Egg production rates of the copepod Calanus marshallae in relation to seasonal and interannual variations in microplankton biomass and species composition in the coastal upwelling zone off Oregon, USA


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Egg production rates of the copepod *Calanus marshallae* in relation to seasonal and interannual variations in microplankton biomass and species composition in the coastal upwelling zone off Oregon, USA

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**Abstract**

In this study, we assessed trophic interactions between microplankton and copepods by studying the functional response of egg production rates (EPR; eggs female \(^{-1}\) day \(^{-1}\)) of the copepod *Calanus marshallae* to variations in microplankton biomass, species composition and community structure. Female *C. marshallae* and phytoplankton water samples were collected biweekly at an inner-shelf station off Newport, Oregon USA for four years, 2011–2014, during which a total of 1213 female *C. marshallae* were incubated in 63 experiments. On average, 80% of the females spawned with an overall mean EPR of 30.4. EPRs were intermediate in winter (Jan–Feb; 32.5). Interannually, EPRs were significantly higher in 2014 than 2011 and 2012. Total chlorophyll a (Chl a) concentration and diatom abundance both were significantly higher in summer while no seasonal differences were found in abundance of dinoflagellates, ciliates or Cryptophytes. Although total Chl a showed no interannual differences in bulk biomass of phytoplankton, community structure analysis indicated differences among years. More diverse diatom communities were observed in 2013 and 2014 compared to 2011 and 2012. Relationships between EPR and potential food variables (phytoplankton and ciliates) were significant by season: a hyperbolic functional response was found between EPR and total Chl a in winter–spring and summer, separately, and between EPR and ciliate abundance in winter–spring; a linear model fit best the functional response of EPR to diatom abundance in summer. The estimate of potential population recruitment rate (the number of females \(\times\) EPR; eggs day \(^{-1}\) m \(^{-2}\)) was highest in spring (Apr–May), and annually was highest in 2013 (11,660), followed by 2011 (6209), 2012 (3172) and 2014 (1480). Our observations of *in situ* EPRs were far higher than published laboratory rates of 23.5, calling into question our past laboratory studies that used mono-algae cultures as food for copepods. Moreover, an increasing number of studies (including this study) are showing an apparent greater importance of ciliates as a nutritious food source for *Calanus* species demonstrating the importance of the microbial loop to secondary production.

**1. Introduction**

*Calanus marshallae*, a sibling species of *Calanus finmarchicus*, *Calanus sinicus* and *Calanus australis* (Frost, 1974), occurs commonly in continental shelf waters of the northern California Current from January through August as well as in the coastal Gulf of Alaska and Bering Sea. This species was thought to be restricted to the North Pacific Ocean and Bering Sea (Frost, 1974) as well as the Chukchi Sea (Hopcroft et al., 2010); however, there is evidence that it has invaded the Arctic Ocean, in waters near Spitsbergen (Melle and Skjoldal, 1998). In the northern California Current, adults usually go through a brief period of diapause during autumn months after which adults re-appear in January. The awakening in winter is presumably timed to take advantage of a winter bloom that occurs frequently in February (Feinberg et al., 2010). A cohort is born at this time, which becomes mature within 7–8 weeks (Peterson, 1986); during the course of annual growing season, at least five generations are produced (Peterson, 1980). This life history is in contrast to that of *C. marshallae* populations in Dabob Bay, Washington (Osgood and Frost, 1994) and the Bering Sea (Baier and Napp, 2003; Smith and Vidal, 1984) which produce only one or two generations per year. The reason that *C. marshallae* can produce multiple generations in the northern California Current is almost certainly due to the availability of sufficient food resources throughout much of the year which in turn supports egg production.
and subsequent development and growth of nauplii and copepodes.

The coastal waters off Oregon are characterized by high phytoplankton production nearly year-round due to four independent "primary production events" – a late winter bloom (in January/February) in some years, a spring bloom (in April/May), multiple summer blooms during the upwelling season (June–September), and a fall bloom after the end of the upwelling season (October/November). These blooms have vastly different magnitudes, ranging from 1 to 2 μg Chl-a L⁻¹ during the winter blooms to 15–30 μg Chl-a L⁻¹ during summer blooms. The fall bloom, when it occurs, is on the order of 10 μg Chl-a L⁻¹ (Du and Peterson, 2014). The natural phytoplankton community contains a mixture of taxa and sizes, from smaller nano- and micro-flagellates to larger long-chain diatoms that exceed 1 mm in length. Moreover, the dominant species are highly variable and ephemeral during bloom events (Du and Peterson, 2014). Thus we were curious as to the ability of C. marshallae to take advantage of the different prey types, bloom magnitude and sizes of the microplankton species as well as their functional response to phytoplankton biomass and species composition.

