion of phytoplankton may not be practical or accurate for grazing experiments with aggregates. For example, measuring ingestion through disappearance requires knowing the concentration of phytoplankton cells, which is not possible in irregularly-shaped

and fragile marine snow particles. In this study, we used two different methods commonly used in grazing experiments and adapted them so they could be applied to quantify the ingestion of marine snow aggregates in a way that is directly comparable to the ingestion of dispersed phytoplankton. In our experiments, the ingestion rates calculated from these two independent methods were very close in magnitude (rarely differing by a factor of more than 2) and often showed similar patterns with respect to treatment and growth phase, suggesting that both methodologies can provide consistent measurements of consumption of marine snow aggregates.

Our choice to compare two methodologies in our experiments necessitated that the number of copepods within our experimental tanks had to be split for the two analyses, with roughly 2/3 of the copepods being used for gut pigment analysis and the remaining 1/3 being used for stable isotope analysis. Even with the smaller sample sizes for stable isotope analysis, we still saw comparable statistical patterns between the two methods. Despite the fact that patterns were very consistent across experiments with *T. weissflogii* (Figure 4), we did not always find statistically significant differences between growth phases (Table 3), which was likely because of variance introduced by high biological variability. Using a single methodology would allow for increased sample size, which will help further reveal relationships in future studies.

Although our study demonstrates that both gut pigment analysis and stable isotope analysis can be used to measure ingestion of marine snow, each method has advantages and limitations. Gut pigment analysis can also be used to measure

marine snow consumption in the field (Möller et al. 2012). However, gut pigment analysis is dependent on the fluorescence of individual cells, which we found can vary between dispersed phytoplankton and aggregates (Table 3). We were able to measure and account for this difference in our calculations of ingestion rate, but this may be difficult or impossible when using this method in the field. Using stable isotope analysis for quantifying ingestion of marine snow requires adding an isotope as a tracer, and so this method may not be as easily adaptable as a shipboard technique. However, this method can be modified by using additional stable isotope tracers to separately quantify ingestion of phytoplankton and aggregates to determine active selection between these two food sources, as was done by Dilling and Brzezinski (2004). In addition, ingestion rate can vary based on the size of the grazer, and it can be easier to account for this variability using stable isotope analysis where the mass of your samples is known. For example, the average ingestion rate normalized per copepod mass for our *T. weissflogii* experiments (obtained by dividing equation 7 by $M_{FedZoop}$) ranged from 0.0016 to 0.0160 μgC/μgC/hour for the dispersed phytoplankton treatment depending on experiment and growth phase, and ranged from 0.0002 to 0.0133 μgC/μgC/hour for the aggregate treatment.

2.4.2 Factors affecting ingestion of aggregates by copepods

Since aggregates are much lower in concentration than individual phytoplankton, it may be surprising that marine snow is readily consumed by copepods. Although aggregates are less likely to be happened upon by a grazer,

these large particles represent a sort of patch of food (Kiørboe 2001). If an individual copepod can find this large source of nutrition for relatively little energy cost because aggregates are abundant, it could be optimal to exploit this food source (Pyke 1984, DeMott 1989). Bacterial activity on marine snow as it sinks also creates a chemical trail that can be used by zooplankton to locate aggregates, making them more exploitable as a resource than if copepods relied on random encounters (Kiørboe 2001, Lombard et al. 2013). It has also been seen that, in situations when phytoplankton are present mostly in the dispersed form, the energy cost to find aggregates may be too high, resulting in the consumption of primarily individual phytoplankton. Though our experimental design did not allow us to test this hypothesis directly since copepods were not given a choice between dispersed and aggregated phytoplankton, a previous study by Dilling and Brzezinski (2004) found substantial consumption of aggregates by *C. pacificus* even in the presence of dispersed phytoplankton. One possible indication of aggregates acting as a food patch would be higher variance of ingestion rates in the aggregate treatment compared to the phytoplankton treatment; however, we did not see this in our results (Table 6).

The observed impact of growth phase on copepod ingestion may be a result of changes in phytoplankton physiology as the cultures grow and deplete nutrients to a point that induces stress. One of these physiological responses could include changes to biochemical composition of phytoplankton, since under nutrient limitation, phytoplankton can display increased carbohydrates and decreased protein (Harrison et al. 1989). In addition, phytoplankton growth phase

might affect the health of cultures, as zooplankton have been shown to discriminate against dead cells in grazing experiments (DeMott 1998). Although the phytoplankton cultures for each treatment were grown for the same amount of time, the culture for the aggregate treatment was subsequently incubated on a rolling table in the dark for 3 days to form aggregates, potentially placing these phytoplankton in a state of senescence and lowering their nutritional value (Harrison et al. 1989). This could also explain the drop in consumption in the late stationary growth phase (measured in Experiments 1 and 2, Figure 4), which coincides with the cultures reaching a state of senescence (Kahl et al. 2008).

The differences in ingestion of marine snow based on phytoplankton growth phase and phytoplankton species may be a result of physical changes to aggregates (i.e. size, density, porosity). Both size and shape of marine snow aggregates can vary based on the phytoplankton that are present (Logan and Wilkinson 1990, Li and Logan 1995). In addition, aggregate density can depend on the growth phase of the phytoplankton cultures (Prairie et al. 2019), potentially explaining our qualitative observations of the changes in the apparent compactness of *S. maranoi* aggregates between growth phases (Figure 6). One difference between the two species used in our experiments is that *T. weissfloggi* does not form chains as commonly as *S. maranoi,* which is a smaller and highly chain-forming diatom. These differences, which have been shown to differently deter predation on single cells (Bergkvist et al. 2012), may also affect how they form aggregates and their subsequent ingestion by copepods.

The presence of TEP, needed for the formation of aggregates from phytoplankton, may also explain the patterns in ingestion rate that we observed. Copepods, specifically our study species *C. pacificus*, have been known to forage on these gel-like particles (Ling and Alldredge 2003), which could potentially provide additional nutritional value based on their composition of carbohydrates (Passow 2002, Ortega-Retuerta et al. 2009). Prieto et al. (2001) suggested that copepods could trigger a higher production of TEP when interacting with diatoms, creating a potential positive feedback loop in nutritional production. Furthermore, production of TEP varies during different stages of the phytoplankton growth curve (Prairie et al. 2019), and for different phytoplankton species (Passow 2002), providing a possible explanation for the variation in ingestion rate we observed. Along with TEP, the bacterial community that grows alongside the phytoplankton likely varies depending on phytoplankton species and growth phase (Pinhassi et al. 2004). These bacteria are not only present but often necessary in the marine snow formation process, including for one of the phytoplankton species, *T. weissflogii*, used in this study (Gärdes et al. 2011). These bacteria-phytoplankton interactions could introduce other factors affecting the consumption of marine snow by zooplankton (Mayor et al. 2014). In our experiments, we presume TEP was present in our phytoplankton cultures as evidenced by the successful formation of marine snow aggregates, though TEP was not quantified in this study. Future research could quantify TEP and bacterial abundance or community composition in relation to zooplankton ingestion of marine snow.

2.4.3 Significance to plankton ecology

Although phytoplankton have always been known to provide food for copepods and other grazers alike, more recently studies have introduced the notion that the aggregation of phytoplankton into marine snow could provide an additional nutritional pathway for copepods (e.g., Steinberg et al. 1994, Dilling and Brzezinski 2004). The results of this study further challenge the classical understanding of pelagic food webs that place phytoplankton (as individual cells) as the sole food source for herbivorous zooplankton, as demonstrated by ingestion of *C. pacificus* on marine snow aggregates being similar to that on dispersed phytoplankton. Thus, the aggregation of smaller phytoplankton into larger marine snow particles could provide a trophic pathway that is often not considered in pelagic food web models.

