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Growth, Grazing and Starvation Survival in Three Heterotrophic Dinoflagellate Species

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**GROWTH, GRAZING AND STARVATION SURVIVAL IN THREE
HETEROTROPHIC DINOFLAGELLATE SPECIES**

BY

SEAN ANDERSON

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

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ABSTRACT

We measured growth and grazing rates of three heterotrophic dinoflagellate species *Oxyrrhis marina*, *Gyrodinium dominans* and *G. spirale* exposed to starved versus continuously-fed prey conditions and assessed their starvation survival ability. All three dinoflagellate species survived long periods (>10 days) without prey, up to 118 days in the case of *O. marina*. After 1-3 weeks, starvation led to a 17-57% decrease in grazer cell volume and cells became more deformed and transparent over time. Starved grazers rapidly ingested new prey within 3 hours as evidenced by increased cell volumes of 4-17%. Grazer cultures that were starved had much lower maximum growth rates (-0.16 - 0.25 d^{-1}) than continuously-fed cultures (0.18 - 0.55 d^{-1}) at an equivalent prey concentration. This suggested a time lag >3 days for all heterotrophic dinoflagellate species to reach their maximum growth rate. Long survival ability coupled with immediate post-starvation ingestion may offer heterotrophic dinoflagellates an advantage over other grazers (e.g. ciliates) in the ability to exploit patchy prey. Delayed grazer population growth and ingestion after starvation has important implications on how we view predator dynamics in a prey-patchy ocean and especially at the onset of seasonal phytoplankton blooms.

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PREFACE

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Manuscript

In Preparation for Submission to the *Journal of Eukaryotic Microbiology*

**Growth, Grazing and Starvation Survival in Three
Heterotrophic Dinoflagellate Species**

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INTRODUCTION

Carbon cycling in the ocean hinges on the level of predation exerted on primary producers and the transfer of organic material through the food web (Worden et al. 2015). Most predation loss of primary production is due to the grazing activity of heterotrophic protists (i.e. <200 μm microzooplankton) (Sherr and Sherr 2002). These organisms, which are ubiquitous in eutrophic and oligotrophic ecosystems, have been estimated to consume an average of 67% of daily primary production, exerting a grazing impact on phytoplankton biomass exceeding that of copepods and other mesozooplankton (Calbet and Landry 2004; Putland 2000; Sherr and Sherr 2002; 2007). Heterotrophic protists play an important role in marine food webs as they link phytoplankton to larger grazers and help drive biogeochemical cycling and carbon flux (Sherr and Sherr 2002).

The success of a single-celled heterotrophic protist largely depends on its ability to encounter prey, which may be difficult at times due to fluctuations in phytoplankton abundance (Menden-Deuer et al. 2005). Patchiness of phytoplankton can occur over wide spatial (μm -m) and temporal (min-wk) scales (McManus et al. 2003). Phytoplankton abundance can fluctuate seasonally and abundance can remain low for several months of the year (e.g. winter-time abundance) in both polar and temperate regions (McManus et al. 2003; Menden-Deuer et al. 2005). Additionally, changes in prey quality may be important, since many heterotrophic protists are known to be selective in regard to prey (Buskey 1997; Hansen and Calado 1999; Jakobsen and Hansen 1997). Patchy prey coupled with predator selection means suitable prey may

be rare at times and once a prey patch has been exploited, protistan predators may be faced with periods of starvation.

The responses of diverse heterotrophic protists to famine-like conditions have been assessed in a variety of laboratory studies (Calbet et al. 2013; Fenchel 1982; 1989; Menden-Deuer et al. 2005). Once starved, initial strategies may involve increased feeding responses coupled with fast digestion times or increased food storage (Fenchel 1989; Menden Deuer et al. 2005; Meunier et al. 2012a, b). Upon entering starvation, some heterotrophic grazers have also been reported to experience an initial cell division without growth, producing smaller and faster daughter cells (termed swimmers) (Fenchel 1982; 1989). Protists are thought to only use a small portion of their energy budget for motility (0.1%), so this adaptive strategy may allow cells to conserve energy while maximizing their search for new prey (Fenchel 1982). Possible secondary responses include the formation of temporary cysts, a reduction in metabolism, or reallocation of cellular reserves such as autophagy of the mitochondria (Calbet et al. 2013; Fenchel 1989; Hansen 1992; Menden-Deuer et al. 2005).

Significant energetic trade-offs associated with survival exist. Heterotrophs must limit metabolism during starvation while maintaining the ability to rapidly resume growth once prey is rediscovered. Ciliated protists have been shown to respond to newly added prey after being starved via immediate ingestion, though predator population growth may lag depending on the duration of starvation (Fenchel 1982; 1989). In a study by Fenchel (1989), the longer a ciliate species was able to survive starvation, the more time (in cell generations) it took to resume population growth. Furthermore, intra- and interspecific variation may exist in the response of predators to

a prey pulse (Calbet et al. 2013; Schmoker et al. 2011). Determining the species-specific response of heterotrophic protists to starvation and the subsequent re-addition of food is critical to understanding predator dynamics in a prey heterogeneous environment.

Heterotrophic dinoflagellates are important phytoplankton grazers and often account for more than half of the total biomass of the microzooplankton community (Kim and Jeong 2004; Lessard 1991; Sherr and Sherr 2007). Though heterotrophic dinoflagellates have lower carbon specific ingestion rates compared with other members of the micro- and mesozooplankton community (e.g. ciliates and copepods), the overall grazing impact of heterotrophic dinoflagellates on natural phytoplankton populations is usually higher than those of other taxonomic groups because of typically higher cell abundances (Jeong et al. 2010). Thus, as this group often constitutes the dominant grazer in terms of total biomass within the plankton assemblage, it is important to understand how heterotrophic dinoflagellates in particular respond to prey fluxes.

The three heterotrophic dinoflagellate species included in this study are quite cosmopolitan, though each thrives in a different habitat. *Gyrodinium* spp. are typically found in coastal and open oceans, while *Oxyrrhis marina* mostly inhabits intertidal pools or estuaries (Hansen 1992; Nakamura et al. 1995; Roberts et al. 2010). All three heterotrophic dinoflagellates are raptorial, ingest their prey via direct engulfment at the cingulum and ingest prey of diverse type and size (Hansen 1992; Hansen and Calado 1999; Jeong et al. 2010; Roberts et al. 2010). Unlike *O. marina* which prefers prey <20 μm , *Gyrodinium* spp. have been shown to ingest prey larger than the

individual's own cell size (Hansen 1992), including chain-forming diatoms (Horner et al. 2005; Saito et al. 2006). Sherr and Sherr (2009) also identified heterotrophic dinoflagellates, particularly *Gyrodinium* sp., as important predators of diatom biomass in polar waters. As diatoms are known to be a major constituent of vertical carbon flux, the ability of *Gyrodinium* spp. to graze on diatoms makes this genus a potentially important mediator of export production (Sarhou et al. 2005).

There has only been one study on starvation and refeed in *G. dominans* and *O. marina* (Calbet et al. 2013), though *G. dominans* had previously been shown to respond to pulsed prey (Schmoker et al. 2011). Calbet et al. (2013) reported both species were able to survive up to 12 d when starved, though significant decreases in body size, respiration and fatty acid content were measured after 5 d; both *O. marina* and *G. dominans* responded to prey post-starvation by increasing cell volume after 10 h, though potential ingestion rates were initially depressed for both species (Calbet et al. 2013). In the field, Saito et al. (2006) reported an increased abundance of *Gyrodinium* sp. over a period of days during an iron-enriched diatom bloom. These studies provide evidence that heterotrophic dinoflagellates are capable of quickly responding to increased prey abundance.

Few responses of heterotrophic dinoflagellates to long-term starvation have been assessed. Common dinoflagellates such as those in the genus *Protoperidinium* have been shown to survive beyond 20 d and up to 71 d at less than 1 $\mu\text{g C liter}^{-1}$ of prey (Menden-Deuer et al. 2005). Strom (1991) also reported a survival time of 30 d within a species of starved *Gymnodinium*. Overall, heterotrophic dinoflagellates are thought to be able to survive in famine-like conditions for longer periods than other major

protistan grazers, namely ciliates, which die within a matter of days when starved (Jackson and Berger 1984; Jeong and Latz 1994).

It has been reported that heterotrophic dinoflagellates feed when exposed to a new prey pulse (Calbet et al. 2013; Schmoker et al. 2011), but to our knowledge there have been no reports of subsequent daily population growth rates after starvation.