Despite the broad distribution of C. marshallae around the margin of the temperate/subarctic waters of the eastern North Pacific, Bering Sea and the Chukchi Sea, few studies ecological exist and most are limited to the northern California Current. Past work on egg production rates (EPR, eggs female⁻¹ day⁻¹) of C. marshallae did not consider the potential effects from microplankton in terms of species and community structure, except that significant relationships were found between chlorophyll-a (Chl a) and EPR. Gómez-Gutiérrez and Peterson (1999) reported a mean EPR of 22.5 during Jul–Aug 1997. These rates were linearly correlated with Chl a concentration. Similarly, Peterson et al. (2002) found a significant linear relationship between EPR and Chl a in June 1996. The range in values of EPR across continental shelf waters was ~20–30 when Chl a >5–10 μg L⁻¹. Shaw et al. (2009) reported an average rate of 20.3 during three cruises in summers 2005 and 2006. There is a somewhat controversial report of EPR by "C. marshallae/glacialis" from the western Arctic Ocean by Plourde et al. (2005) who found average EPRs of 14 and 20 in spring and summer 2002, respectively, but Lane et al. (2008) who sampled copepods on the same cruise as Plourde et al. (op.cit) claimed that there were no C. marshallae in samples.

This study considers several new topics related to EPR of C. marshallae and the in situ food environment in the shelf waters off Newport, Oregon. The first topic considers reasons why a wide range of EPRs were observed during incubation experiments over a four-year period and how that was affected by the potential microplankton food resources. For example, in our experiments, individual female C. marshallae were found to be capable of producing up to 97 eggs per spawn with a maximum population average EPR of 68.5. These rates are far higher than any of our previous studies (Peterson, 1988; Gómez-Gutiérrez and Peterson, 1999; Peterson et al., 2002; Shaw et al., 2009). Meanwhile, the present study also observed very low EPRs of <10. Thus we wanted to determine if such a wide range of EPR values were simply a matter of seasonal variations in microplankton biomass as opposed to variations in abundance of specific functional groups (diatoms, dinoflagellates, Cryptophytes and ciliates), community structure and/or species diversity. Second, on a few occasions, EPRs were depressed during certain diatom blooms, noteworthy because past studies have indicated that some diatom blooms may be detrimental to copepod egg production and egg hatching success (Paffenhofer, 2002; Paffenhofer et al., 2005). Thus we tried to determine if the reduced EPRs could be attributed to specific diatom taxa. Third, we compared results of EPR based on incubation of freshly-caught females (this study) with EPR measured under controlled laboratory conditions (Peterson, 1988) during which females were fed a single diatom species Thalassiosira weissflogii. We discuss reasons why the rates measured in the lab were less than half of the rates from in situ incubation. Finally, we discuss the potential reproduction rates (as the product of EPR on a given day × abundance of females in situ on the same day) to determine if there were seasonal and interannual differences in potential 'recruitment'.

### 2. Methods

Female C. marshallae for egg production experiments and phytoplankton were collected biweekly from a station located 5 nautical miles (9 km) from shore, in 62 m of water along the Newport Hydrographic line (station NH05, 124.2°W, 44.6°N) off central Oregon, USA over a four-year period of 2011–2014. C. marshallae were collected mostly at night from the upper 20 m of the water column using a 60 cm diameter Bongo net (333 μm mesh). Live samples were placed in a 20 L cooler filled with surface seawater and were transported to a temperature-controlled room in our shore-based laboratory within 2–3 h of collection. To collect sample for analysis of abundance of female C. marshallae, we used a ½ m 202 μm mesh net hauled vertically from a few meters above the sea floor to the sea surface. The contents of the cod-end were preserved in formalin (final concentration of 5–10%).

#### 2.1. Copepod egg production experiments

Individual female C. marshallae were sorted with the aid of a stereomicroscope and a wide-bore pipette. Healthy-appearing individuals were placed into 60 ml jars prefilled with sieved (200 μm mesh) surface seawater. The goal was to pick 20–25 females for each experiment but this depended on the availability of female C. marshallae. Individual jars were incubated for 24 h in the dark at a constant temperature 10 ± 0.5 °C, chosen because it is the average year-around value observed for the upper 20 m of the water column over the inner and middle shelf off Oregon (winter is 10.3 °C; summer 10.1 °C, Peterson, unpublished data). Eggs were first counted in the late morning, about 6–8 h after setting up each experiment. Individuals that had not spawned by that time were reexamined every 4–5 h until the end of the experiment. Most females spawn eggs between 0400 and 1100 h (Peterson, pers. obs. for C. marshallae), thus enumerating the eggs shortly after they were spawned reduces the potential for egg cannibalism following recommendations of Runge and Roff (2000). Also Peterson (1988) discussed why cannibalism is not likely to be a problem in these types of experiments. Egg production rate (EPR; eggs female⁻¹ day⁻¹) is calculated from the total eggs in an experiment divided by the number of females in that experiment, thus it is an estimate of the "population" egg production rate, not an average of only those females that spawned during each experiment.