In addition to the impacts on trophic dynamics, zooplankton interactions with marine snow alter aggregate sinking rates through the process of fragmentation (Dilling and Alldredge 2000, Goldthwait et al. 2005), with important consequences for the efficiency of the biological pump. Moreover, the observed effect of growth phase on the ingestion of marine snow by copepods suggests that this effect on carbon flux may be regulated by the seasonality of phytoplankton growth. For example, based on our results, earlier stages of blooms of *T. weissflogii* might experience higher relative ingestion of aggregates, potentially resulting in lower carbon export compared to later stages of the bloom. Our findings demonstrate that understanding how different phytoplankton characteristics can affect the ingestion of both individual phytoplankton and

aggregates is important for predicting zooplankton grazing during different times and for different regions of the ocean.

Given the diversity of feeding strategies among zooplankton, we would expect very different feeding responses to aggregates for other groups of zooplankton, and future studies with other organisms could help elucidate these interactions. Our study emphasizes that understanding trophic dynamics as a whole depends on knowing the organisms that are present and their physiological state, since both can affect zooplankton grazing and the transfer of energy up the food chain. Including aggregate foraging behavior can also improve our understanding of how carbon is exported to the deep ocean, by including an important interaction that is currently neglected in models of the biological carbon pump. The complexity of the ocean necessitates an understanding of how multiple simultaneous factors affect different interactions in the ocean to be able to predict the larger-scale impacts of these interactions on global processes.

Table 1. Description of the copepod grazing experiments, including duration that each culture was grown for each grown phase, dates of the experiment for each growth phase, sample size for gut pigment analysis and stable isotope analysis (given for control, phytoplankton, and aggregates treatment, respectively, with the sample sizes combined for the two replicate tanks), and the phytoplankton species used in each experiment. Growth phases are abbreviated as Early Exp (for early exponential), Late Exp (for late exponential) and Late Stat (for late stationary).

Exp.	Growth Phase	Experiment Date	Sample Size for Gut Pigment	Sample Size for Stable Isotope	Phytoplankton Species
1	Early Exp (5 Days)	6/13/18	20, 18, 19	8, 8, 8	*T. weissflogii*
	Late Exp (11 Days)	6/19/18	16, 15, 17	7, 7, 7	
	Late Stat (17 Days)	6/25/18	6, 8, 7	N/A	
2	Early Exp (5 Days)	7/25/18	20, 19, 19	8, 8, 8	*T. weissflogii*
	Late Exp (11 Days)	7/31/18	19, 19, 20	8, 8, 8	
	Late Stat (17 Days)	8/6/18	9, 18, 15	4, 8, 8	
3	Early Exp (5 Days)	9/23/19	19, 19, 20	10, 10, 10	*T. weissflogii*
	Late Exp (12 Days)	9/30/19	20, 19, 17	10, 10, 10	
4	Early Exp (5 Days)	10/7/19	18, 18, 18	10, 10, 10	*S. marinoi*
	Late Exp (12 Days)	10/14/19	19, 18, 18	10, 10, 10	
5	Early Exp (5 Days)	11/11/19	19, 17, 17	10, 10, 10	*T. weissflogii*
	Late Exp (12 Days)	11/18/19	17, 20, 18	10, 10, 10	
6	Early Exp (5 Days)	11/25/19	20, 18, 20	10, 10, 10	*S. marinoi*
	Late Exp (12 Days)	12/9/19	17, 16, 20	10, 10, 10	

Table 2. Fluorescence per cell (*F*) values for each growth phase and treatment (with Phyto representing treatment with dispersed phytoplankton and Agg representing treatment with aggregates) of each experiment as calculated from equation 2.

Experiment	Growth Phase	Treatment	Fluorescence Per Cell (10^{-6} μg pigment/cell)
1	Early Exp	Phyto	4.461
	Early Exp	Agg	2.084
	Late Exp	Phyto	3.470
	Late Exp	Agg	3.722
	Late Stat	Phyto	2.485
	Late Stat	Agg	1.848
2	Early Exp	Phyto	1.902
	Early Exp	Agg	1.174
	Late Exp	Phyto	2.547
	Late Exp	Agg	2.650
	Late Stat	Phyto	2.259
	Late Stat	Agg	1.699
3	Early Exp	Phyto	1.763
	Early Exp	Agg	1.487
	Late Exp	Phyto	2.055
	Late Exp	Agg	2.709
4	Early Exp	Phyto	1.475
	Early Exp	Agg	0.821
	Late Exp	Phyto	2.105
	Late Exp	Agg	1.836
5	Early Exp	Phyto	1.775
	Early Exp	Agg	1.543
	Late Exp	Phyto	3.698
	Late Exp	Agg	3.278
6	Early Exp	Phyto	2.428
	Early Exp	Agg	1.989
	Late Exp	Phyto	2.958
	Late Exp	Agg	2.046

Table 3. Results of the two-way mixed-effect ANOVA tests run for each experiment for ingestion rates calculated from both gut pigment and stable isotope data. P-values are provided for the fixed effect of treatment, fixed effect of growth phase, and interaction effect. Asterisks indicate $p<0.05$.

		Gut Pigment	Stable Isotope
Exp. 1	Treatment	p = 0.000*	p = 0.002*
	Growth Phase	p = 0.534	p = 0.003*
	Interaction Effect	p = 0.006*	p = 0.071
Exp. 2	Treatment	p = 0.002*	p = 0.007*
	Growth Phase	p = 0.105	p = 0.073
	Interaction Effect	p = 0.084	p = 0.252
Exp. 3	Treatment	p = 0.005*	p = 0.023*
	Growth Phase	p = 0.082	p = 0.248
	Interaction Effect	p = 0.006*	p = 0.069
Exp. 4	Treatment	p = 0.005*	p = 0.002*
	Growth Phase	p = 0.956	p = 0.736
	Interaction Effect	p = 0.535	p = 0.992
Exp. 5	Treatment	p = 0.003*	p = 0.004*
	Growth Phase	p = 0.567	p = 0.013*
	Interaction Effect	p = 0.114	p = 0.077
Exp. 6	Treatment	p = 0.001*	p = 0.017*
	Growth Phase	p = 0.049*	p = 0.582
	Interaction Effect	p = 0.056	p = 0.039*

Table 4. P-values for Tukey-Kramer post-hoc pairwise comparisons of ingestion rates as calculated from gut pigment data between treatments (A = Aggregate, C = Control, P = Phytoplankton) for each growth phase and experiment. Asterisks indicate $p<0.05$.

	Early Exponential	Late Exponential
Experiment 1	A-C: p = 0.000* A-P: p = 0.002* P-C: p = 0.066	A-C: p = 0.174 A-P: p = 0.134 P-C: p = 0.013*
Experiment 2	A-C: p = 0.020* A-P: p = 0.186 P-C: p = 0.331	A-C: p = 0.033* A-P: p = 0.107 P-C: p = 0.001*
Experiment 3	A-C: p = 0.077 A-P: p = 0.274 P-C: p = 0.601	A-C: p = 0.484 A-P: p = 0.004* P-C: p = 0.001*
Experiment 4	A-C: p = 0.013* A-P: p = 0.073 P-C: p = 0.366	A-C: p = 0.028* A-P: p = 0.549 P-C: p = 0.109
Experiment 5	A-C: p = 0.301 A-P: p = 0.697 P-C: p = 0.103	A-C: p = 0.218 A-P: p = 0.019* P-C: p = 0.003*
Experiment 6	A-C: p = 0.040* A-P: p = 0.955 P-C: p = 0.064	A-C: p = 0.002* A-P: p = 0.012* P-C: p = 0.201

Table 5. P-values for Tukey-Kramer post-hoc pairwise comparisons of ingestion rates as calculated from stable isotope data between treatments (A = Aggregate, C = Control, P = Phytoplankton) for each growth phase and experiment. Asterisks indicate $p<0.05$.