Heterotrophic protists are thought to grow at rates similar to the phytoplankton cells they eat, allowing their numbers to respond quickly to an increase in available prey (Sherr et al. 2003). How does the potential delay to feeding after the reintroduction of prey impact the daily division rates of dinoflagellates? A significant delay in feeding or population growth after starvation may have direct implications on how grazers survive between prey patches and on grazing success once a prey patch is found. If there is a lag, as we expect based on previous studies (Calbet et al. 2013; Fenchel 1989), grazing pressure by heterotrophic dinoflagellates would be initially reduced relative to rates of primary production. Ultimately, reduced grazing may enable bloom-like conditions to develop or persist until grazers resume their maximum growth rates.

The main focus of this study was to compare the functional and numerical responses of three heterotrophic dinoflagellate species *Oxyrrhis marina*, *Gyrodinium spirale* and *G. dominans* in response to different food conditions (continuously-fed vs. starved) and over different time scales (h vs. d). More specifically, we were motivated to (1) assess whether heterotrophic dinoflagellates are capable of instantaneously ingesting prey after starvation and (2) to test if this ingestion results in immediate population growth, or whether a time lag exists for heterotrophic dinoflagellates to

resume population growth, as suggested by work from Fenchel (1989). A better understanding of how heterotrophic dinoflagellates respond to periods of starvation and to recurring prey will offer insight into the impact of predation during times of realistic prey fluctuations in the marine environment.

MATERIALS AND METHODS

Culture maintenance

I. Predators

Three heterotrophic dinoflagellate species were used in growth, grazing and starvation experiments. Clonal cultures of *Gyrodinium spirale* (PA300413), *G. dominans* (SPMC 103) and *Oxyrrhis marina* (SPMC 107) were established by single cell isolation. *G. spirale* originated from Narragansett Bay, RI (2013), while strains of *G. dominans* and *O. marina* have origins as described in Strom et al. (2013). All heterotroph cultures were maintained in 1-liter polycarbonate (PC) bottles on a 12 h:12 h light-dark cycle at 14.5 °C, salinity of ~30 psu, and a light intensity of 8-15 $\mu\text{mol photon m}^{-2}\text{s}^{-1}$. Unless specified otherwise, heterotrophs were fed twice per wk with the prey culture *Heterocapsa triquetra* to a final concentration of ($\sim 2,000 \text{ cells ml}^{-1}$) and refreshed with new filtered seawater biweekly.

II. Prey

Three phytoplankton species were cultured for use in feeding experiments. The mixotrophic dinoflagellate *Heterocapsa triquetra* (CCMP 448), the diatom *Skeletonema marinoi* (CCMP 1332), and the prymnesiophyte *Isochrysis galbana* (CCMP 1323) were cultured in autotrophic f/2 media and transferred every 3-5 d to maintain exponential growth (Guillard 1975). All prey cultures were maintained in

250-500-ml PC bottles on a 12 h:12 h light-dark cycle at 15 °C, salinity of ~30 psu, and a light intensity of 70-80 $\mu\text{mol photon m}^{-2}\text{s}^{-1}$.

Cell abundance and biomass

To determine changes in cell abundance over time, 3-ml samples from plankton (both predator and prey) cultures were fixed and counted. Samples were fixed with Lugol's iodine solution (1%) and mounted on a 1-ml Sedgwick-Rafter counting chamber. To establish comparable biomass estimates of the differently-sized species, the biovolume of predators and prey were also measured. Cell counts and measurements were made on a Nikon Eclipse E800 light microscope equipped with image capture (Coriander) and analysis (ImageJ) software. Cell volumes of *Gyrodinium* spp. were calculated assuming an ellipsoid sphere using the following equation: $\text{volume} = 1/3[\pi(\text{cell width}/2)^2](\text{cell length})$ (Kim and Jeong 2004). Cell volume estimates for *Oxyrrhis marina* were calculated assuming a prolate spheroid using the equation: $\text{volume} = (\pi/6)(\text{cell width})(\text{cell length})$ (Hillebrand et al. 1999). Carbon biomass (ng C cell^{-1}) was determined for each predator using the species-specific cell volume estimates and published C to volume conversion equations (Menden-Deuer and Lessard 2000).

Growth and grazing experiments

Incubation experiments were performed to determine growth and ingestion rates of the three heterotrophic dinoflagellate species fed the same prey, *Heterocapsa triquetra*. Fifteen prey concentrations of *H. triquetra* culture (50-6,000 cells ml^{-1} or 18-2,145 ng C ml^{-1}) were added to duplicate 150-ml bottles of known initial predator concentration (80-313 cells ml^{-1}). Duplicate controls were included, consisting of a

known concentration of only prey to control for prey abundance changes in the absence of a predator. Heterotrophic dinoflagellates were starved for 2-3 d prior to grazing experiments to ensure no residual prey remained at the onset of experiments (this was verified microscopically). Duplicate samples of 3 ml were fixed daily for 3 d for abundance and biomass estimates.

Due to the observation of *Gyrodinium* spp. as a potential diatom grazer, the feeding response of *G. spirale* on *Skeletonema marinoi* was also tested. Feeding experiments with *S. marinoi* were placed on a rotating wheel under the same experimental conditions as described above. In addition, a strain of *G. spirale* (isolated from the Western Antarctica Peninsula) was fed with *Thalassiosira weissflogii* (CCMP 1336) and incubated on a rotating wheel at 2 °C. Seven prey concentrations (2,857-19,049 cells ml⁻¹ or 214-1,430 ng C ml⁻¹) were used and duplicate samples were taken daily for 4 d.

The numerical response of the predators was determined by the growth rate equation used by Hansen (1992):

$$\mu = [\ln(N_t/N_0)]/\Delta t \quad (1)$$

where N_0 and N_t represent the concentration of cells at the beginning and end of the experiment, respectively and Δt is the difference in incubation time (d). Growth rates were calculated cumulatively over the 3 d from an initial cell abundance. The highest specific growth rate over that incubation period was determined for each predator. Final growth curves for *G. spirale* represented 72 h of incubation, while *O. marina* and *G. dominans* represented 48 h. Michaelis-Menten enzyme kinetics were used as it provides an accurate approximation of feeding in heterotrophic protists and has

become standard in the field (Jeong et al. 2008; Kim and Jeong 2004; Montagnes and Berges 2004). The growth constant (μ) as a function of each of the 15 prey concentrations was fitted to the Michaelis-Menten equation used by Kim and Jeong (2004), which accounts for negative predator growth:

$$\mu = \mu_{\max} (\mathbf{x} - \mathbf{x}') / \mathbf{K}_{\text{Gr}} + (\mathbf{x} - \mathbf{x}') \quad (2)$$

where $\mu_{\max}$ is the maximum growth rate (d^{-1}), $\mathbf{x}$ is the prey concentration, $\mathbf{x}'$ is the threshold prey concentration where $\mu = 0$, and $\mathbf{K}_{\text{Gr}}$ is the prey concentration sustaining $1/2\mu_{\max}$. For *O. marina*, a threshold prey concentration was not detected and thus $\mathbf{x}'$ was not accounted for in the Michaelis-Menten fit. Data were fitted to this model using SigmaPlot.

Ingestion and clearance rates were calculated using the equations of Frost (1972) and Heinbokel (1978) to account for predator growth. Incubation time used to calculate these rates was the same as for estimating growth rates. Ingestion rates for the predators were also fitted to Michaelis-Menten:

$$\mathbf{IR} = \mathbf{I}_{\max} (\mathbf{x}) / \mathbf{K}_{\text{IR}} + (\mathbf{x}) \quad (3)$$

where $\mathbf{I}_{\max}$ is the maximum ingestion rate of the predator ($\text{cells predator}^{-1} \text{d}^{-1}$ or $\text{ng C predator}^{-1} \text{d}^{-1}$), $\mathbf{x}$ is the prey concentration (cells ml^{-1} or ng C ml^{-1}), and $\mathbf{K}_{\text{IR}}$ is the prey concentration sustaining half of the $\mathbf{I}_{\max}$ (Kim and Jeong 2004).

Gross growth efficiency

Gross growth efficiency (GGE), defined as grazer biomass produced or lost per prey biomass ingested ($\mu/\text{IR} \times 100$), was calculated for mean *Heterocapsa triquetra* concentrations where predator growth was saturated.

Starvation experiments

The response of each heterotrophic dinoflagellate species to prolonged starvation was tested. Starved cultures were transferred to triplicate 2-liter PC bottles and fed *Heterocapsa triquetra* at a final concentration of 2,000 cell ml⁻¹. The starvation period began once *H. triquetra* concentrations reached threshold levels at which predator growth equaled zero ($\mu = 0$) as determined from numerical response curves. For *Oxyrrhis marina*, this period began once all food was cleared. Triplicate experimental bottles were maintained in low light to minimize *H. triquetra* growth. Initially, samples were taken from each bottle every 1-2 d. After 2-3 wk, sampling frequency was decreased to twice per wk. All samples were fixed and predator abundance counted microscopically. Triplicate cell volume measurements were made for each predator at discrete time points to assess changes in predator size over the starvation period. Duplicate 350-ml tissue cultures of starved predators were included in parallel to allow for live inspection of predator morphology and swimming behavior under a dissecting microscope.