#### 2.2. Phytoplankton sample analysis

On each cruise, surface water samples were collected for the analyses of phytoplankton species and chlorophyll-a (Chl a). Total Chl a and >5 μm size fraction subsamples (each of 100 ml) were filtered through GF/F filters and >5 μm polycarbonate filters, respectively, extracted in 90% acetone for 24 h in the dark, and then fluorescence was measured using a Turner 10-AU fluorometer. The concentration of Chl a was calculated following equations in Strickland and Parsons (1972). The <5 μm Chl a concentration was the difference between total Chl a and the >5 μm fraction. Phytoplankton samples were fixed with acid Lugol's solution in 125 ml bottles (2% final concentration) immediately after collection. To identify and enumerate species, a subsample of 50 ml was taken.
from the full sample and then settled in a 50 ml tissue culture flask (Falcon) for at least 24 h prior to counting. The wide flat side of the flask was placed on the stage of an inverted light microscope (Leica DM IRB); species identification and enumeration were carried out at the magnifications of 200× or 400×. The portion of the 50 ml sub-sample that was examined varied with species density. To meet a minimum total count of 500 cells, between 2 and 20 transects across the wide side of the flask were counted. For diatoms and dinoflagellates, species identification was made primarily following Horner (2002). A group of smaller dinoflagellates (length <20–30 μm) were relatively consistent and abundant in our samples but difficult to be identified to species, thus we use “small dinoflagellates” to name this group. Ciliates, a common group of microzooplankton in our phytoplankton water samples, were included for this study and are important because during the non-diatom bloom season they comprised a significant fraction of total microplankton biomass. For ciliates, identification to species level was not made, instead, the major subgroup of naked ciliates were recorded as size categories, small ciliates (length <30 μm) and large ciliates (>30 μm). Loricate ciliates were named as “tintinnids”. Cryptophytes were enumerated because they are morphologically easier to be recognized, whereas other nanoflagellates were not included for quantitative analysis due to the identification limitation of light microscopy. Cell abundance is expressed as cells per liter (cells L⁻¹).

2.3. Statistical analysis

To compare interannual and seasonal variations in EPR and microplankton biomass, Analysis of Variance (ANOVA) was implemented on EPR, abundance of four functional groups (diatom, dinoflagellate, ciliate and Cryptophyte) and Chl a concentration data series, respectively. Tukey HSD pairwise comparisons were used to test for the differences in pairs. Phytoplankton abundance and Chl a concentration were log₁₀(x + 1) transformed before analyses so as to achieve normality and equal variance. The functional response of EPR (dependent variable) to the various measures of microplankton biomass (taxa and chlorophyll) was characterized by ordinary linear regression and by hyperbolic (Michaelis–Menten) models. The equation for the hyperbolic model is

\[ y = \frac{a x}{b + x}, \]

where \( a \) is the maximum EPR and \( b \) the half-saturation constant value of the independent variable where one-half of the maximum value of the dependent variable (EPR) is reached.

Differences in phytoplankton community structure were examined using hierarchical cluster analysis (with SIMPROF significance test at 0.05) and nonmetric multidimensional scaling (NMDS). Two-way crossed ANOSIM (Analysis of Similarity) was used to test for differences in phytoplankton community structure by year and season. The statistic R (scaled to a range of values from 0 to 1) in ANOSIM is a measure of community separation between groups with 0 indicating no separation and 1 indicating complete separation. Similarity of percentage (SIMPER) analysis provides a list of species in rank order of high to low species percent contribution to the overall similarity of a specific group. The length of the species list presented in Results was determined when cumulative individual species percent contribution added up to 80% of total. The above multivariate analyses were conducted in PRIMER-E software (Clarke and Gorley, 2006). Other statistical comparisons and correlation analysis were performed in JMP 11 (SAS).

3. Results

3.1. Egg production rate (EPR)

A total of 1213 females were incubated in 63 experiments, an average of 19 females per experiment. EPRs (eggs female⁻¹ day⁻¹) were highly variable (Fig. 1A). The three lowest values were 3.9 (27 Jul 2011), 4.0 (7 Mar 2012) and 7.7 (20 July 2012) and the three highest 68.5 (1 Apr 2014), 62.0 (29 Jul 2013) and 55.1 (16 Jan 2014). The overall mean EPR was 30.4 ± 15 (standard error). It was unusual for all females to spawn in an experiment (only 12 out of 63 experiments). The average percentage of females which spawned during the 24 h incubation was 80%, and by season, 84% in winter–spring (Jan–May) and 75% in summer (Jun–Oct). The average percentage of females that spawned was related to Chl a concentration: 81% when Chl a concentrations were high (5–30 μg L⁻¹), 77% when less than 5 μg L⁻¹ and 74% when below 1 μg L⁻¹. Because not all females spawned during a 24 h incubation, per capita rates (egg production rate calculated using only those females that spawned) were higher than the population rates (mean 39.7 vs. 30.4, t = 5.77, p < 0.001). However, the two ‘rates’ were significantly correlated (R² = 0.87, not illustrated) suggesting results of comparing EPR rates in response to various food environments are not dependent upon which method is used to calculate EPR. We chose the ‘population’ rates as this method is most often used by copepodologists.

3.2. Monthly EPR and Chl a concentration

The correspondence between monthly average EPR and Chl a was poor. Monthly average EPRs (Fig. 2) were higher in Jan–Feb and Apr–May than Aug–Oct, and lower in Mar, Jun–Jul and Nov–Dec. Total Chl a concentration ranged from 0.5 to 28.8 μg L⁻¹ (Fig. 1B) and was much higher from July to September and lower in the other months (Fig. 2). The >5 μm Chl a size fraction followed the same seasonal patterns as total Chl a, whereas monthly averaged percentage of Chl a <5 μm size fraction was relatively constant, ranging from 50.3% to 70.8%.