	Early Exponential	Late Exponential
Experiment 1	A-C: p = 0.002* A-P: p = 0.300 P-C: p = 0.007*	A-C: p = 0.145 A-P: p = 1.00 P-C: p = 0.146
Experiment 2	A-C: p = 0.001* A-P: p = 0.233 P-C: p = 0.007*	A-C: p = 0.084 A-P: p = 0.383 P-C: p = 0.015*
Experiment 3	A-C: p = 0.250 A-P: p = 0.809 P-C: p = 0.516	A-C: p = 0.552 A-P: p = 0.039* P-C: p = 0.011*
Experiment 4	A-C: p = 0.308 A-P: p = 0.068* P-C: p = 0.010*	A-C: p = 0.311 A-P: p = 0.082 P-C: p = 0.010*
Experiment 5	A-C: p = 0.988 A-P: p = 0.234 P-C: p = 0.193	A-C: p = 0.094 A-P: p = 0.020* P-C: p = 0.002*
Experiment 6	A-C: p = 0.033* A-P: p = 0.132 P-C: p = 0.544	A-C: p = 0.305 A-P: p = 0.101 P-C: p = 0.014*

Table 6. P-values for F-tests to determine differences in variance of ingestion rates as calculated from gut pigment data between phytoplankton and aggregate treatments for each experiment and growth phase. Asterisks represents significant differences (using a significance level of α=0.05), and Phyto or Agg represents which treatment had higher sample variance in ingestion.

	Early Exponential	Late Exponential
Experiment 1	p-value = 0.329, Agg	p-value = 0.554, Phyto
Experiment 2	p-value = 0.733, Phyto	p-value = 0.001, Phyto*
Experiment 3	p-value = 0.002, Agg*	p-value = 0.001, Phyto*
Experiment 4	p-value = 0.000, Agg*	p-value = 0.266, Agg
Experiment 5	p-value = 0.185, Phyto	p-value = 0.256, Agg
Experiment 6	p-value = 0.279, Agg	p-value = 0.348, Agg

Figure 1. Schematic of experimental treatments in the grazing experiments. Control tanks have no food (filtered seawater only), phytoplankton tanks contain dispersed individual phytoplankton, and aggregate tanks contain marine snow formed from phytoplankton (of the same species and at the same growth phase as in the phytoplankton tanks),

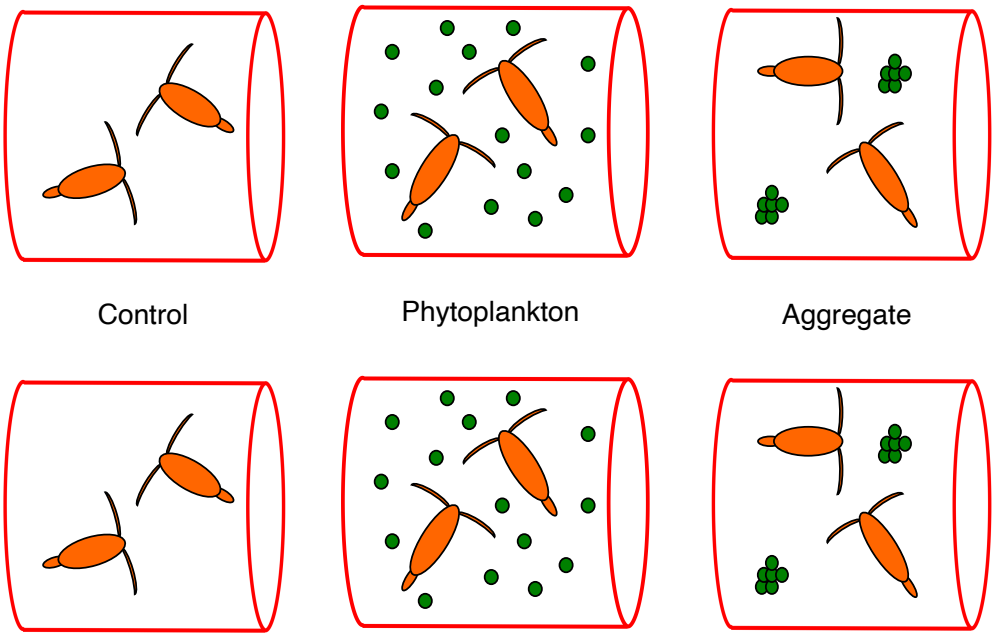

Control
Phytoplankton
Aggregate

Figure 2. Cell concentration (cells/mL) of the culture of *T. weissflogii* grown for the aggregate treatment over time (in days after the culture was started) for Experiment 2 (top), Experiment 3 (middle) and of *S. marinoi* for Experiment 6 (bottom). Blue lines represents early exponential growth phase, black lines represent late exponential growth phase, and the pink line represents the late stationary growth phase (which was only done for Experiments 1 and 2).