Refeed experiments

Given the starvation response, we tested the capacity of starved heterotrophic dinoflagellates to exploit a recurring prey pulse. Heterotroph cultures fed with *Heterocapsa triquetra* were transferred to triplicate 500-ml PC bottles and sampled daily to monitor abundance and biomass. Predators were starved for 1-3 wk until significant decreases in abundance or cell volume were apparent. Heterotrophs were exposed to a pulse of prey by spiking the starved cultures with a known amount of *H. triquetra* or *Isochrysis galbana*. Prey type was chosen for logistical reasons to ensure

appropriate resolution between predator and prey size measurements on the coulter counter. The smaller predators, *Oxyrrhis marina* and *Gyrodinium dominans*, were given $\sim 75,000$ cells ml^{-1} ($\sim 1,006$ ng C ml^{-1}) of *I. galbana*. The chosen concentration of *I. galbana* was shown to induce positive growth rates of *O. marina* under similar temperature conditions (Kimmance et al. 2006). The larger *G. spirale* was given $\sim 4,000$ cells ml^{-1} ($\sim 1,430$ ng C ml^{-1}) of *H. triquetra*, equal to the concentration supporting maximum growth rate (μ_{max}) of *G. spirale* (this study). Predator and prey-only controls were included in triplicate.

Cell volume and abundance of triplicate predator cultures was measured at intervals of 5 to 30 min for the first 2 h and again after 3 h on a MultisizerTM 3 Coulter Counter[®] (version 3.53, Beckman Coulter). The coulter counter provided a more rapid sampling approach than microscopy and allowed repeat measurements for 3 h at 5-30 min intervals. Microscope samples were taken and preserved throughout the refeed to verify coulter counter measurements. Size was initially measured on the coulter counter as ESD (estimated spherical diameter), which was later converted to cell volume (μm^3). Refed heterotroph cultures were allowed to incubate for up to 3 d after the refeed to determine the long term (daily) growth and grazing response. Growth, grazing and clearance rates of all three heterotrophic dinoflagellate species were measured as described above.

RESULTS

Growth rates

All three heterotrophic dinoflagellate species were able to ingest and achieve positive growth rates on *Heterocapsa triquetra* (Fig. 1). Of the dinoflagellate species, *Oxyrrhis marina* exhibited the highest maximum specific growth rate of 0.58 d^{-1} at approximately 322 ng C ml^{-1} . Specific growth rates of *O. marina* increased rapidly at low prey concentrations and became saturated $>143 \text{ ng C ml}^{-1}$ (Fig. 1). *O. marina* growth rates were positive, even at the lowest prey concentration (18 ng C ml^{-1}), resulting in a good approximation of Michaelis-Menten (Eq. 2; $r^2 = 0.85$).

The specific growth rates of *Gyrodinium spirale* feeding on *H. triquetra* increased with increasing prey concentration, reaching a maximum of 0.45 d^{-1} at $1,429 \text{ ng C ml}^{-1}$ (Fig. 1). *G. spirale* growth rate decreased at the highest prey concentration ($2,145 \text{ ng C ml}^{-1}$). At lower prey concentrations ($<245 \text{ ng C ml}^{-1}$), *G. spirale* exhibited negative growth which was proportional to the prey concentration, with near linear decreases in predator growth rate at diminishing prey concentrations.

G. dominans had the lowest maximum growth rate of the three dinoflagellate species on *H. triquetra* (0.24 d^{-1}); however, *G. dominans* growth rates were on average 160% higher than *G. spirale* at lower prey concentrations ($18\text{-}179 \text{ ng C ml}^{-1}$). Specific growth rates of *G. dominans* increased with increasing prey concentration, becoming saturated above 536 ng C ml^{-1} (Fig. 1). Growth-prey abundance relationships were well approximated by Michaelis-Menten kinetics for both *G. dominans* and *G. spirale* (Eq. 2; $r^2 = 0.76; 0.71$).

G. spirale grew poorly on the chain-forming diatom *Skeletonema marinoi*.

Specific growth rates on *S. marinoi* were negative at all prey concentrations except for 1,072 ng C ml⁻¹ (45,816 cells ml⁻¹; Appendix Fig. 1). At this concentration, *G. spirale* exhibited a maximum growth rate of 0.20 d⁻¹. At 2 °C, growth rates of *G. spirale* on *Thalassiosira weissflogii* were negative at concentrations <715 ng C ml⁻¹ and reached a maximum of 0.23 d⁻¹ at the highest prey concentration (1,430 ng C ml⁻¹).

Ingestion and clearance rates

All three heterotrophic dinoflagellate species had positive ingestion rates on *Heterocapsa triquetra* regardless of prey concentration (Fig. 2). Ingestion rates of *Oxyrrhis marina* on *H. triquetra* increased with increasing prey concentration until reaching a maximum ingestion rate of 1.91 ng C grazer⁻¹ d⁻¹ (5.3 cells grazer⁻¹ d⁻¹) at 6,000 cells ml⁻¹ or 2,144 ng C ml⁻¹. Ingestion rates of *O. marina* were well approximated by Michaelis-Menten (Eq. 3; $r^2 = 0.92$). Clearance rates of *O. marina* on *H. triquetra* decreased with increasing prey concentrations, following a Holling type-2 feeding response. The maximum clearance rate of *O. marina* was 0.56 µl grazer⁻¹ h⁻¹ at approximately 36 ng C ml⁻¹ (Appendix Fig. 2).

The functional response curve of *Gyrodinium dominans* was similar to *O. marina* and ingestion rates steadily increased without saturation (Fig. 2). *G. dominans* reached a maximum ingestion rate of 1.43 ng C grazer⁻¹ d⁻¹ (4 cells grazer⁻¹ d⁻¹) at 2,144 ng C ml⁻¹ (Eq. 3; $r^2 = 0.88$). Maximum clearance of *G. dominans* was 0.24 µl grazer⁻¹ h⁻¹, approximately half the maximum clearance of *O. marina* (Appendix Fig. 2). Ingestion rates of *G. spirale* increased steadily with increasing prey concentration, saturating above 1,072 ng C ml⁻¹. The maximum ingestion rate of *G. spirale* on *H. triquetra* was

3.37 ng C grazer⁻¹ d⁻¹ (9.4 cells grazer⁻¹ d⁻¹) (Eq. 3; $r^2 = 0.87$) and the maximum clearance rate was 0.34 $\mu\text{l grazer}^{-1} \text{ h}^{-1}$.

Though *G. spirale* did not sustain positive growth on *Skeletonema marinoi*, ingestion was seen at all prey concentrations (Appendix Fig. 3a). This may indicate *G. spirale* was unable to process ingested *S. marinoi* within the sampling duration (3 d) and required a longer incubation time. Ingestion rates increased with increasing prey concentration, reaching a maximum ingestion rate of 3.57 ng C grazer⁻¹ d⁻¹ (155 cells grazer⁻¹ d⁻¹). Ingestion rates fit Michaelis-Menten kinetics (Eq. 3; $r^2 = 0.87$). The maximum clearance rate of *G. spirale* on *S. marinoi* was 0.36 $\mu\text{l grazer}^{-1} \text{ h}^{-1}$ (Appendix Fig. 3b), which was comparable to the maximum clearance on the mixotroph *H. triquetra*. At 2 °C, *G. spirale* fed *Thalassiosira weissflogii* reached a maximum ingestion rate of 6.82 ng C grazer⁻¹ d⁻¹ (91 cells grazer⁻¹ d⁻¹) and clearance rate of 0.70 $\mu\text{l grazer}^{-1} \text{ h}^{-1}$.

Growth gross efficiency

At *Heterocapsa triquetra* concentrations above predator growth rate saturation, average GGE of *Oxyrrhis marina* was 75%, while *Gyrodinium spirale* and *G. dominans* had lower GGEs of 18% and 13%, respectively.

Starvation

All three dinoflagellate species were able to survive extended periods of time (at least 18 and up to 118 d) in the absence of prey or at very low phytoplankton prey concentrations (below threshold levels; Fig. 3). The no-net-growth threshold concentration corresponded to 179 ng C ml⁻¹ and 18 ng C ml⁻¹ for *Gyrodinium spirale* and *G. dominans*, respectively. *Oxyrrhis marina* never reached a threshold on

Heterocapsa triquetra and starvation experiments began once all prey was cleared, typically within 2 d.

The population size of *G. spirale* dropped more than half (59%) in the first 13 d of starvation from 81 ± 2 to 33 ± 1 cells ml^{-1} . The population size of *G. dominans* decreased from 749 ± 20 to 180 ± 3 cells ml^{-1} in the first 12 d (76%), a more pronounced decrease than *G. spirale* over the same period (Fig. 3). Populations of *G. dominans* decreased steadily after 2 wk of starvation and remained at cell concentrations >0.5 cells ml^{-1} for 63 d. *G. spirale* remained at concentrations >0.5 cells ml^{-1} for 18 d, but were undetectable thereafter.

