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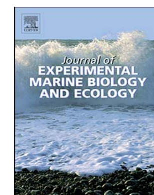

Incubation duration effects on copepod naupliar grazing estimates

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ABSTRACT

Copepod naupliar grazing estimates often appear to represent an insignificant fraction of prey community mortality, despite high naupliar abundances and weight-specific ingestion rates. To address this seeming paradox, the impact of incubation time on grazing by nauplii of the subtropical copepod *Parvocalanus crassirostris* on natural prey assemblages was evaluated. Measurements of prey removal during feeding experiments were taken every 6-h over a 24-h period during two experiments (E1, E2), where the initial 2–35 μm natural prey biomass differed by 3-fold, i.e., 66 μg C L^{-1} (E1) vs. 198 μg C L^{-1} (E2). Results showed that total prey ingestion rate estimates decreased over the course of incubation by up to 75% after 24-h, with highest ingestion rates obtained during the initial 6-h. In no predator controls, total prey biomass also decreased significantly during the 24-h incubation particularly in the smallest prey size groups despite the absence of nauplii, and positive non-significant trends were observed in prey > 10 μm in the experiment with higher initial prey abundances. Predator density effects in this experiment provided additional evidence for trophic cascades. Results of this work suggest that in communities with rapidly changing natural prey assemblages and predators with short development times, short incubations minimize bottle effects and reduce the risk of measuring grazing rates on prey communities that no longer resemble the *in situ* populations.

1. Introduction

Year-round copepod reproduction in subtropical ecosystems (e.g., Webber and Roff, 1995) can lead to large fluctuations in copepod naupliar abundance that are linked to local environmental variation (Hopcroft and Roff, 1998; McKinnon and Duggan, 2003; Hoover et al., 2006). During peaks of abundance, nauplii can exert significant grazing pressure on their prey, for example *A. tonsa* nauplii accounted for up to 50% of the zooplankton grazing in Chesapeake Bay (White and Roman, 1992). Daily carbon rations of nauplii can be much greater than those of adult conspecifics, and many species are capable of consuming well over 100% of their body carbon per day (Paffenhöfer, 1971; White and Roman, 1992; Saiz and Calbet, 2007; Böttjer et al., 2010). In the subtropics, nauplii often do not contribute significantly to plankton biomass (e.g. Roff et al., 1995; Hopcroft et al., 1998). Nevertheless, due to high growth rates (e.g., Kiørboe and Sabatini, 1995; McKinnon and Duggan, 2003) and rapid development times (Hart, 1990; Kiørboe and Sabatini, 1995; Peterson, 2001), nauplii may be an important pathway transferring microbial production to higher trophic levels, given their ability to feed on pico- and nano-sized prey (Roff et al., 1995).

A standard method used to measure zooplankton ingestion rates is using a bottle incubation experiment to study prey removal relative to controls with no predators (Gauld, 1951; Frost, 1972; Paffenhöfer, 1988). Other methods to study zooplankton grazing, which do not require incubations, include gut fluorescence methods (e.g., Mackas and Bohrer, 1976; Kleppel and Pieper, 1984; Vogt et al., 2013), microscopic examination of gut contents or fecal pellets (e.g., Harding, 1974; Turner, 1986; Kleppel et al., 1988), radioisotope tracers (Chipman, 1959; Roman and Gauzens, 1997), and, most recently, molecular methods (Nejstgaard et al., 2008; Craig et al., 2014); and while each method has strengths, most of them do not account for the full spectrum of potential prey. While there are drawbacks to bottle incubations to estimate grazing rates, this method is one of few that measures feeding rates on both pigmented (phytoplankton) and non-pigmented (heterotrophic protist) prey directly, which are important to measure in subtropical environments where both prey types are common (e.g. Takahashi and Bienfang, 1983). This type of experiment requires optimization of incubation conditions to avoid artificial