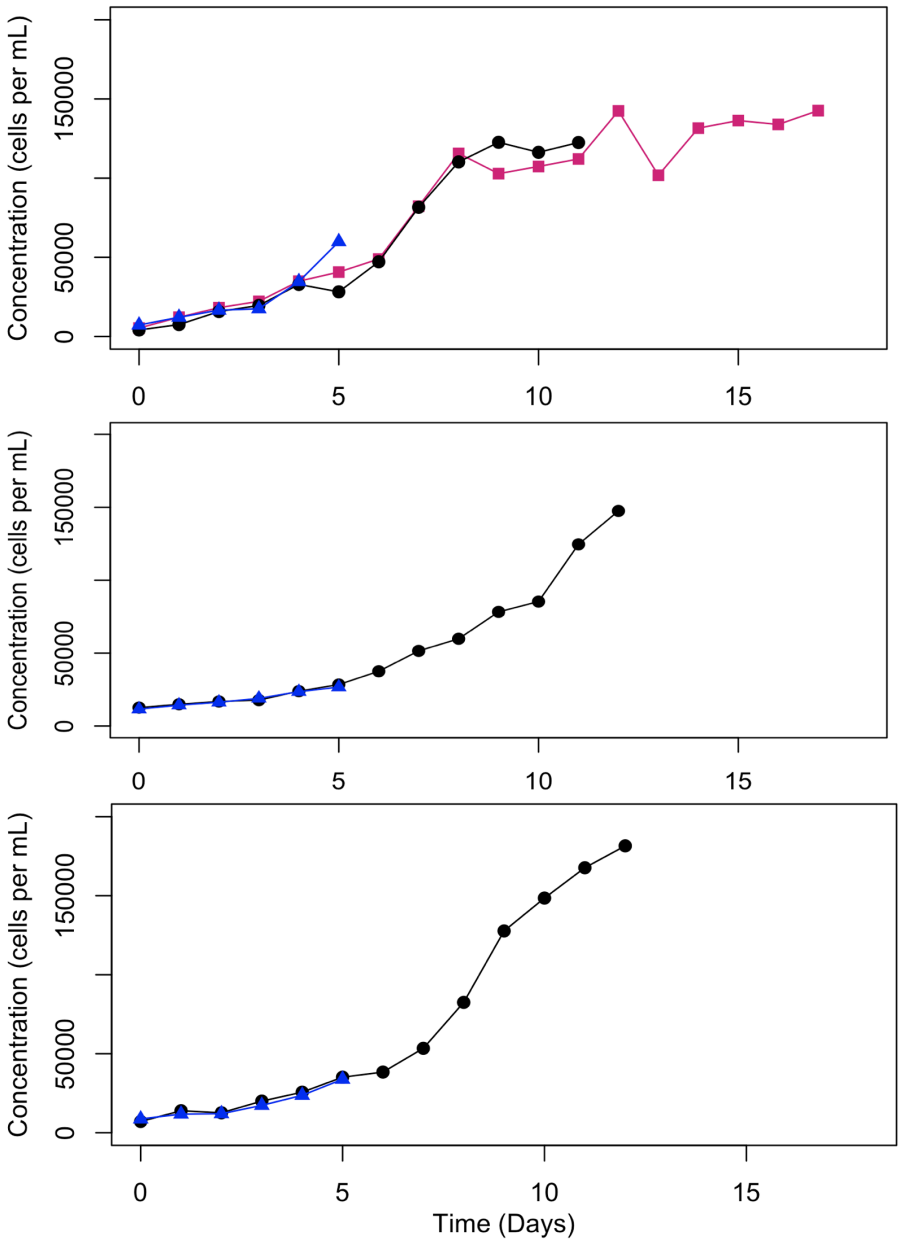

Concentration (cells per mL)
0
50000
150000
0
5
10
15
Concentration (cells per mL)
0
50000
150000
0
5
10
15
Concentration (cells per mL)
0
50000
150000
0
5
10
15
Time (Days)

Figure 3. Interaction plots of gut pigment content (A), and ingestion rate calculated from gut pigment data (B), versus growth phase and treatment for Experiment 2. Error bars represent standard error. Dashed blue lines represents aggregate treatment, solid green lines represent phytoplankton treatment, and the dotted brown line represents control treatment. (C) and (D) are the same as (A) and (B) but for Experiment 3.

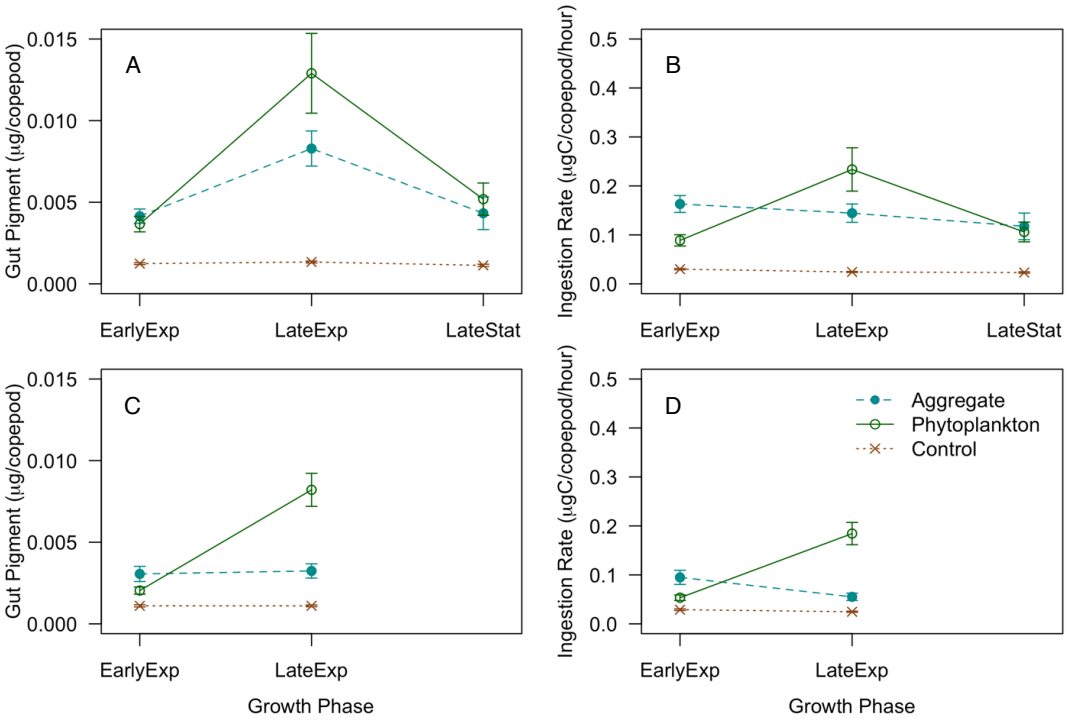

A
B
C
D
Gut Pigment (µg/copepod)
Ingestion Rate (µgC/copepod/hour)
0.015
0.010
0.005
0.000
0.5
0.4
0.3
0.2
0.1
0.0
EarlyExp
LateExp
LateStat
Growth Phase
Aggregate
Phytoplankton
Control

Figure 4. Interaction plots of ingestion rate as calculated from gut pigment data (first column). and stable isotope analysis data (second column) versus growth phase and treatment for the four experiments using the phytoplankton species *T.weissflogii*: (A and B) Experiment 1, (C and D) Experiment 2, (E and F) Experiment 3, and (G and H) Experiment 5. Error bars represent standard error. Colors and symbols represent the same treatments as in Figure 3.