Cell volumes of *G. dominans* decreased substantially (31%) within the first 24 h of starvation ($1,048 \pm 9$ to 721 ± 4 μm^3) and even further (57%) after 10 d (441 ± 4 μm^3 ; Fig. 4). Average cell volume of *G. spirale* increased after 2 d ($5,865 \pm 75$ to $6,488 \pm 91$ μm^3), but decreased to $3,479 \pm 52$ μm^3 after 6 d (41%). Cultures of *G. dominans* appeared to swim faster within the first 2 wk of starvation. *G. spirale* appeared to swim faster within the first 6 d of starvation, but appeared sluggish after 10 d. Throughout the starvation, *G. spirale* cells became increasingly deformed in cell shape, often appearing more elongated.

In contrast to *Gyrodinium* spp., population size of *O. marina* remained fairly stable within the first 15 d of starvation and even slightly increased (915 ± 27 to $1,004 \pm 32$ cells ml^{-1}). After 32 d, cell abundance of *O. marina* was 213 ± 11 cells ml^{-1} , representing a decrease of 77% from initial concentrations (Fig. 3). Cell abundance continued to gradually decrease over time and *O. marina* was able to survive for 118 d (> 1 cell ml^{-1}), longer than either species of *Gyrodinium*. Unlike *Gyrodinium* spp., *O.*

marina did not appear to swim faster at the onset of starvation. Cell volume of *O. marina* increased after 2 d ($3,869 \pm 69$ to $4,474 \pm 45 \mu\text{m}^3$) and decreased at a slower rate (17%) than *Gyrodinium* spp. over the first 15 d ($3,199 \pm 56 \mu\text{m}^3$; Fig. 4). *O. marina* and *G. dominans* became increasingly transparent throughout their respective starvation periods.

Refeed short-term cell volume response

Both *Oxyrrhis marina* and *Gyrodinium dominans* responded to an *Isochrysis galbana* prey pulse ($\sim 75,000$ cells ml^{-1} or $1,006$ ng C ml^{-1}) with increases in cell volume over time (Fig. 5). *O. marina* was refed after cultures were starved for 16 d, while *G. dominans* was refed after 8 d of starvation. It is worth noting that average cell volume of starved *O. marina* ($1,233 \mu\text{m}^3$) was similar to *G. dominans* ($1,265 \mu\text{m}^3$) at the beginning of the refeed. Average cell volume of *O. marina* increased to $1,358 \pm 6 \mu\text{m}^3$ or by 9% after 5 min of feeding. Over the course of the next 2 h, cell volume of *O. marina* remained similar, before increasing to $1,444 \pm 8 \mu\text{m}^3$ after 3 h of feeding (Fig. 5a). During the short-term feeding period (3 h total), average body size of *O. marina* increased by 17%. *O. marina* that were unfed (predator-only control on continued starvation) did not undergo a significant increase in cell volume and were 16% smaller after 3 h than refed *O. marina* ($1,213 \pm 4 \mu\text{m}^3$). Only when prey was available were increases in predator volume observed, suggesting that prey uptake resulted in the observed cell volume changes, which was also corroborated by microscope observations (Fig. 6).

G. dominans did not respond as quickly to added prey compared to *O. marina* (Fig. 5b). Average cell volume of *G. dominans* showed signs of volume increase after

45 min of incubation, reaching $1,309 \pm 19 \mu\text{m}^3$ or $\sim 3\%$ relative to initial starved cell volume. After 3 h, cell volume of refed *G. dominans* was $1,355 \pm 5 \mu\text{m}^3$, which was a 7% increase in cell volume from starved cultures. Control cultures of *G. dominans* that were kept under starvation conditions remained smaller and identical in cell size at initiation of the refeed experiment ($1,269 \pm 5 \mu\text{m}^3$). Cell abundance varied by ± 121 and 248 cells ml^{-1} over the 3 h for *O. marina* and *G. dominans*, respectively.

The larger dinoflagellate, *G. spirale* responded to a new prey pulse of *Heterocapsa triquetra* ($\sim 4,000$ cells ml^{-1} or $1,430$ ng C ml^{-1}) after 6 d of starvation. During the short-term sampling period, the largest increase in *G. spirale* cell volume occurred within the first 5 min; cell volume increased from $6,414 \pm 129$ to $6,935 \pm 114 \mu\text{m}^3$, equal to an 8% increase (Fig. 5c). This increase in cell volume was not sustained and fluctuated for the rest of the sampling period. *G. spirale* cell size increased by only 4% after 3 h. *G. spirale* that were unfed, did not show much fluctuation in cell volume over the short-term refeed. However, unlike the other two dinoflagellates, the unfed *G. spirale* cultures were at times higher in cell volume than refed cultures (Average: $6,676 \pm 20 \mu\text{m}^3$), and thus, it was impossible to attribute changes in cell size to a feeding response. Overall, *G. spirale* had a much wider range in cell volume compared with the other two dinoflagellates, which may have added to this discrepancy. Cell abundance of *G. spirale* varied by ± 21 cells ml^{-1} over the 3-h sampling period.

Refeed daily growth and grazing rates

Cell volume of all three dinoflagellate species continued to increase after the short-term sampling period (Fig. 7). *Oxyrrhis marina* reached a maximum cell volume after

48 h ($2,835 \pm 124 \mu\text{m}^3$), representing a 56% increase from the initial refeed. *O. marina* cell volume decreased to $2,364 \pm 15 \mu\text{m}^3$ after 72 h. Average cell volume of *Gyrodinium dominans* reached a maximum of $2,616 \pm 46 \mu\text{m}^3$ (51% increase) after 72 h, while *G. spirale* increased to $9,863 \pm 292 \mu\text{m}^3$ over the same duration (35%; Fig. 7). Concentrations of *G. spirale* at the start of the refeed (64 cells ml^{-1}) were much lower than either *G. dominans* ($732 \text{ cells ml}^{-1}$) or *O. marina* ($1094 \text{ cells ml}^{-1}$). Even when in continuous laboratory cultures, *G. spirale* did not grow as fast on *Heterocapsa triquetra* and could not reach concentrations $>250 \text{ cells ml}^{-1}$, whereas the smaller heterotrophic dinoflagellate species could reach $>1,000 \text{ cells ml}^{-1}$ on the same prey.

After a pulsed refeeding, all heterotrophic dinoflagellate species resumed slow growth and were unable to reach maximum growth rates of continuously-fed cultures (Fig. 8). After 24 h, *O. marina* refed with *Isochrysis galbana* did not show positive growth in population abundance due to a negative population growth rate after prolonged starvation ($\mu = -0.10 \text{ d}^{-1}$). The specific growth rate of *O. marina* reached 0.04 d^{-1} after 48 h and a substantive, positive growth rate of 0.25 d^{-1} was measured after 72 h of incubation. Specific growth rate of *G. dominans* was similar to that of *O. marina* after the first 48 h of incubation and *G. dominans* also reached its maximum growth after 72 h (0.09 d^{-1}). *G. spirale* had the lowest maximum growth rate after 72 h of all three dinoflagellates, which remained negative ($\mu = -0.16 \text{ d}^{-1}$). Though refed with a different prey (*I. galbana*), growth rates of continuously-fed *O. marina* and *G. dominans* at an equivalent biomass of *H. triquetra* ($\sim 1,006 \text{ ng C ml}^{-1}$) were much higher at 0.55 and 0.18 d^{-1} , respectively. The maximum growth rate of continuously-

fed *G. spirale* was much higher (0.45 d^{-1}) on $1,430 \text{ ng C ml}^{-1}$ of *H. triquetra* compared to starved cultures fed the same amount of *H. triquetra* (-0.16 d^{-1}) (Fig. 8).

The maximum ingestion rate of *O. marina* refed *I. galbana* was $0.34 \text{ ng C grazer}^{-1} \text{ d}^{-1}$ ($34 \text{ cells grazer}^{-1} \text{ d}^{-1}$) after 48 h and ingestion became saturated after 72 h.

Ingestion rates of *G. dominans* also increased throughout the refeed period, reaching a maximum rate of $0.37 \text{ ng C grazer}^{-1} \text{ d}^{-1}$ ($37 \text{ cells grazer}^{-1} \text{ d}^{-1}$) after 72 h. At $\sim 1,006 \text{ ng C ml}^{-1}$ of *H. triquetra*, ingestion rates of continuously-fed *O. marina* and *G. dominans* were more than 2-fold higher than starved cultures at 1.09 and $0.99 \text{ ng C grazer}^{-1} \text{ d}^{-1}$, respectively (Appendix Fig. 4). Clearance rates of *O. marina* and *G. dominans* on newly added *I. galbana* were static and remained low ($<0.05 \mu\text{l grazer}^{-1} \text{ h}^{-1}$) over the refeed incubation period. Unlike the other two dinoflagellate species, positive ingestion or clearance on the refed prey pulse was not distinguishable for *G. spirale* and thus rates were not presented.