Abbreviations: Chl *a*, chlorophyll *a*; CV, coefficient of variation (%); DW, dry weight; E1, experiment 1; E2, experiment 2; E, excretion rate (μg N $animal^{-1}$ h^{-1}); ESD, equivalent spherical diameter (μm); *F*, clearance rate (mL $grazer^{-1}$ h^{-1}); *I*, ingestion rate (ng C $grazer^{-1}$ h^{-1}); P_{high}, high predator treatment (100 nauplii L^{-1}); P_{low}, low predator treatment (50 nauplii L^{-1}); t_0, initial timepoint; TL, total length

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estimates of grazing due to 'bottle effects', or conditions that occur in the bottle that may differ from the natural environment. Potential bottle effects include changes in predator grazing or prey growth over time due to differing nutrient, light, or turbulence regimes, crowding of grazers, interactions with container walls, and trophic cascades or food web effects (see Båmstedt et al., 2000). Roman and Rublee (1980) recommended shorter incubations to reduce the impact of bottle effects that can be inconsistent over time based on several grazing indicators such as ATP, chlorophyll *a*, and particle concentration.

Negative relationships between ingestion rates and the duration of the incubation have been reported previously (Mullin, 1963; Roman and Rublee, 1980; Tackx and Polk, 1986). Multiple factors contribute to the reduction in ingestion estimates over long incubations and these include diel rhythms in grazing rates (Kiørboe et al., 1985), a decline in food quality over time (Roman and Rublee, 1980), and trophic interactions in control bottles (Calbet and Landry, 1999; Nejstgaard et al., 2001). This can be a particular concern in studies with natural microzooplankton as prey items, which can directly compete with nauplii for prey, requiring extra consideration of potential trophic interactions in bottle incubations (Nejstgaard et al., 2001).

Thus, grazing rate measurements for nauplii based on 24-h incubations are highly variable. In some temperate regions, naupliar grazing removed a large portion of specific prey items (e.g. 56% ciliates in Chesapeake Bay, [Merrell and Stoecker, 1998]; 54% nanoplankton off the Chilean coast, [Böttjer et al., 2010]) while other grazing estimates from a range of latitudes suggest that nauplii were insignificant grazers on prey (Castellani et al., 2008; Verity et al., 1996; Almeda et al., 2011). While the impact of naupliar grazers may vary by habitat and season, there may be confounding results from the methods used to measure grazing impact.

The primary goal of this work was to optimize conditions and duration of incubation for measurements of naupliar grazing. Naupliar grazing experiments were performed on the natural prey community in order to (1) evaluate the magnitude of changes in the enclosed prey community over the course of 24-h bottle incubation experiments (controls), (2) determine how the length of the incubation affects the resulting estimates of ingestion rates and grazing impacts by copepod nauplii, and (3) test how different predator concentrations affect ingestion rate estimates and the potential for trophic cascades during the experiment. Results of this work suggest that shorter incubation times minimize bottle artifacts, such as declining ingestion rate estimates over time, and give a more clear view of trophic interactions within bottles when compared with longer incubations.

2. Materials and methods

Naupliar grazing rates were measured on field-collected prey assemblages in bottle incubation experiments in the laboratory. Nauplii used in these experiments were derived from laboratory culture populations of *Parvocalanus crassirostris*, originally established from animals collected in Kane'ohe Bay. This species is a dominant component of tropical, neritic plankton communities (Hopcroft et al., 1998; McKinnon and Klumpp, 1998; Hoover et al., 2006; Jungbluth and Lenz, 2013) and has relatively short development times (e.g. McKinnon et al., 2003); it is capable of completing naupliar development within 3 days and reaching the adult stage (C6) in approximately 8 days (at 23–25 °C). Use of these monospecific cultures enabled us to produce naupliar cohorts of a specific stage for use in grazing incubations. At 18-h prior to the start of each experiment, adults were isolated and fed *Tisochrysis lutea* (formerly *Isochrysis galbana* Tahitian strain [Bendif et al., 2013]) at a concentration of 10^5–10^6 cells mL^{-1} After 6-h, adults were removed, and eggs and nauplii were allowed to develop for 12-h in order to produce a cohort of mid-stage nauplii (N3-N4) with a narrow age-range at the beginning of each experiment. Sets of approximately 50 nauplii were isolated into small volumes (< 10 mL) of 0.2 µm filtered seawater 1–2 h prior to the start of each grazing experiment.