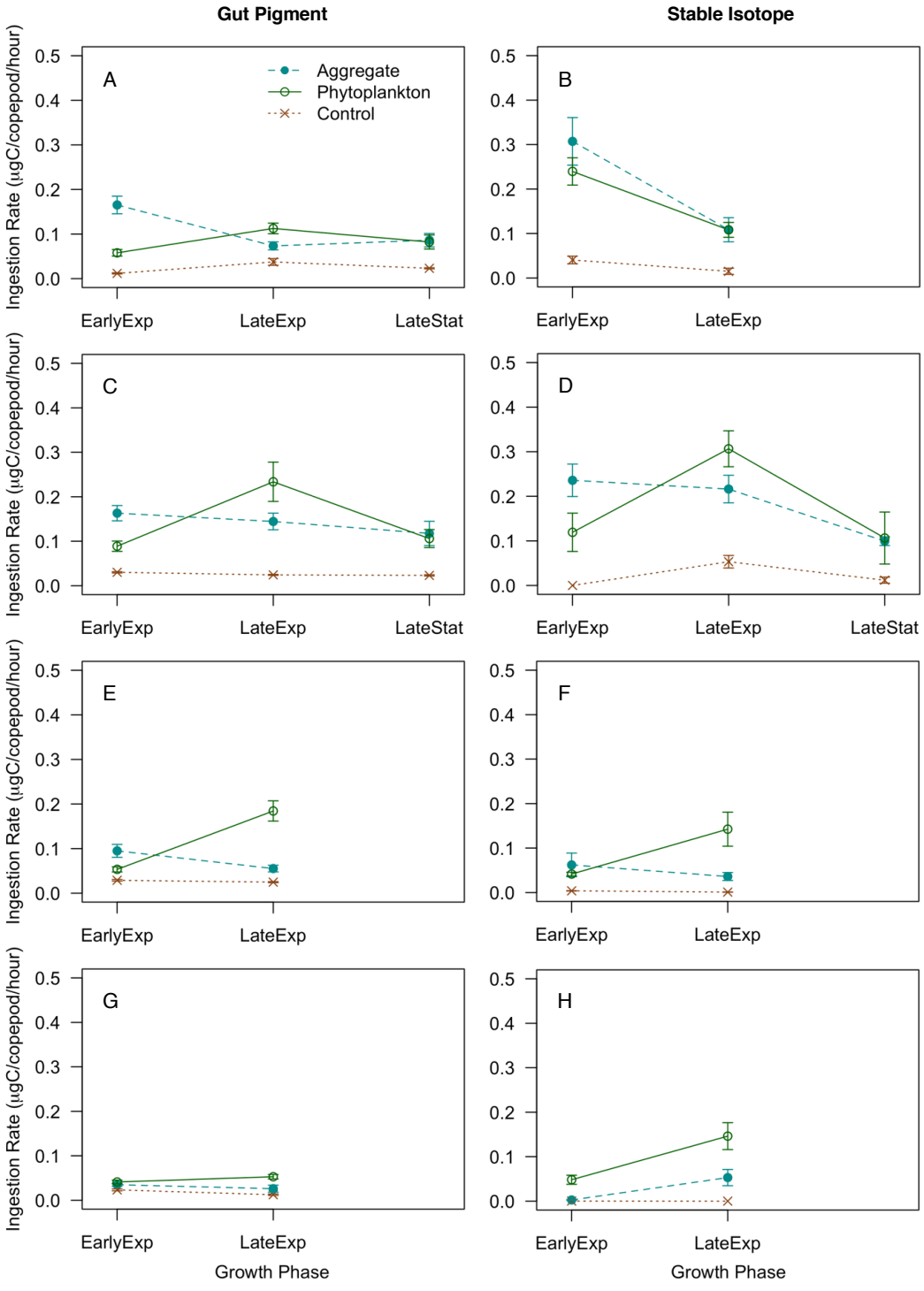

Gut Pigment
Stable Isotope
Aggregate
Phytoplankton
Control
A
B
C
D
E
F
G
H
Ingestion Rate (µgC/copepod/hour)
0.0
0.1
0.2
0.3
0.4
0.5
EarlyExp
LateExp
LateStat
Growth Phase

Figure 5. Interaction plots of ingestion rate as calculated from gut pigment data (first column). and stable isotope analysis data (second column) versus growth phase and treatment for the two experiments using the phytoplankton species *S. maranoi*: (A and B) Experiment 4, (C and D) Experiment 6. Error bars represent standard error. Colors and symbols represent the same treatments as in Figure 3.

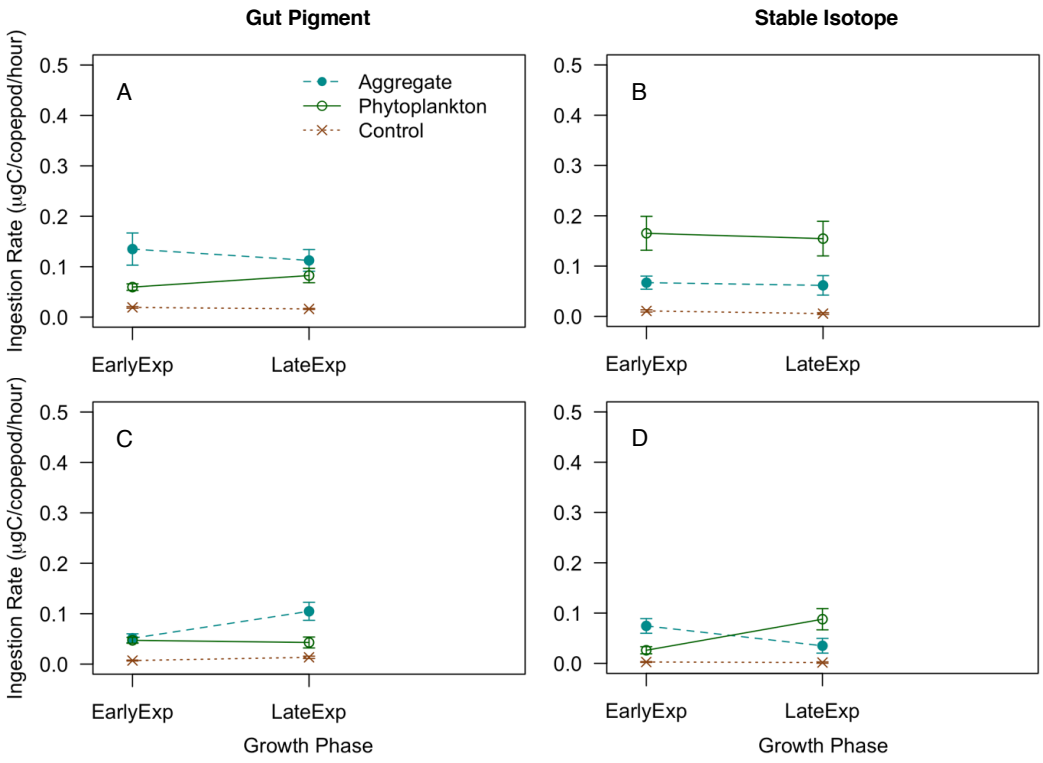
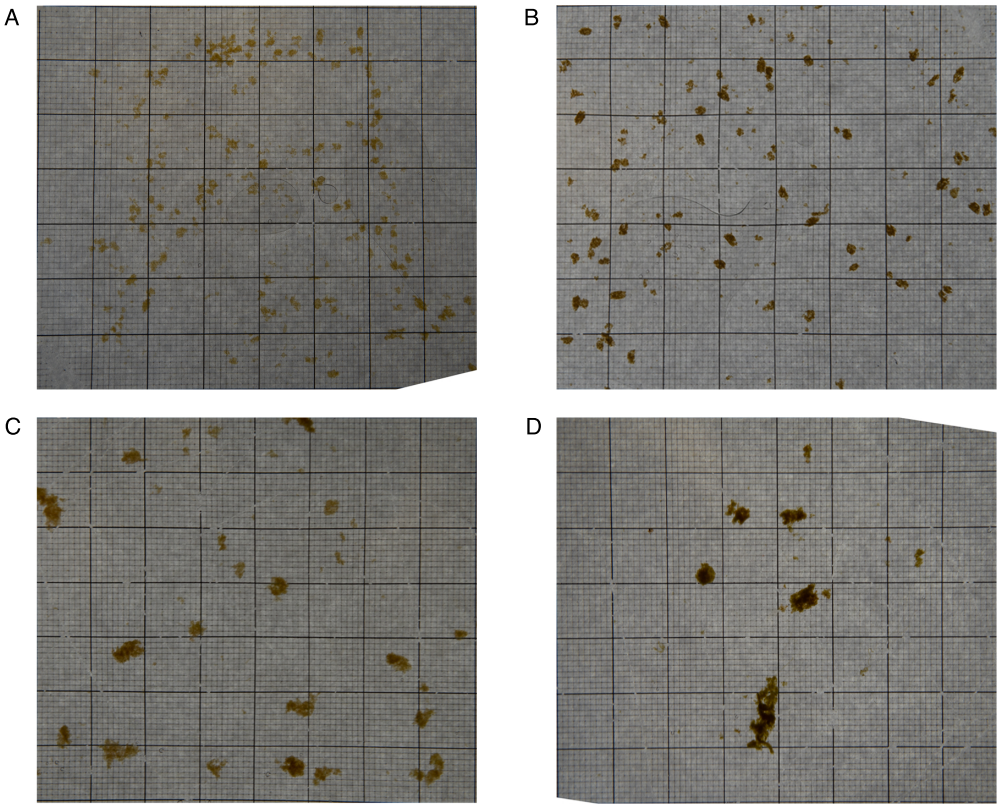

Gut Pigment
Stable Isotope
A
B
C
D
Aggregate
Phytoplankton
Control
Ingestion Rate (µgC/copepod/hour)
0.0
0.1
0.2
0.3
0.4
0.5
EarlyExp
LateExp
Growth Phase

Figure 6. Images of *S. maranoi* aggregates from one of the two aggregate treatment tanks for Experiment 4 early exponential growth phase (A), Experiment 4 late exponential growth phase (B), Experiment 6 early exponential growth phase (C), and Experiment 6 late exponential growth phase (D). Small square grids in images measure 1 mm^2.