DISCUSSION

Heterotrophic protists are considered particularly important to the structure and function of marine food webs. The growth rates of these heterotrophs are on par with the growth of their phytoplankton prey, resulting in considerable biogeochemical impacts (Sherr et al. 2003; Sherr and Sherr 2009). Here we investigated whether the maximum specific growth and grazing rates of prominent dinoflagellate predators were impacted by fluctuations in the presence and quantity of prey. Thus, our experiments sought to mimic the conditions in a patchy ocean, with spatio-temporal fluctuations in prey abundance in a heterogeneous environment. We were able to capture an instantaneous (within min-h) feeding response after a period of 1-3 wk of

starvation, though all three heterotrophic dinoflagellate species were unable to reach their maximum daily growth and ingestion rates over 3 d. Our results confirm the already well-established ability of heterotrophic dinoflagellates to withstand extended periods of starvation (Hansen 1992; Menden-Deuer et al. 2005; Strom 1991) and report, to date, the longest observed starvation duration of 118 d for any protistan grazer (*Oxyrrhis marina*), astonishing for a single-celled organism.

Though starved, predators maintained the ability to ingest and remove phytoplankton biomass. In 2 out of 3 predator species starved for 6-16 d, increases in cell volume were measurable on the order of min-h. Within 3 h, cell volume of *O. marina* and *Gyrodinium dominans* increased from initial starved volumes by 17% and 7%, respectively, while cell volume was too variable in *G. spirale* to support measurable changes in cell volume. This agrees with the one prior study investigating refeeding capacity in dinoflagellates, in which cell volume of refeed *O. marina* increased by ~72%, while *G. dominans* increased by ~38%, over 10 h (Calbet et al. 2013). Though potential ingestion rates were high at the onset of the refeed, ingestion rates stabilized after 100 min for both dinoflagellate species (Calbet et al. 2013). In both of these refeed studies, rapid increases in cell volume of heterotrophic dinoflagellates suggested they were able to immediately ingest new prey post-starvation. The ability of heterotrophic dinoflagellates to rapidly ingest prey implies they are not completely inert after experiencing starvation, though rates of predator ingestion and in turn growth may be limited.

A predator's ability to resume cell division after starvation is critical for survival and has been quantified in a collection of ciliates (Fenchel 1982; 1989), but not in

heterotrophic dinoflagellates. Thus, we incorporated measurements of predator population growth rates after starvation to address this gap in knowledge. Only after 2-3 d did refeed *O. marina* and *G. dominans* populations show positive growth, while *G. spirale* was unable to attain positive growth or measurable ingestion throughout the refeed period. Though some grazers experienced positive population growth as soon as 2 d after starvation, the time to reach maximum growth or ingestion was delayed, in this case longer than 3 d. This discrepancy in starved vs. continuously-fed predator growth rates implies that starved predators have a relatively lower potential to remove phytoplankton production than continuously-fed predators. Variability in the feeding response after starvation suggests this response is species-specific among heterotrophic dinoflagellates. Hence, predator species composition is an important factor influencing the response to patchy prey and should be considered when modelling this response.

This observed time lag before commencement of positive population growth may reflect a type of energetic trade-off between starvation and ability to resume predation, as proposed by Fenchel (1989). When a predator undergoes starvation, over time metabolic rates will decrease, the extent of this reduction is determined by how long the predator starves (Fenchel 1989). After 5 d of starvation, Calbet et al. (2013) recorded decreases in respiration rates of *O. marina* and *G. dominans* strains by up to 70% and 50%, respectively. Heterotrophic dinoflagellates in our study were subjected to longer starvation times (6-16 d) before new prey was added and thus significant decreases in respiration and in turn metabolism were likely to have occurred prior to the refeed, though no measurements were made. We did measure alterations to cell

morphology in starved heterotrophic dinoflagellates (e.g. 17-57% lower cell volume or cell deformation), which may have signified a shift in metabolism (Fenchel 1989).

Predators in this study did not initially grow after starvation and in some cases continued to decrease in daily population abundance, despite the occurrence of new prey. Delayed or even negative population growth after starvation may implicate the ability of these heterotrophic dinoflagellates to grow at rates comparable to their phytoplankton prey. As a result of heterotrophic dinoflagellates' role as dominant plankton grazers, a reduction in grazing pressure after starvation may directly impact predator-prey dynamics and carbon flow when prey is patchy.

It is also important to better understand the capacity of heterotrophic dinoflagellates to survive periods of famine, as prey patchiness can occur over a wide range of spatial and temporal scales (Jakobsen and Hansen 1997; McManus et al. 2003). All three heterotrophic dinoflagellate species survived for at least 18 d and up to 118 d in the case of *O. marina*, representing to our knowledge the longest published starvation duration for a heterotrophic dinoflagellate. Long starvation periods have been observed previously for dinoflagellates (Menden-Deuer et al. 2005; Strom 1991) but not for ciliates, which die rapidly within hr-d (Jackson and Berger 1984; Jakobsen and Hansen 1997). Though ciliates may exhibit faster rates of ingestion and cell division compared to heterotrophic dinoflagellates (Jeong et al. 2010; Strom and Morello 1998), they are less equipped for long periods of starvation. Heterotrophic dinoflagellates may have an advantage over ciliates in their ability to allocate part of their energy into storage products to prolong survival (Menden-Deuer et al. 2005). The difference in survival vs. feeding between these two grazer types may follow an *r*- vs.

k-strategy, where generally heterotrophic dinoflagellates (*k-strategy*) are slower to divide, but can survive longer under starvation. In contrast, ciliates (*r-strategy*) have fast division times, but are unable to survive beyond 2-3 d without prey. In order to accurately compare the starvation and feeding response of these different grazer types and their respective predation impact on phytoplankton biomass, measures of both feeding and survival ability must be considered.

Assuming constant mortality, Calbet et al. (2013) predicted a survival time of ~50 d for an *O. marina* strain used in their study, but measurements presented here suggest *O. marina* was capable of much longer starvation. *O. marina* has a slow digestion time (Klein et al. 1986) and large capacity for food storage which allows cell division of the organism, even when food is absent (Calbet et al. 2013; Flynn and Mitra 2009; this study). While cannibalism has been shown to occur in *O. marina* populations, the probability even at high predator abundance is low ($\leq 2\%$) and even less likely as predator abundance decreases (Montagnes et al. 2010). Though no evidence of bacterial ingestion was found, it may be reasonable to assume *O. marina* was ingesting bacteria in our cultures, as cells continued to divide during the first 2 wk of starvation. Moreover, *O. marina* has been shown to survive on the uptake of dissolved organic molecules (Lowe et al. 2010; Roberts et al. 2010), which it may rely on to survive incredible lengths. Together, these adaptations may increase the probability of *O. marina* encountering new prey and enable the species to survive in fluctuating habitats such as tide pools and salt marshes.

Cyst formation was not observed during starvation in any of the three studied species, though it is a well-known, long-term survival strategy for other dinoflagellate

genera like *Alexandrium* (Anderson et al. 2014). However, production of swarmer cells may have occurred in species of *Gyrodinium*. Interestingly, there was no immediate decrease in cell volume of *O. marina*, which may relate to its propensity to maximize food storage instead of devoting energy to quickly digest prey and increase swimming when starved. Within the first 2 wk of starvation, *Gyrodinium* spp. cell volume decreased by >50% and cells appeared to swim faster compared to initial populations, a morphological change also reported by Fenchel (1989) in ciliate species. These morphological changes may represent a strategy to maximize swimming at the onset of starvation to help expedite the search for new prey, particularly as *Gyrodinium* spp. reside in open waters with patchy prey.

As starvation time increased, all three heterotrophic dinoflagellate species became increasingly transparent, especially in the case of *O. marina* and *G. dominans*. These two heterotrophs survived much longer, suggesting that transparency likely indicates the use of cellular reserves, a response also seen in starving *Protoperidinium* (Menden-Deuer et al. 2005). Overall, some generalizations can be made in regards to the response of heterotrophic dinoflagellates to starvation, which include the ability to avoid cyst production, become smaller in size, and more transparent over time. Responses of dinoflagellates to starvation may be the result of a strategy to preserve cellular metabolic demands in the absence of food, enabling long starvation survival.