3.3. Seasonal and interannual variations in EPR, phytoplankton biomass and functional groups

Fewer experiments were carried out in November (1 experiment) and December (2 experiments) due to low numbers and/ or lack of females in the Bongo net samples. Although there was a high number of females (average of 19) per experiment in March, the dramatically decreased monthly average EPR compared to February and April (Fig. 2) was likely related to a small sample size (2 experiments). We were concerned that the low sample size in March, November and December might bias quantitative evaluation of EPR. Therefore, the three months with small sample sizes were not included in the seasonal/interannual quantitative analyses.

Interannual differences in EPR were significant (p = 0.023). There were higher EPRs in 2014 than 2011 and 2012 (p = 0.05), respectively (Table 1). Seasonal changes were also significant (p = 0.018) and showed higher EPRs in Apr–May (spring) than Jun–Oct (summer) but similar rates between winter (Jan–Feb) and spring.

In contrast, no significant interannual changes were shown in either total Chl a or size-fractionated Chl a concentrations (p = 0.9), but seasonal variations were significant (p < 0.01) and the overall difference was the result of higher Chl a concentrations in summer than winter.

Interannual variations were not consistent for the four functional groups (two-way ANOVA, year and season): diatoms were significantly more abundant in 2013 than 2012, and Cryptophytes were more abundant in 2013 than in 2011, but interannual differences were insignificant for dinoflagellates and ciliates. There were seasonal differences for diatoms only (p < 0.05): higher abundance in summer than in spring and winter, and spring higher than winter. The abundances of dinoflagellates, ciliates and Cryptophytes
were similar among seasons. One-way ANOVA for testing interannual differences in each of the phytoplankton functional groups was also run for the winter and spring seasons, separately. In winter, significantly higher abundances were found for ciliates in 2014 than 2011 and 2012 (p = 0.02), and for dinoflagellates in 2014 than 2011 (p = 0.04), but no significant differences in diatoms and Cryptophytes among years. As for spring, diatoms and ciliates were similar among years; dinoflagellates were again more abundant in 2014 than 2011 and Cryptophytes were more abundant in 2013 than 2011 (p < 0.05).

3.4. Phytoplankton community structure and EPR

Cluster analysis identified five different communities (Fig. 3, upper panel), among which cluster “a” represents a community type characterized by the lowest average diatom abundance (174,040 cells L$^{-1}$) and lowest species richness (13 diatom species). Most of the samples were from January, February and April with some from September and October. Cluster “c” represents a summer community type with the highest average diatom abundance (1,740,800 cells L$^{-1}$) and species richness (31 diatom species) and includes samples mostly from May to October. Notice that nearly all samples in “c” were from 2013 and 2014. Clusters “d” and “e” include spring and fall samplings, respectively, with moderately

Table 1

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<th>Jan–Feb</th>
<th>Apr–May</th>
<th>Jun–Oct</th>
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<td>28.6 ± 7.3(3)</td>
<td>24.2 ± 3.8(11)</td>
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<td>26.3 ± 3.8(13)</td>
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<td>38.0 ± 6.0(5)</td>
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<td>44.7 ± 5.7(5)</td>
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<td>51.4 ± 10.3(2)</td>
<td>44.9 ± 6.5(5)</td>
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<td>Mean</td>
<td>31.8 ± 2.0(57)</td>
<td>32.5 ± 4.7(10)</td>
<td>40.2 ± 3.0(18)</td>
<td>26.4 ± 2.6(29)</td>
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high diatom abundance (721,380 and 583,970 cells L\(^{-1}\), respectively) and species richness (27 and 17 diatom species, respectively). The three sampling dates in “d” were from 2013 to 2014 and the six sampling dates in “e” were from 2011 to 2012. The cluster “b” included two sampling dates and was distinguished from “c” by some unusually abundant species (a silicoflagellate \textit{Dictyocha speculum} and a dinoflagellate \textit{Protoperidinium depressum}) in addition to the common ones in “c”. The ordination analysis (Fig. 3, left lower panel) also showed a clear separation of phytoplankton community types. Cluster “a” and “c” had no overlap, “b” was associated with “c” whereas “d” and “e” appeared as transitional communities.
Changes of phytoplankton community structure were significant across years (ANOSIM, $R = 0.408$, $p < 0.001$, Table 2) and seasons ($R = 0.352$, $p < 0.001$). Pair-wise year comparisons were significant except 2013 vs 2014 ($p = 0.2$) and all the pair-wise seasonal comparisons were significant as well (e.g. winter vs spring, $p = 0.005$).