A
B
C
D

REFERENCES

Alldredge, Alice L., and Chris Gotschalk. "In situ settling behavior of marine snow 1." *Limnology and Oceanography* 33, no. 3 (1988): 339-351.

Alldredge, Alice L., and Mary W. Silver. "Characteristics, dynamics and significance of marine snow." *Progress in oceanography* 20, no. 1 (1988): 41-82.

Armengol, Laia, Gara Franchy, Alicia Ojeda, Ángelo Santana-del Pino, and Santiago Hernández-León. "Effects of copepods on natural microplankton communities: do they exert top-down control?." *Marine Biology* 164, no. 6 (2017): 136.

Bautista, B., and R. P. Harris. "Copepod gut contents, ingestion rates and grazing impact on phytoplankton in relation to size structure of zooplankton and phytoplankton during a spring bloom." *Marine Ecology Progress Series*(1992): 41-50.

Barofsky, Alexandra, Paolo Simonelli, Charles Vidoudez, Christofer Troedsson, Jens C. Nejstgaard, Hans H. Jakobsen, and Georg Pohnert. "Growth phase of the diatom Skeletonema marinoi influences the metabolic profile of the cells and the selective feeding of the copepod Calanus spp." *Journal of Plankton Research* 32, no. 3 (2009): 263-272.

Bergkvist, Johanna, Peter Thor, Hans Henrik Jakobsen, Sten-Åke Wängberg, and Erik Selander. "Grazer-induced chain length plasticity reduces grazing risk in a marine diatom." *Limnology and Oceanography* 57, no. 1 (2012): 318-324.

Bochdansky, Alexander B., and Gerhard J. Herndl. "Ecology of amorphous aggregations (marine snow) in the Northern Adriatic Sea. 111. Zooplankton interactions with marine snow." *Mar Ecol Prog Ser* 87 (1992): 135-146.

Bruland, K. W., and M. W. Silver. "Sinking rates of fecal pellets from gelatinous zooplankton (salps, pteropods, doliolids)." *Marine Biology* 63, no. 3 (1981): 295-300.

Christaki, U., and France Van Wambeke. "Simulated phytoplankton bloom input in top-down manipulated microcosms: comparative effect of zooflagellates, ciliates and copepods." *Aquatic microbial ecology* 9, no. 2 (1995): 137-147.

Dagg, Michael. "Sinking particles as a possible source of nutrition for the large calanoid copepod Neocalanus cristatus in the subarctic Pacific

Ocean." *Deep Sea Research Part I: Oceanographic Research Papers* 40, no. 7 (1993): 1431-1445.

Dam, Hans G., and William T. Peterson. "The effect of temperature on the gut clearance rate constant of planktonic copepods." *Journal of Experimental Marine Biology and Ecology* 123, no. 1 (1988): 1-14.

DeMott, William R. "Discrimination between algae and detritus by freshwater and marine zooplankton." *Bulletin of Marine Science* 43, no. 3 (1988): 486-499.

DeMott, William R. "Optimal foraging theory as a predictor of chemically mediated food selection by suspension-feeding copepods." *Limnology and Oceanography* 34, no. 1 (1989): 140-154.

De Troch, Marleen, Pascal Boeckx, Clio Cnudde, Dirk Van Gansbeke, Ann Vanreusel, Magda Vincx, and Maria José Caramujo. "Bioconversion of fatty acids at the basis of marine food webs: insights from a compound-specific stable isotope analysis." *Marine Ecology Progress Series* 465 (2012): 53-67.

Dilling, Lisa, Jacqueline Wilson, Deborah Steinberg, and Alice Alldredge. "Feeding by the euphausiid Euphausia pacifica and the copepod Calanus

pacificus on marine snow." *Marine Ecology Progress Series* 170 (1998): 189-201.

Dilling, Lisa, and Alice L. Alldredge. "Fragmentation of marine snow by swimming macrozooplankton: A new process impacting carbon cycling in the sea." *Deep Sea Research Part I: Oceanographic Research Papers* 47, no. 7 (2000): 1227-1245.

Dilling, Lisa, and Mark A. Brzezinski. "Quantifying marine snow as a food choice for zooplankton using stable silicon isotope tracers." *Journal of plankton research* 26, no. 9 (2004): 1105-1114.

Engel, Anja, Jennifer Szlosek, Lynn Abramson, Zhanfei Liu, and Cindy Lee. "Investigating the effect of ballasting by CaCO3 in Emiliania huxleyi: I. Formation, settling velocities and physical properties of aggregates." *Deep Sea Research Part II: Topical Studies in Oceanography* 56, no. 18 (2009): 1396-1407.

Frost, B. W. "Effects Of Size And Concentration Of Food Particles On The Feeding Behavior of the Marine Planktonic Copepod Calanus Pacificus 1." *Limnology and oceanography* 17, no. 6 (1972): 805-815.

Green, E. P., and M. J. Dagg. "Mesozooplankton associations with medium to large marine snow aggregates in the northern Gulf of Mexico." *Journal of Plankton Research* 19, no. 4 (1997): 435-447.

Gibbs, Ronald J. "Estuarine flocs: their size, settling velocity and density." *Journal of Geophysical Research: Oceans* 90, no. C2 (1985): 3249-3251.

Goldthwait, Sarah, Jeannette Yen, Jason Brown, and Alice Alldredge. "Quantification of marine snow fragmentation by swimming euphausiids." *Limnology and Oceanography* 49, no. 4 (2004): 940-952.

Goldthwait, S. A., C. A. Carlson, G. K. Henderson, and A. L. Alldredge. "Effects of physical fragmentation on remineralization of marine snow." *Marine Ecology Progress Series* 305 (2005): 59-65.

Hansen, Benni, Peter Koefoed Bjornsen, and Per Juel Hansen. "The size ratio between planktonic predators and their prey." *Limnology and oceanography* 39, no. 2 (1994): 395-403.

Harrison, P. J., P. A. Thompson, and G. S. Calderwood. "Effects of nutrient and light limitation on the biochemical composition of phytoplankton." *Journal of Applied Phycology*2, no. 1 (1990): 45-56.

Iversen, Morten, and Helle Ploug. "Ballast minerals and the sinking carbon flux in the ocean: carbon-specific respiration rates and sinking velocity of marine snow aggregates." *Biogeosciences 7:* (2010): 2613-2624.

Kahl, L. Alex, Assaf Vardi, and Oscar Schofield. "Effects of phytoplankton physiology on export flux." *Marine Ecology Progress Series* 354 (2008): 3-19.

Kiko, R., A. Biastoch, P. Brandt, S. Cravatte, H. Hauss, R. Hummels, I. Kriest, F. Marin, A. M. P. McDonnell, A. Oschlies, M. Picheral, F. U. Schwarzkopf, A. M. Thurnherr, and L. Stemmann. "Biological and physical influences on marine snowfall at the equator." *Nature Geoscience* 10, no. 11 (2017): 852.

Kiørboe, Thomas. "Formation and fate of marine snow: small-scale processes with large-scale implications." *Scientia marina*65, no. S2 (2001): 57-71.

Kiørboe, Thomas. *A mechanistic approach to plankton ecology*. Princeton University Press, 2008.

Lampitt, R. S., K. F. Wishner, C. M. Turley, and M. V. Angel. "Marine snow studies in the Northeast Atlantic Ocean: distribution, composition and role as a food source for migrating plankton." *Marine Biology* 116, no. 4 (1993): 689-702.

Landry, M. R. "A review of important concepts in the trophic organization of pelagic ecosystems." *Helgoländer Wissenschaftliche Meeresuntersuchungen* 30, no. 1-4 (1977): 8-17.