The feeding response of heterotrophic dinoflagellates under continuously-fed conditions offered a comparison to pulsed feeding studies of starved predators and allowed us to establish a baseline feeding behavior. All three heterotrophic dinoflagellate species ingested and grew on *Heterocapsa triquetra*, though *O. marina*

had the highest maximum growth rate (0.58 d^{-1}) and was the most efficient grazer in terms of assimilating carbon (highest GGE). Feeding of *O. marina* on *H. triquetra* had not been investigated prior to this experiment, though *O. marina* had been shown to feed on a wide range of pico- to nanoplankton, including flagellates $\geq 4\text{ }\mu\text{m}$ (Roberts et al. 2010). Though growth rates of *Gyrodinium* spp. on *H. triquetra* matched well with previous studies (Table 1; Hansen 1992; Nakamura et al. 1995), rates of ingestion were markedly lower, which may be attributed to strain-specific variation in the predator (Calbet et al. 2013; Nakamura et al. 1995).

Measurements of *G. spirale* feeding on the chain-forming diatom *Skeletonema marinoi* allowed us to compare feeding behavior on multiple prey items. Though *G. spirale* ingested the diatom at a similar rate as *H. triquetra*, positive population growth was not supported over a wide range of prey concentrations and growth rates were much lower than on *H. triquetra*. This provides additional evidence that heterotrophic dinoflagellates do not have an all-encompassing feeding response and are selective in their feeding (Hansen 1992; Jakobsen and Hansen 1997; Jeong and Latz 1994). Even within the genera of *Gyrodinium* and *Oxyrrhis*, growth and ingestion rates have been shown to vary based on prey type (Table 1). The ability of heterotrophic dinoflagellates to ingest certain prey depends on a number of factors including prey size and motility, nutritional value, and chemical composition (Hansen and Calado 1999; Tillmann 2004). The preferred prey of many heterotrophic dinoflagellates remains unknown (Hansen and Daugbjerg 2004), thus laboratory measurements of growth and grazing on a range of prey types are useful in effectively culturing these predators and in understanding prey selection.

Significant intra-genaric variation existed amongst *Gyrodinium* spp. in both their ability to survive starvation and exploit recurring prey. *G. dominans* had been shown to adapt to prey fluctuations (Schmoker et al. 2011; this study), while the larger *G. spirale* died rapidly when starved and had a much weaker post-starvation feeding response. The feeding behavior of these two species had been directly compared in a study by Kim and Jeong (2004), where they measured higher swimming speeds and growth rates at low prey concentrations in *G. dominans* relative to *G. spirale*. In contrast, *G. spirale* may have an advantage over *G. dominans* during eutrophic conditions characterized by high prey abundances (Kim and Jeong 2004; this study). *G. dominans* was not refed with the similar-sized *H. triquetra* in large part due to constraints associated with distinguishing cell volume changes using rapid coulter counter sampling. As a result of this sampling constraint, our studies were not optimized to choose the prey species supporting the highest specific growth rate and thus predators did not all receive the same prey. This variation in prey type may have contributed to the difference in growth after starvation amongst the two species of *Gyrodinium* we tested.

It has been argued that food particles are rarely exhausted in the ocean and protist grazers never experience complete starvation as simulated in our study (Paffenhöfer et al. 2007). However, phytoplankton are rare or absent over wide spatio-temporal scales and grazers have been shown to be selective in the prey they consume (Jakobsen and Hansen 1997; McManus et al. 2003; this study), thus grazers are likely faced with periods of low prey abundance. Nevertheless, *O. marina* and *G. dominans* exhibited tremendous ability to survive without prey, which may offer a competitive advantage

over other heterotrophic dinoflagellate species like *G. spirale*, and certainly heterotrophic protists with lower starvation capacity (e.g. ciliates). Grazers that can adapt to prey fluctuations will be better equipped when a new prey patch is encountered.

We observed that a considerable time lag existed before heterotrophic dinoflagellate populations could resume growth after extended periods of starvation. This is contrary to the common assumption that protistan grazers exert rapid increases in predator population growth to match growth of their prey (Sherr et al. 2003). If our laboratory observations apply to *in situ* conditions and predator populations undergo starvation, ingestion is indeed immediate, but predator population abundance and thus predation pressure would not necessarily increase within the first 2-3 d. Ultimately, bloom conditions may be able to persist, as the predation impact from grazers would be initially low. This supports suggestions from Sherr and Sherr (2009), in which they discussed the inability of herbivorous protists to control the initiation and development of global phytoplankton blooms. A lag time in population growth may help explain observations of high phytoplankton growth and low grazing at the onset of a bloom event and be a determining factor for carbon transport during these times.

Building on previous studies (Calbet et al. 2013; Menden-Deuer et al. 2005; Strom 1991), we were able to quantify the response to starvation and recurring prey in a set of important heterotrophic dinoflagellate species. Though species-specific variation existed, we were able to identify some generalized responses to starvation, which included a reduction in cell size, an increase in transparency and a remarkable survival ability on the order of wk-mo. All three heterotrophic dinoflagellates could

immediately engulf prey after starvation, but positive population growth and ingestion was delayed by 2-3 d. This finding has implications on how we view predator-prey dynamics in a seemingly prey-patchy ocean and especially at the onset of seasonal phytoplankton blooms. Heterotrophic dinoflagellates are one of the dominant groups of phytoplankton grazers and as a result can be potentially important conduits of carbon transport in marine food webs. Therefore, further measures of growth and ingestion coupled with an understanding of survival capacity in heterotrophic dinoflagellates will be critical in assessing the impact of predation in a heterogeneous environment.

CONCLUSION

To date, only a handful of studies have investigated the response of heterotrophic dinoflagellates to starvation (Menden-Deuer et al. 2005; Strom 1991) and to the addition of new prey post-starvation (Calbet et al. 2013; Hansen 1992; this study). Among the heterotrophic dinoflagellate species we tested, the response to prolonged starvation was relatively consistent and survival times reached 118 d in the case of *Oxyrrhis marina*. After periods of 1-3 wk, which seem reasonable for *in situ* conditions (e.g. between blooms; low winter-time prey abundances), starvation elicited decreases in heterotrophic dinoflagellate abundance, altered cell morphology and even impacted future growth of grazer populations. Even though heterotrophic dinoflagellates may be capable of rapid ingestion after starvation, they exhibited delayed growth. Variability among heterotrophic dinoflagellate species in terms of daily growth and grazing after starvation, may indicate long-term effects are harder to predict and are likely species-specific. We were able to provide additional evidence

suggesting differences in feeding and survival strategy between ciliates and heterotrophic dinoflagellates (Jackson and Berger 1984; Menden-Deuer et al. 2005), which should be considered when assessing the impact of herbivory on primary production. Additional measurements of feeding during various prey conditions and across a wider grazer taxa are critical to accurately model the impact of starvation on heterotrophic dinoflagellate feeding and the associated implications this may have on larger food web processes.

REFERENCES

- Anderson, D. M., Keafer, B. A., Kleindinst, J. L., McGillicuddy, D. J., Martin, J. L., Norton, K., Pilskaln, C. H., Smith, J. L., Sherwood, C. R. & Butman, B. 2014. *Alexandrium fundyense* cysts in the Gulf of Maine: long-term time series of abundance and distribution, and linkages to past and future blooms. *Deep Sea Res., Part II*, 103:6-26.
- Buskey, E. J. 1997. Behavioral components of feeding selectivity of the heterotrophic dinoflagellate *Protoperidinium pellucidum*. *Mar. Ecol. Prog. Ser.*, 153:77-89.
- Calbet, A. & Landry, M. R. 2004. Phytoplankton growth, microzooplankton grazing, and carbon cycling in marine systems. *Limnol. Oceanogr.*, 49:51-57.
- Calbet, A., Isari, S., Martínez, R. A., Saiz, E., Garrido, S., Peters, J., Borrat, R. M. & Alcaraz, M. 2013. Adaptations to feast and famine in different strains of the marine heterotrophic dinoflagellates *Gyrodinium dominans* and *Oxyrrhis marina*. *Mar. Ecol. Prog. Ser.*, 483:67-84.
- Dodge, J. D. & Hart-Jones, B. 1982. *Marine dinoflagellates of the British Isles*. HMSO.
- Fenchel, T. 1982. Ecology of heterotrophic microflagellates. II. Bioenergetics and growth. *Mar. Ecol. Prog. Ser.*, 8:225-231.
- Fenchel, T. 1989. Adaptations to a feast and famine existence in protozoa. In: Wieser W, Gnaiger G (eds) *Energy transformation in cells and organisms*. Georg Thieme Verlag, Stuttgart, pp. 290-295.
- Flynn, K. J. & Mitra, A. 2009. Building the “perfect beast”: modelling mixotrophic plankton. *J. Plankton Res.*, 31:965-992.
- Frost, B. W. 1972. Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. *Limnol. Oceanogr.*, 17:805-815.
- Goldman, J. C., Dennett, M. R. & Gordin, H. 1989. Dynamics of herbivorous grazing by the heterotrophic dinoflagellate *Oxyrrhis marina*. *J. Plankton Res.*, 11:391-407.
- Guillard, R. R. 1975. Culture of phytoplankton for feeding marine invertebrates. In *Culture of marine invertebrate animals*, pp. 29-60.
- Hansen, P. J. 1992. Prey size selection, feeding rates and growth dynamics of heterotrophic dinoflagellates with special emphasis on *Gyrodinium spirale*. *Mar. Biol.*, 114:327-334.
- Hansen, B. W., Bjørnsen, P. K. & Hansen, B. W. 1997. Zooplankton grazing and growth: Scaling within the 2-2,000-~ III body size range. *Limnol. Oceanogr.*, 42:687-704.
- Hansen, P. J. & Calado, A. J. 1999. Phagotrophic Mechanisms and Prey Selection in Free-living Dinoflagellates. *J. Eukaryotic Microbiol.*, 46:382-389.
- Hansen, G. & Daugbjerg, N. 2004. Ultrastructure of *Gyrodinium spirale*, the type species of *Gyrodinium* (Dinophyceae), including a phylogeny of *G. dominans*, *G. rubrum* and *G. spirale* deduced from partial LSU rDNA sequences. *Protist*, 155:271-294.