Although there were significantly different phytoplankton community clusters, the corresponding EPRs were similar ($p = 0.6$, one-way ANOVA). For example, the sampling dates of two most contrasting clusters “a” (winter) and “c” (summer) had very similar EPRs on average, 32.9 ± 3.1 and 32.6 ± 4.4, respectively. Furthermore, patterns of the five clusters shown in the ordination plot were not mirrored by the values of EPR represented as quartile levels (Fig. 3, right lower panel). Thus phytoplankton community clusters, the corresponding EPRs were similar among years and seasons, large fluctuations in 2011 were

3.5. Relationships between EPR and Chl α, phytoplankton functional groups

When EPRs were plotted against total Chl α concentrations in winter–spring (Jan–May) and summer (Jun–Oct), respectively, hyperbolic models (Michaelis–Menton) best represented their correlations at $p < 0.01$ (Fig. 4). The estimated maximum EPR was 50.4 in winter–spring with a 0.4 μg L$^{-1}$ Chl α half-saturation constant, higher than the summer maximum EPR of 39.7 with a 2.6 μg L$^{-1}$ Chl α half-saturation constant.

In the 10 experiments in Jan–Feb (Table 3), we collected female C. marshallae either before or during the small winter bloom (Chl α = 1-2 μg L$^{-1}$). An average EPR for winter was 33.9 (maximum of 55.1). Thus it is clear that significant numbers of eggs are produced annually during winter months regardless of the presence of significant phytoplankton blooms.

To further explore this idea, EPRs were regressed against ciliate and diatom abundance by season, respectively (Fig. 5). A significant correlation was found between EPR and ciliate abundance in winter–spring (hyperbolic model, $p = 0.008$) with an estimated maximum EPR of 44.6, while in summer EPR and diatom abundance was significantly correlated (linear model, $p = 0.01$, Fig. 5). A hyperbolic model was also applied for EPR versus diatom abundance in summer but the result was less significant ($p = 0.07$) with a maximum EPR of 40.6.

Non-significant relationships were found between ciliates and EPR in summer (hyperbola, $p = 0.64$), and between diatoms and EPR in winter–spring (hyperbola, $p = 0.19$). Neither dinoflagellates nor Cryptophytes were correlated significantly with EPR in either season (not illustrated). An exception for dinoflagellates is that the subgroup – small dinoflagellates, was found significantly correlated with EPRs in winter–spring ($R^2 = 0.24$, $p = 0.02$) and this correlation was strengthened when only winter data were considered ($R^2 = 0.65$, $p = 0.005$).

3.6. Diatom community composition and species richness

While the abundance of dinoflagellates, ciliates and Cryptophytes were similar among years and seasons, large fluctuations were seen within diatom functional group in terms of species richness and composition (Table 4). Species composition was generally similar between 2013 and 2014, and in both years species richness was far higher (17 and 18 species, respectively) than 2011 and 2012 (7 and 9 species, respectively).

The most abundant taxa contributing to the interannual differences in the diatom community in 2011 were Nitzschia, Dactylosolen fragilissimus and Pseudo-nitzschia delicata in 2012, Chaetoceros debilis, D. fragilissimus and Nitzschia in 2013, three Chaetoceros species (debilis, socialis and contortus); in 2014, Leptocylindrus minimus, D. fragilissimus and C. debilis. The full list is shown in Table 3.

By season, species richness increased gradually from winter (4 species) to summer (15 species) with only three species common in all seasons Cylindrotheca closterium, Thalassionema nitzschioides and Asterionellosis glacialis (Table 3). There were 9 common diatom species between spring (Apr–May) and summer and the ones more likely to be present in both seasons are the genera Chaetoceros, Pseudo-nitzschia and Thalassiosira.

EPRs were significantly correlated with species richness of those species which dominate in a given sample (those species which had an abundance >10$^4$ cells L$^{-1}$ in summer from June to August). Species richness accounted for 32% of the variance (Fig. 6); however, this relationship became insignificant ($p = 0.26$) when the test period was expanded from June to October (plot not shown).

3.7. EPR and summer diatom blooms

In 5 of 21 experiments in summer (Jun–Oct), EPRs were lower than expected, determined by the data points falling outside of the lower 95% confidence interval (CI) band in the linear regression plot (Fig. 5C). These observations raise the question as to what might have been different in the phytoplankton community when Chl α concentrations are high and/or when large diatom blooms

Table 3

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<tr>
<th>Date</th>
<th>Chl α</th>
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<th>Cryptophytes</th>
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**Fig. 4.** Egg production rates (EPR; eggs female$^{-1}$ day$^{-1}$) of female Calanus marshallae as a function of total Chl α concentrations (μg L$^{-1}$). Two hyperbolic curves are the regressions of EPR versus total Chl α in Jan to May (green and grey symbols) and Jun to Oct (red symbols), respectively. Coefficients (a and b) are 50.4 and 0.4 for Jan to May, 39.7 and 2.6 for Jun to Oct, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
were observed in this season. Thus we inspected closely relationships between EPRs and diatoms on those few dates (Table 5).

- 22 July 2011 (EPR = 3.8). Chl a concentrations were moderately high (3.6 μg L\(^{-1}\)) but the small <5 μm size fraction was 81% of total. Total diatom abundance was very low (31,903 cells L\(^{-1}\)) while the small dinoflagellates group was the highest observed during our study (99,767 cells L\(^{-1}\), 99% of total dinoflagellate abundance on this date). Since both diatoms and Chl a >5 μm size fraction had low concentrations, this suggests that on this date small dinoflagellates alone, though abundant, might not be a suitable food resource to induce high EPRs.