Seawater for the prey assemblage was collected from the central basin of the southern semi-enclosed region of Kane'ohe Bay, Oahu, Hawai'i (21°25′56″N, 157°46′47″W) on two dates: 10 March 2015 (Experiment: E1) and 22 April 2015 (Experiment: E2). Seawater was collected from ~2 m depth using a 5 L General Oceanics Niskin bottle deployed by hand line, and gently transferred using acid-washed silicon tubing directly from the Niskin bottle into 20 L covered (dark) polycarbonate carboys. The seawater was transported to the laboratory within 2-h of collection. The collected water was gently pre-screened (35 µm Nitex mesh), which was intended to remove all *in situ* nauplii and other large grazers, so that the only metazoan grazers in the bottles were the added nauplii. The < 35 µm incubation water was added to pre-washed (10% HCL rinse, followed by 3 rinses with experimental seawater) 1 L polycarbonate bottles (total volume: 1120 mL).

Nutrients were not amended in control or treatment bottles due to the expected low rates of excretion by these small biomass nauplii over the incubation duration as compared with baseline levels in Kane'ohe Bay, and also in order to minimize development of artificially high nutrients given prevailing oligotrophic conditions in the study area. Excretion rates of copepods are a function of biomass (Vidal and Whitledge, 1982; Mauchline, 1998), with excretion by nauplii roughly an order of magnitude lower than conspecific adults. At a nauplius grazer concentration of 50 nauplii in a 1 L volume, excretion rates result in values 2 to 3 orders of magnitude below the average nitrogen concentrations of 0.2–1.0 µM in Kane'ohe Bay (Drupp et al., 2011). Therefore, excretion rates in bottle incubations were expected to have negligible impacts on prey growth rates in experimental bottles, and nutrient amendment would have only altered the prey community further away from *in situ* conditions.

The isolated N3-N4 nauplii were transferred into triplicate < 35 µm incubation water bottles (grazing treatments) and placed on a bottle roller (4–6 rpm) to maintain prey in suspension for the duration of the incubation period. Parallel triplicate control treatments (incubation water without added nauplii) were also placed on the bottle roller. Grazing rates were measured using two densities of naupliar grazers: high (N = 92–97 nauplii L^{-1}) and moderate (N = 45–50 nauplii L^{-1}) densities. All incubations were run for a total of 24-h in the dark, with subsamples taken every six hours to examine changes in ingestion rates over time. Experiments were run at 21 °C, which is at the low end of the range of annual temperature fluctuations for this region of Kane'ohe Bay (20–29 °C in prior 5 years [Franklin et al., 2015]).

During the course of the incubation, triplicate 2-mL volumes of each subsample were measured with a Coulter Counter (Beckman-Coulter Multisizer III) with a 100 µm orifice tube, yielding a spectrum of particle sizes from 2 to 35 µm ESD, as well as quantitative abundance data. In a diverse environment with a variety of autotrophic and heterotrophic pico- to microplankton, standard cell quantification methods (e.g. epifluorescence microscopy, inverted microscopy) do not reliably preserve some components of the community (Omori and Ikeda, 1984; Sherr and Sherr, 1993), requiring a patchwork of methods to quantify the full potential suite of prey items. In the absence of large cells or of abiotic particles that may result in unreliable quantification (e.g. Harbison and McAlister, 1980), the Coulter Counter is an appropriate and more reliable means of describing how the abundance of different sized cells change over the duration of grazing incubations (Paffenhöfer, 1984), with results comparable to methods based on gut fluorescence and egg production (Kiørboe et al., 1985). Water subsamples for Coulter Counter measurements were taken directly from experimental bottles upon addition of nauplii at the start of each experiment (time 0) and at each six-hour time point, being careful to retain nauplii as experimental grazers by recovery of animals on a 35 µm cap filter and washing them back into bottles during subsampling with a small volume of filtered seawater.

Data on prey size (ESD) and abundance from the Coulter Counter were further processed using R (Core Team, 2013). Prey ESD was converted to biovolume (BV, μm^3), then to carbon (C, pg C $cell^{-1}$)

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