- Heinbokel, J. F. 1978. Studies on the functional role of tintinnids in the Southern California Bight. II. Grazing rates of field populations. *Mar. Biol.*, 47:191-197.
- Hillebrand, H., Dürselen, C. D., Kirschtel, D., Pollinger, U. & Zohary, T. 1999. Biovolume calculation for pelagic and benthic microalgae. *J. Phycol.*, 35:403-424.
- Horner, R. A., Postel, J. R., Halsband-Lenk, C., Pierson, J. J., Pohnert, G. & Wichard, T. 2005. Winter-spring phytoplankton blooms in Dabob Bay, Washington. *Prog. Oceanogr.*, 67:286-313.
- Jackson, K. M. & Berger, J. 1984. Survival of ciliate protozoa under starvation conditions and at low bacterial levels. *Microb. Ecol.*, 10:47-59.
- Jakobsen, H. H. & Hansen, P. J. 1997. Prey size selection, grazing and growth response of the small heterotrophic dinoflagellate *Gymnodinium* sp. and the ciliate *Balanion comatum*-a comparative study. *Mar. Ecol. Prog. Ser.*, 158:75-86.
- Jeong, H. J. & Latz, M. I. 1994. Growth and grazing rates of the heterotrophic dinoflagellates *Protoperidinium* spp. on red tide dinoflagellates. *Mar. Ecol. Prog. Ser.*, 106:173-185.
- Jeong, H. J., Kim, S. K., Kim, J. S., Kim, S. T., Yoo, Y. D. & Yoon, J. Y. 2001. Growth and Grazing Rates of the Heterotrophic Dinoflagellate *Polykrikos kofoidii* on Red-Tide and Toxic Dinoflagellates. *J. Eukaryotic Microbiol.*, 48:298-308.
- Jeong, H. J., Kim, J. S., Yoo, Y. D., Kim, S. T., Kim, T. H., Park, M. G., Lee, H. G., Seong, K. H., Kang, N. S. & Shim, J. H. 2003. Feeding by the heterotrophic dinoflagellate *Oxyrrhis marina* on the red-tide raphidophyte *Heterosigma akashiwo*: a potential biological method to control red tides using mass-cultured grazers. *J. Eukaryotic Microbiol.*, 50:274-282.
- Jeong, H. J., Seong, K. A., Yoo, Y. D., Kim, T. H., Kang, N. S., Kim, S., Park, J. Y., Kim, J. S., Kim, G. H. & Song, J. Y. 2008. Feeding and grazing impact by small marine heterotrophic dinoflagellates on heterotrophic bacteria. *J. Eukaryotic Microbiol.*, 55:271-288.
- Jeong, H. J., Du Yoo, Y., Kim, J. S., Seong, K. A., Kang, N. S. & Kim, T. H. 2010. Growth, feeding and ecological roles of the mixotrophic and heterotrophic dinoflagellates in marine planktonic food webs. *Ocean Sci. J.*, 45:65-91.
- Kim, J. S. & Jeong, H. J. 2004. Feeding by the heterotrophic dinoflagellates *Gyrodinium dominans* and *G. spirale* on the red-tide dinoflagellate *Prorocentrum minimum*. *Mar. Ecol. Prog. Ser.*, 280:85-94.
- Kimmance, S. A., Atkinson, D. & Montagnes, D. J. 2006. Do temperature-food interactions matter? Responses of production and its components in the model heterotrophic flagellate *Oxyrrhis marina*. *Aquat. Microb. Ecol.*, 42:63-73.
- Klein, B., Gieskes, W. W. & Krray, G. G. 1986. Digestion of chlorophylls and carotenoids by the marine protozoan *Oxyrrhis marina* studied by hplc analysis of algal pigments. *J. Plankton Res.*, 8:827-836.
- Lessard, E. J. 1991. The trophic role of heterotrophic dinoflagellates in diverse marine environments. *Mar. Microb. Food Webs* 5:49-58.

- Lowe, C. D., Martin, L. E., Roberts, E. C., Watts, P. C., Wootton, E. C. & Montagnes, D. J. 2010. Collection, isolation and culturing strategies for *Oxyrrhis marina*. *J. Plankton Res.*, 00:1-10.
- McManus, M. A., Alldredge, A. L., Barnard, A. H., Boss, E., Case, J. F., Cowles, T. J., Donaghay, P. L., Eisner, L. B., Gifford, D. J., Greenlaw, C. F., Herren, C. M., Holliday, D. V., Johnson, D., MacIntyre, S., McGehee, D. M., Osborn, T. R., Perry, M. J., Pieper, E. R., Rines, J. E. B., Smith, D. C., Sullivan, J. M., Talbot, M. K., Twardowski, M. S., Weidemann, A. & Zaneveld, J. R. 2003. Characteristics, distribution and persistence of thin layers over a 48 hour period. *Mar. Ecol. Prog. Ser.*, 261:1-19.
- Menden-Deuer, S. & Lessard, E. J. 2000. Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. *Limnol. Oceanogr.*, 45:569-579.
- Menden-Deuer S., Lessard, E. J., Satterberg, J. & Grünbaum, D. 2005. Growth rates and starvation survival of the pallium-feeding, thecate dinoflagellate genus *Peridinium*. *Aquat. Microb. Ecol.*, 41:145-152.
- Meunier, C. L., Haafke, J., Oppermann, B., Boersma, M. & Malzahn, A. M. 2012a. Dynamic stoichiometric response to food quality fluctuations in the heterotrophic dinoflagellate *Oxyrrhis marina*. *Mar. Biol.*, 159:2241-2248.
- Meunier, C. L., Hantzsche, F. M., Cunha-Dupont, A. Ö., Haafke, J., Oppermann, B., Malzahn, A. M. & Boersma, M. 2012b. Intraspecific selectivity, compensatory feeding and flexible homeostasis in the phagotrophic flagellate *Oxyrrhis marina*: three ways to handle food quality fluctuations. *Hydrobiologia*, 680:53-62.
- Montagnes, D. J. S. & Berges, J. A. 2004. Determining parameters of the numerical response. *Microb. Ecol.*, 48:139-144.
- Montagnes, D. J., Lowe, C. D., Martin, L., Watts, P. C., Downes-Tettmar, N., Yang, Z., Roberts, E. C. & Davidson, K. 2010. *Oxyrrhis marina* growth, sex and reproduction. *J. Plankton Res.*, 00:1-13.
- Nakamura, Y., Yamazaki, Y. & Hiromi, J. 1992. Growth and grazing of a heterotrophic dinoflagellate, *Gyrodinium dominans*, feeding on a red tide flagellate, *Chattonella antiqua*. *Mar. Ecol. Prog. Ser.*, 82:275-279.
- Nakamura, Y., Suzuki, S. Y. & Hiromi, J. 1995. Growth and grazing of a naked heterotrophic dinoflagellate, *Gyrodinium dominans*. *Aquat. Microb. Ecol.*, 9:157-164.
- Paffenhöfer, G. A., Sherr, B. F. & Sherr, E. B. 2007. From small scales to the big picture: persistence mechanisms of planktonic grazers in the oligotrophic ocean. *Mar. Ecol.*, 28:243-253.
- Putland, J. N. 2000. Microzooplankton herbivory and bacterivory in Newfoundland coastal waters during spring, summer and winter. *J. Plankton Res.*, 22:253-277.
- Roberts, E. C., Wootton, E. C., Davidson, K., Jeong, H. J., Lowe, C. D. & Montagnes, D. J. 2010. Feeding in the dinoflagellate *Oxyrrhis marina*: linking behavior with mechanisms. *J. Plankton Res.*, 00:1-12.
- Sarthou, G., Timmermans, K. R., Blain, S. & Tréguer, P. 2005. Growth physiology and fate of diatoms in the ocean: a review. *J. Sea Res.*, 53:25-42.