- 27 July 2011 (EPR = 10.1). Chl a concentration was higher (6.3 μg L\(^{-1}\)) than on 22 July but with 84% in the <5 μm size fraction. A mono-species bloom of the diatom *D. fragilissimus* occurred (691,827 cells L\(^{-1}\), 98% of total diatom abundance) which might explain the low EPR.

- 20 July 2012 (EPR = 7.7). Chl a concentration was 3.0 μg L\(^{-1}\) with 48% in the <5 μm size fraction. EPR should have been higher because a typical summer diatom bloom was observed on this date, with total diatom abundance of 960,511 cells L\(^{-1}\) and a diverse set of dominants: *D. fragilissimus*, *Skeletonema costatum*, *Guinardia delicatula*, *Chaetoceros* (Ch. contortus, Ch. debilis, Ch. socialis, Ch. affinis, Ch. lorenzianus and similis) and *Thalassiosira nordenskioldii*. The most abundant species was again *D. fragilissimus* (564,302 cells L\(^{-1}\), 51% of total diatom abundance), the same species associated with the low EPR on 27 July 2011. However, for all experiments, the relationship between EPR and *D. fragilissimus* was not statistically significant (Hyperbolic model, \(n = 25, r = 0.2, p = 0.4\)).

- 7 Aug 2012 (EPR = 5.7). Chl a concentration was 3.8 μg L\(^{-1}\) with 66% in the <5 μm size fraction. The low EPR was similar to the earlier observation on 20 July 2012. Diatoms developed another typical bloom (324,688 cells L\(^{-1}\)) with co-existed dominants such as *Coscinodiscus*, *Pseudo-nitzschia*, *G. delicatula* and *Ch. debilis*. However, the large dinoflagellate *Protoperidinium compressum* and silicoflagellate *D. speculum* both were unusually abundant at the same time (31,388 and 162,944 cells L\(^{-1}\), respectively) compared to their long term average amount in our study area (510 and 3808 cells L\(^{-1}\), respectively). The low EPR might be related to these two unusually abundant species.

- 7 June 2013 (EPR = 13.7). Chl a concentration was again at a moderate level, 4.2 μg L\(^{-1}\), with 39% in the <5 μm size fraction. Diatom blooms were characterized by both high species richness and abundance in June and July 2013. The diatom bloom on 7 June 2013 (1,205,789 cells L\(^{-1}\)) was in a similar magnitude to the other June and July observations and characterized by a common set of genera/species including *Chaetoceros* (e.g. Ch. contortus and debilis), Asterionellopsis, *S. costatum*, *Pseudo-nitzschia* and *C. closterium*. This gives an example of the apparent mismatch between a seemingly ideal food supply and a low EPR.

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**Fig. 5.** Egg production rates of female *Calanus marshallae* as a function of total diatom (left panel A and C) and ciliate abundance (right panel B and D) by season (winter–spring, summer). In plot C, the blue lines indicate the 95% confidence intervals (CI). Below the lower 95% CI, empty circles indicate dates with very low EPRs and above the upper 95% CI, filled red circles indicate dates with very high EPRs. Note that the hyperbola model for diatom abundance versus EPR in summer was less significant (\(r = 0.41, p = 0.07\), not illustrated here) than linear model. Coefficients (a and b) in each of the hyperbola models are: 41.7 and 61046.2 in (A); 44.6 and 544.2 in (B); 24.5 and 297.9 in (D). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
In comparison, five dates in summer had extraordinarily high EPRs and fell above the upper 95% CI. These dates were in 2011 (17 Aug, 29 Sep and 26 Oct) and 2013 (23 and 29 July).

- **17 Aug 2011 (EPR = 30.9)**. Chl a value was very high, 17.3 μg L\(^{-1}\) (76% in the <5 μm fraction). Total diatom abundance was moderately high (571,849 cells L\(^{-1}\)) with a 41% contribution from *Thalassiosira*.

- **29 Sep 2011 (EPR = 48.6)**. Chl a value was low as 2.6 μg L\(^{-1}\) (65% for <5 μm fraction) but EPR was high. Diatoms were dominant (487,118 cells L\(^{-1}\)) and *Ch. debilis* alone made up 83% of total abundance.

- **26 Oct 2011 (EPR = 24.9)**. The Chl a value was very low, only 1.3 μg L\(^{-1}\) (62% for <5 μm fraction) and EPR was close to the average. Diatom abundance was similarly low, 14,065 cells L\(^{-1}\), whereas small dinoflagellates were more abundant (23,670 cells L\(^{-1}\)).

- **23 and 29 July 2013 (EPR = 35.6 and 61.7, respectively)**. A bloom developed during these two sampling dates, with Chl a ranging from 7.5 μg L\(^{-1}\) to 15.9 μg L\(^{-1}\) and <5 μm size fraction comprising 11% and 77%, respectively. Diatom abundances were exceptionally high (1.1 and 1.6 × 10\(^{6}\) cells L\(^{-1}\)). Dominance within the diatom community was shared between *Chaetoceros* (61% and 44%, respectively) and *Thalassiosira* (24% and 44%, respectively).