Li, Xiaoyan, and Bruce E. Logan. "Size distributions and fractal properties of particles during a simulated phytoplankton bloom in a mesocosm." *Deep Sea Research Part II: Topical Studies in Oceanography* 42, no. 1 (1995): 125-138.

Logan, Bruce E., and Daniel B. Wilkinson. "Fractal geometry of marine snow and other biological aggregates." *Limnology and Oceanography* 35, no. 1 (1990): 130-136.

Lombard, Fabien, Marja Koski, and Thomas Kiørboe. "Copepods use chemical trails to find sinking marine snow aggregates." *Limnology and oceanography* 58, no. 1 (2013): 185-192.

Long, Jeremy D., and Mark E. Hay. "When intraspecific exceeds interspecific variance: effects of phytoplankton morphology and growth phase on copepod feeding and fitness." *Limnology and oceanography* 51, no. 2 (2006): 988-996.

McDonnell, Andrew MP, and Ken O. Buesseler. "Variability in the average sinking velocity of marine particles." *Limnology and Oceanography* 55, no. 5 (2010): 2085-2096.

Möller, Klas O., Michael St John, Axel Temming, Jens Floeter, Anne F. Sell, Jens-Peter Herrmann, and Christian Möllmann. "Marine snow, zooplankton and thin layers: indications of a trophic link from small-scale sampling with the Video Plankton Recorder." *Marine Ecology Progress Series* 468 (2012): 57-69.

Parsons, T. R., R. J. LeBrasseur, and J. D. Fulton. "Some observations on the dependence of zooplankton grazing on the cell size and concentration of phytoplankton blooms." *J. Oceanogr. Soc. Japan* 23, no. 1 (1967): 10-17.

Passow, Uta. "Transparent exopolymer particles (TEP) in aquatic environments." *Progress in oceanography* 55, no. 3-4 (2002): 287-333.

Pinhassi, Jarone, Maria Montserrat Sala, Harry Havskum, Francesc Peters, Oscar Guadayol, Andrea Malits, and Celia Marrasé. "Changes in bacterioplankton composition under different phytoplankton regimens." *Applied and Environmental Microbiology* 70, no. 11 (2004): 6753-6766.

Prairie, Jennifer C., Kai Ziervogel, Carol Arnosti, Roberto Camassa, Claudia Falcon, Shilpa Khatri, Richard M. McLaughlin, Brian L. White, and Sungduk Yu. "Delayed settling of marine snow at sharp density transitions driven by fluid entrainment and diffusion-limited retention." *Marine Ecology Progress Series* 487 (2013): 185-200.

Prairie, J.C.; Montgomery, Q.W.; Proctor, K.W.; Ghiorso, K.S. Effects of Phytoplankton Growth Phase on Settling Properties of Marine Aggregates. *J. Mar. Sci. Eng.* 2019, *7*, 265.

Pyke, Graham H. "Optimal foraging theory: a critical review." *Annual review of ecology and systematics* 15, no. 1 (1984): 523-575.

Sigman, Daniel Mikhail, K. L. Karsh, and K. L. Casciotti. "Ocean process tracers: nitrogen isotopes in the ocean." (2009).

Shanks, A. L., and E. W. Edmondson. "Laboratory-made artificial marine snow: a biological model of the real thing." *Marine Biology* 101, no. 4 (1989): 463-470.

Shanks, Alan L., and Elizabeth W. Edmondson. "The vertical flux of metazoans (holoplankton, meiofauna, and larval invertebrates) due to their association with marine snow." *Limnology and Oceanography* 35, no. 2 (1990): 455-463.

Shanks, Alan L., and Keith Walters. "Holoplankton, meroplankton, and meiofauna associated with marine snow." *Marine Ecology Progress Series* 156 (1997): 75-86

Steinberg, Deborah K., Mary W. Silver, Cynthia H. Pilskaln, Susan L. Coale, and Jennifer B. Paduan. "Midwater zooplankton communities on pelagic detritus (giant larvacean houses) in Monterey Bay, California." *Limnology and Oceanography* 39, no. 7 (1994): 1606-1620.

Thornton, Daniel CO, and Brenda Thake. "Effect of temperature on the aggregation of Skeletonema costatum (Bacillariophyceae) and the implication for carbon flux in coastal waters." *Marine Ecology Progress Series* 174 (1998): 223-231.

Turner, Jefferson T. "The importance of small planktonic copepods and their roles in pelagic marine food webs." *Zoological studies* 43, no. 2 (2004): 255-266.

Turner, Jefferson T., and John G. Ferrante. "Zooplankton fecal pellets in aquatic ecosystems." *BioScience* 29, no. 11 (1979): 670-677.

Turner, Jefferson T., and Patricia A. Tester. "Toxic marine phytoplankton, zooplankton grazers, and pelagic food webs." *Limnology and Oceanography* 42, no. 5part2 (1997): 1203-1213.

Uttal, L., and K. R. Buck. "Dietary study of the midwater polychaete Poeobius meseres in Monterey Bay, California." *Marine Biology* 125, no. 2 (1996): 333-343.

Verschoor, Antonie M., Harry Boonstra, and Thijs Meijer. "Application of stable isotope tracers to studies of zooplankton feeding, using the rotifer Brachionus calyciflorus as an example." *Hydrobiologia* 546, no. 1 (2005): 535-549.

Wilson, S. E., and D. K. Steinberg. "Autotrophic picoplankton in mesozooplankton guts: evidence of aggregate feeding in the mesopelagic

zone and export of small phytoplankton." *Marine Ecology Progress Series* 412 (2010): 11-27.

Yamada, Yosuke, Hideki Fukuda, Katsuyuki Inoue, Kazuhiro Kogure, and Toshi Nagata. "Effects of attached bacteria on organic aggregate settling velocity in seawater." *Aquatic microbial ecology* 70, no. 3 (2013): 261-272.

CHAPTER 3: Conclusion

This study showed that phytoplankton properties do impact the ingestion of marine snow aggregates by *C. pacificus*. We also found that both gut pigment and stable isotope analyses are methods that can be used to effectively quantify the ingestion rate of aggregates, which is notable since previous studies described challenges with measuring marine snow ingestion using some classical plankton grazing methods (Dilling et al. 1998). Our results demonstrated that phytoplankton growth phase affected not only the consumption of aggregates by *C. pacificus*, but also the relative consumption of individual phytoplankton and aggregates. Understanding factors like this that change zooplankton foraging patterns help provide insight into the role of marine snow in trophic dynamics, which has not always been considered in planktonic food webs. This is particularly important since factors like temperature and nutrient availability are not consistent across ocean basins and can vary by season (Takahaski et al. 1993, Martiny et al. 2013). This variation can lead to different phytoplankton regimes being present at different times and regions, such as small phytoplankton dominating in areas of low nutrients, which can then impact zooplankton grazing on aggregates.

Our understanding of zooplankton ingestion of marine snow within planktonic food webs is also beneficial in how it informs the development and parameterization of models which allow the prediction of energy exchange between predator and prey. In addition, planktonic ecosystem models can predict

seasonal variation in carbon export based on the understanding of zooplankton interacting with various food source under different conditions (Yool et al. 2011).

Future studies can build on the findings of this study to determine how the ingestion and fragmentation of marine snow impacts the density and size of particles, therefore impacting its sinking and contribution to carbon sequestration. Also, it will be important to learn how the potential variation in the microbial communities could impact aggregates as they form and the extent they are grazed upon.