- Saito, H., Ota, T., Suzuki, K., Nishioka, J. & Tsuda, A. 2006. Role of heterotrophic dinoflagellate *Gyrodinium* sp. in the fate of an iron induced diatom bloom. *Geophys. Res. Lett.* 33: L09602, doi: 10.1029/2005GL025366.
- Schmoker, C., Thor, P., Hernández-León, S. & Hansen, B. W. 2011 Feeding, growth and metabolism of the marine heterotrophic dinoflagellate *Gyrodinium dominans*. *Aquat. Microb. Ecol.*, 65:65-73.
- Sherr, E. B. & Sherr, B. F. 2002. Significance of predation by protists in aquatic microbial food webs. *Antonie van Leeuwenhoek*, 81:293-308.
- Sherr, E. B., Sherr, B. F., Wheeler, P. A. & Thompson, K. 2003. Temporal and spatial variation in stocks of autotrophic and heterotrophic microbes in the upper water column of the central Arctic Ocean. *Deep Sea Res. I*, 50:557-571.
- Sherr, E. B. & Sherr, B. F. 2007. Heterotrophic dinoflagellates: a significant component of microzooplankton biomass and major grazers of diatoms in the sea. *Mar. Ecol. Prog. Ser.*, 352:187-197.
- Sherr, E. B. & Sherr, B. F. 2009. Capacity of herbivorous protists to control initiation and development of mass phytoplankton blooms. *Aquat. Microb. Ecol.*, 57:253-262.
- Strom, S. L. 1991. Growth and grazing rates of the herbivorous dinoflagellate *Gymnodinium* sp. from the open subarctic Pacific Ocean. *Mar. Ecol. Prog. Ser.*, 78:103-113.
- Strom, S. L. & Strom, M. W. 1996. Microzooplankton growth, grazing and community structure in the northern Gulf of Mexico. *Mar. Ecol. Prog. Ser.*, 130:229-240.
- Strom, S. L. & Morello, T. A. 1998. Comparative growth rates and yields of ciliates and heterotrophic dinoflagellates. *J. Plankton Res.*, 20:571-584.
- Strom, S. L., Harvey, E. L., Fredrickson, K. A. & Menden-Deuer, S. 2013. Broad salinity tolerance as a refuge from predation in the harmful raphidophyte alga *Heterosigma akashiwo* (Raphidophyceae). *J. Phycol.*, 49:20-31.
- Tillmann, U. 2004. Interactions between planktonic microalgae and protozoan grazers. *J. Eukaryotic Microbiol.*, 51:156-168.
- Worden, A. Z., Follows, M. J., Giovannoni, S. J., Wilken, S., Zimmerman, A. E. & Keeling, P. J. 2015. Rethinking the marine carbon cycle: Factoring in the multifarious lifestyles of microbes. *Science*, 347:6223.

TABLES

Table 1: Comparison of growth, ingestion and clearance rates in the genera *Gyrodinium* and *Oxyrrhis*. Rates are corrected to 14.5 °C using $Q_{10} = 2.8$ (Hansen et al. 1997). $\mu_{\max}$ = maximum growth rate (d^{-1}); $I_{\max}$ = maximum ingestion rate (ng C predator $^{-1} d^{-1}$); $C_{\max}$ = maximum clearance rate (μl predator $^{-1} h^{-1}$); DN = Dinoflagellate; DIA = Diatom; RA = Raphidophyte; PRM = Prymnesiophyte

HTD	Prey	$\mu_{\max}$ (d^{-1})	$I_{\max}$ (ng C predator $^{-1} d^{-1}$)	$C_{\max}$ (μl predator $^{-1} h^{-1}$)	Reference
<i>G. spirale</i>	<i>H. triquetra</i> (DN)	0.45	3.37	0.34	This study
<i>G. spirale</i>	<i>H. triquetra</i> (DN)	0.58	7.24	0.28	Hansen 1992
<i>G. spirale</i>	<i>S. marinoi</i> (DIA)	0.20	3.57	0.36	This study
<i>G. spirale</i>	<i>P. minimum</i> (DN)	0.45	7.59	3.02	Kim and Jeong 2004
<i>G. dominans</i>	<i>H. triquetra</i> (DN)	0.24	1.43	0.24	This study
<i>G. dominans</i>	<i>H. triquetra</i> (DN)	0.27	2.75	0.07	Nakamura et al. 1995
<i>G. dominans</i>	<i>P. minimum</i> (DN)	0.65	0.69	0.51	Kim and Jeong 2004
<i>G. dominans</i>	<i>C. antiqua</i> (RA)	0.30	1.29	NA	Nakamura et al. 1992
<i>O. marina</i>	<i>H. triquetra</i> (DN)	0.58	1.91	0.56	This study
<i>O. marina</i>	<i>H. akashiwo</i> (RA)	0.81	0.72	0.17	Jeong et al. 2003
<i>O. marina</i>	<i>I. galbana</i> (PRM)	0.45	3.98	0.20	Goldman et al. 1989
<i>O. marina</i>	<i>A. carterae</i> (DN)	0.66	1.58	1.35	Jeong et al. 2001

Figure 1: Specific growth rates of three heterotrophic dinoflagellate species fed *Heterocapsa triquetra* as a function of mean prey concentration. Both prey abundance (cells mL⁻¹) and biomass (ng C mL⁻¹) are stated. Error bars represent ± 1 standard deviation of duplicate means. Curves represent fits of the Michaelis-Menten equation (Eq. 2) to growth rates at each of up to 15 prey concentrations.

Figure 2: Ingestion rates of each predator species fed *Heterocapsa triquetra* as a function of mean prey concentration. Other details as in Figure 1.

Figure 3: Heterotrophic dinoflagellate abundance as a function of time in starvation experiments where prey was either absent (*O. marina*) or at subthreshold prey concentrations at initial sampling. Unless otherwise stated, symbols represent triplicate treatment means $\pm$ 1 standard deviation.

Figure 4: Percent change in cell volume (μm^3) of heterotrophic dinoflagellate species over the initial starvation period (3 wk). Error bars are within symbols and represent the mean cell volume of 24-72 grazer cells (measured via light microscope).

Figure 5: Cell volume changes of (A) *Oxyrrhis marina*, (B) *Gyrodinium dominans*, or (C) *G. spirale* after addition of a pulse of *Isochrysis galbana* ($\sim 75,000$ cells ml^{-1} or $1,006$ ng C ml^{-1}) or *Heterocapsa triquetra* ($\sim 4,000$ cells ml^{-1} or $1,430$ ng C ml^{-1}) shown as short-term predator cell volume change. Increase in predator volume was due to uptake of prey cells, except for *G. spirale*. Predator-only controls that were not refed (gray) are shown at different time intervals.

Figure 6: (A) Empty *Oxyrrhis marina* cells after 20 d of starvation. After a refeed, (B) 1-3, (C) 4-6 and up to (D) 8-12 prey cells were distinguishable within *Oxyrrhis marina*, verifying rapid ingestion (within 2 h). Scale bars represent 20 μm and all images were taken on a light microscope at 20x magnification.

Figure 7: Percent increase in cell volume (μm^3) of heterotrophic dinoflagellate species after being refed with new prey.

Figure 8: Daily specific growth rates of each refed heterotrophic dinoflagellate species over 3 d. Colored lines correspond to the maximum specific growth rate of a continuously-fed predator culture growing at an equivalent prey biomass. For *Gyrodinium dominans* and *Oxyrrhis marina*, lines represent growth rate at ~1,006 ng C ml⁻¹ of *Heterocapsa triquetra* (purple for *G. dominans* and orange for *O. marina*). The line for *G. spirale* is a direct comparison to ~1,430 ng C ml⁻¹ of *H. triquetra* (green).

Appendix Figure 1: Specific growth rates of *Gyrodinium spirale* fed *Skeletonema marinoi* as a function of mean prey concentration. Both prey abundance (cells mL⁻¹) and biomass (ng C mL⁻¹) are stated. Unless otherwise stated, error bars represent ± 1 standard deviation of duplicate means.

Appendix Figure 2: Clearance rates of the three heterotrophic dinoflagellate species on *Heterocapsa triquetra* as a function of mean prey concentration.

Appendix Figure 3: (A) Ingestion and (B) clearance rates of *Gyrodinium spirale* as a function of mean *Skeletonema marinoi* concentration.

Appendix Figure 4: Daily ingestion rates of refeed *Gyrodinium dominans* and *Oxyrrhis marina* over 3 d. Colored lines correspond to the maximum ingestion rate of a continuously-fed predator culture growing at an equivalent prey biomass. Symbols represent triplicate treatment means $\pm$ 1 standard deviation. *G. spirale* (not shown) did not exhibit positive ingestion after the refeed.