Note that (Table 5) EPRs were high on days when *Thalassiosira* were abundant during summer. Regression analysis (Fig. 7) between EPR and *Thalassiosira* abundance showed a significant hyperbolic relationship (n = 17, r = 0.64, p = 0.0057).

### 4. Discussion

#### 4.1. Spawning of Calanus copepods

Multiple years of egg production experiment demonstrate that the *C. marshallae* population off Oregon produces eggs from winter through late summer. Eggs are produced in winter months soon after the termination of diapause and mostly before the first phytoplankton bloom supporting the idea that there must be triggers other than the bloom that act to awaken them from diapause (Miller et al., 1991).

Spawning before the first bloom of the year, either in winter or spring, is not an exclusive trait for *C. marshallae*. Many studies of other *Calanus* species also found that eggs were produced before...
Rhizosolenia blooms during the summer upwelling season (Jun–Oct) in 2011–2014. S (N > 10^4) the number of species with abundance > 10^4 cells L^-1. Aster: Asterionellopsis; Chae: Chaetoceros; Rhiz: Rhizosolenia + Dactyliosolen + Guinardia + Proboscia; Lept: Leptocylindrus; Pennate: Navicula + Nitzschia + Pinnularia + Thalassionema; P-n: Pseudo-nitzschia; Thal: Thalassiosira. "*" means N > 10^4; "**" means N > 10^5.

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Fig. 7. Relationship (hyperbolic model, coefficients a = -40.6, b = 24794.9) between egg production rate (EPR) and diatom genus Thalassiosira abundance (cells L^-1).

the first bloom of the year, although the numbers of eggs were substantially lower than those observed during or post-blooms. As examples, Plourde and Runge (1993) working on C. finmarchicus in the Gulf of St. Lawrence reported EPR < 10^4 pre-bloom, 22–82 during the bloom and 30–50 post-bloom. Niehoff et al. (1999) for C. finmarchicus in the Norwegian Sea reported EPR of 8 pre-bloom and 44 during the spring bloom. Gislason and Astthorsson (2000) reported a maximum EPR for C. finmarchicus of 7 pre-bloom and 46 during the bloom in waters off Iceland. A similar result for C. glacialis (Melle and Skjoldal, 1998; Niehoff and Hirche, 2005) was found in a Norwegian fjord (see Table 2 in Niehoff and Hirche, 2005). Stenevik et al. (2007) reported for C. finmarchicus in the Norwegian Sea EPR of 10, 22 and 18 with pre-, during and post-bloom conditions, respectively. Madsen et al. (2008) found both C. finmarchicus and C. glacialis spawned before the spring bloom in Disco Bay Greenland, as did Runge et al. (2006) for C. finmarchicus on Georges Bank, U.S. Conversely, Head et al. (2013) found that EPRs of C. finmarchicus in the central Labrador Sea were not different under pre-, during, and post-bloom conditions. Most of these observations cited for other Calanus species are in stark contrast to our work off Oregon where EPRs were often higher in winter before any blooms and lower during summer when large diatom blooms were frequent.

As for Calanus species in the Pacific Ocean, Baier and Napp (2003) reported that C. marshallae in the Bering Sea also spawned well before the spring bloom, as early as February based on back-calculated temperature-dependent development rates of the age-structure observed in April and May. The same is true for C. pacificus in Dabob Bay, WA (Leising et al., 2005; Pierson et al., 2005), and for C. pacificus in the coastal waters off Oregon (Peterson, unpublished data). Spawning by C. chilensis in the southern/central Chile (Hidalgo and Escobedo, 2007) is seasonal whereas C. chilensis spawn year round in waters off northern Chile (Hidalgo and Escobedo, 2008). C. sinicus can spawn year round in the Yellow Sea, P.R. China, although most females do not produce eggs during summer months but go through ‘over-summering’ at depth because temperature of the upper mixed layer is very high, exceeding 18 °C (Huo et al., 2008; Kang et al., 2011; Wang et al., 2009; Zhang et al., 2005). Uye and Murase (1997) reported maximum EPRs of 22–24 for C. sinicus from coastal waters off Japan (Seto Inland Sea) in spring, similar to the rates reported by the above cited studies in the Yellow Sea.

4.2. EPR and phytoplankton concentrations

Significant functional relationships were found between EPR and total Chl a concentration in our study. Our past work in June 1996 and July–September 1997 also found strong correlations between EPR and Chl a (Gomez-Gutierrez and Peterson, 1999; Peterson et al., 2002). This result is common to studies of other species in the genus of Calanus. However, the uniqueness of our findings is that we noted differences in season-specific functional response of C. marshallae EPR to phytoplankton availability. Hyperbolic models provided the best fit for both winter–spring and summer and the parameters from the models inform clearly different biological behaviors and are consistent with in situ observations. The very high EPRs in winter before any blooms and lower during summer when large diatom blooms were frequent.
lower than the magnitude of summer blooms (10–20 \(\mu g L^{-1}\)) suggesting the amount of phytoplankton (mostly diatoms) was not limiting egg production and instead other factors (prey types or environment) caused the high variability in EPR. The significant correlation between EPR and Chl a were obscured when the data from all seasons were combined for the test (\(r = 0.17, p = 0.2\)), which again highlights the different functional response in egg production at different times of the year.