Since processes in the ocean are highly connected, small changes can have large impacts. Understanding small-scale interactions is crucial to develop larger-scale predictions of the effects of a changing climate. Determining how zooplankton interact with their surroundings and their prey not only provides insight into the study of plankton ecology, but also into carbon cycling.

REFERENCES

Dilling, Lisa, Jacqueline Wilson, Deborah Steinberg, and Alice Alldredge. "Feeding by the euphausiid Euphausia pacifica and the copepod Calanus pacificus on marine snow." *Marine Ecology Progress Series* 170 (1998): 189-201.

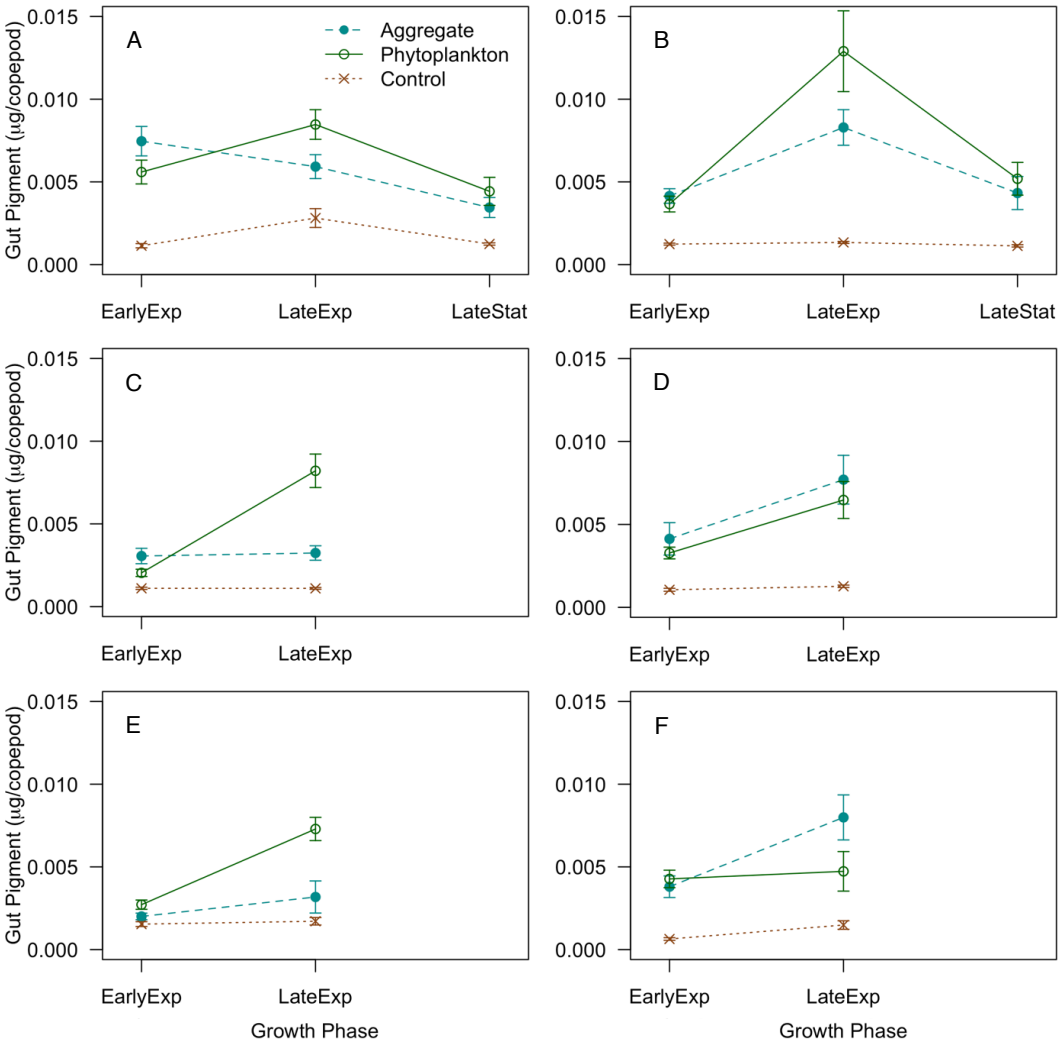
Martiny, A. C., Jasper A. Vrugt, Francois W. Primeau, and Michael W. Lomas. "Regional variation in the particulate organic carbon to nitrogen ratio in the surface ocean." *Global Biogeochemical Cycles* 27, no. 3 (2013): 723-731.

Takahashi, Taro, Jon Olafsson, John G. Goddard, David W. Chipman, and S. C. Sutherland. "Seasonal variation of CO2 and nutrients in the high-latitude surface oceans: A comparative study." *Global Biogeochemical Cycles* 7, no. 4 (1993): 843-878.

Yool, A., E. E. Popova, and T. R. Anderson. "Medusa-1.0: a new intermediate complexity plankton ecosystem model for the global domain." *Geoscientific Model Development* 4, no. 2 (2011): 381-417.

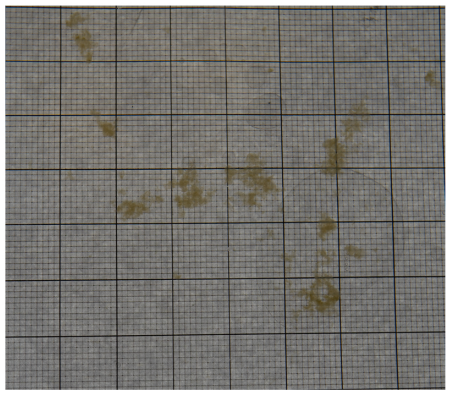
APPENDICES

APPENDIX A. Interaction plots of ingestion rate as calculated from raw gut pigment data versus growth phase and treatment for all six experiments: (A) Experiment 1, (B) Experiment 2, (C) Experiment 3, (D) Experiment 4, (E) Experiment 5, and (F) Experiment 6. Error bars represent standard error. Colors and symbols represent the same treatments as in Figure 3.

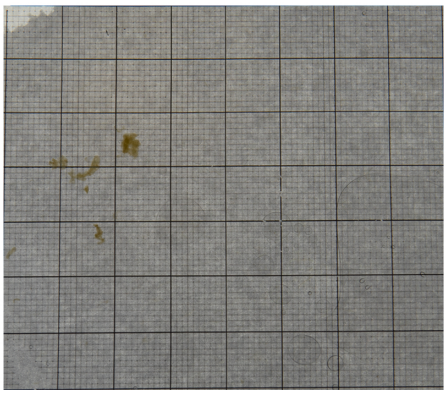

A
B
C
D
E
F
Aggregate
Phytoplankton
Control
Gut Pigment (µg/copepod)
0.015
0.010
0.005
0.000
EarlyExp
LateExp
LateStat
Growth Phase

APPENDIX B. Images of *T. weissflogii* aggregates from one of the two aggregate treatment tanks for Experiment 3 early exponential growth phase (A), Experiment 3 late exponential growth phase (B), Experiment 5 early exponential growth phase (C), and Experiment 5 late exponential growth phase (D). Small square grids in images measure 1 mm^2.

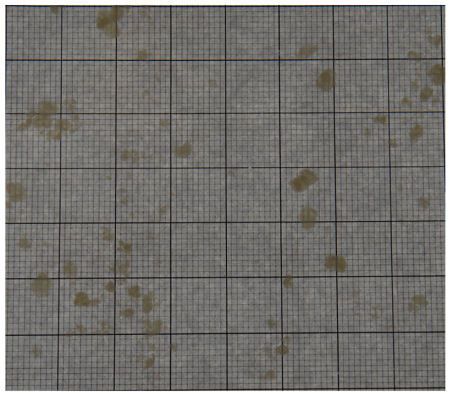
A



B



C



D