The significance of EPRs for *C. marshallae* responding to the abundance of different microplankton functional groups also differed by season: EPRs in winter/spring were best correlated with ciliate abundance, whereas in summer were best correlated with diatom abundance. Similar findings were reported by Vargas et al. (2006) for the copepods *Acartia tonsa* and *Centropages brachiatius* from the coastal upwelling zone off Dichato, Chile where copepods fed on dinoflagellates and ciliates in winter but diatoms in summer.

Some of our winter EPRs were the highest in a given year and occurred before the winter bloom. We thus hypothesize that in winter energetic requirements for egg production and embryo development may come from a combination of lipid reserves and from feeding omnivorously on both phytoplankton and microzooplankton. At this time of the year, EPR ranged from 4 to 68.5 over a range of energetic requirements for egg production and embryo development occurred before the winter bloom. We thus hypothesize that in winter *C. finmarchicus* to quality of phytoplankton (indexed through fatty acid analysis) in shelf waters off Iceland and found that diatom fatty acid markers were correlated with EPRs. Vargas et al. (2010) reached the same conclusion for copepods from the upwelling zone off Dichato, Chile. Ceballos and Alvarez-Marques (2006) also found significant correlations between diatom concentration and egg production of both *C. helgolandicus* and *Calanoides carinatus*, in the coastal upwelling zone off northern Spain (Cantabrian Sea). EPR of *C. pacificus* in Dabob Bay, WA was significantly correlated with diatom concentration in winter and spring (Pierson et al., 2005; Leising et al., 2005). The study by Janora et al. (2015) observed higher in situ EPRs of *Acartia clausi* and *C. helgolandicus* in the years when diatoms were more productive in Adriatic Sea.

### 4.3. EPR and phytoplankton community structure

We had hypothesized that the patterns of phytoplankton community structure from traditional cluster and ordination analysis would explain the variation in EPR due to the combined information on species composition and abundance, functional groups and species richness. However, our results did not show any covariation between the five significantly different community types and EPR values (as compared in Fig. 3). On the other hand, species richness alone seemed to inform better variation in EPR which supports the hypothesis presented by others (e.g., Irigoien et al., 2000b; Leising et al., 2005; Paffenhofer et al., 2005 and reference therein) that mono-specific blooms (lower species diversity) may result in a decline in EPR. In contrast, a mixture of microplankton species may provide a more balanced diet for copepods (Poulet et al., 2006; Uye, 1996; Vehmaa et al., 2011).

### 4.4. Why were there some cases of exceptionally low EPR in summer?

Average EPRs were lower in summer as compared to spring or winter. Our analyses of individual cases in summer (see Section 3.7) provide the following empirical understanding. First, in the trivial case, when both Chl a and diatom concentration were low, EPRs were low; second, mono-species blooms of certain diatoms and dinoflagellates were associated with low EPR; third, high abundance of *Thalassiosira* along with a diverse set of other abundant diatoms were associated with high EPRs. The second and third points are consistent with positive association of higher species richness with higher EPR (Fig. 6). Limited evidence was shown about the possibility of detrimental effects caused by mono-species blooms of certain phytoplankton species (as in 2 of 21 experiments). Recall cases on both 27 July 2011 and 20 July 2012, the dominant diatom was *D. fragilissimum* whereas EPRs were exceptionally low. The same observation was made by Ceballos and Alvarez-Marques (2006) that EPRs were decreased during a bloom of *D. fragilissimum* for both *C. helgolandicus* and *Calanoides carinatus* in July 2002. Further, the bloom observed by us on 7 Aug 2012 showed that an unusually high concentration of the silicoflagellate *D. speculum* was associated with very low EPR, and this too has been found previously by Nejstgaard et al. (2001) in their mesocosm experiments with *C. helgolandicus*. The dinoflagellate *Gyrodiscium aureleum* was shown to have a negative influence on egg production of *C. helgolandicus* (Irigoien et al., 2000a) as was the haptophyte *Phaeocystis* sp. (Bautista et al., 1994) on egg production of the same species. Others have shown reduced EPRs during dense diatom blooms but either species were not enumerated or there were no dominant species that could be associated with reduced EPR (Nejstgaard et al., 2001; Paffenhofer, 2002; Poulet et al., 2007, 2006). Pierson et al. (2005) noted that EPRs of *C. pacificus* in Dabob Bay, WA were reduced during a bloom of *Thalassiosira*; however, we did not detect such negative effects (Fig. 7) by this species. Some studies did not report negative effects from mono-species blooms on egg production: these include blooms of *Phaeocystis* (Irigoien
et al., 2000b; Jonasdottir et al., 2011) and Skeletonema (Irigoien et al., 2005).

Many factors are suspected for the depressed EPR during diatom blooms, such as growth rate and phase of the bloom/taxa during a bloom, fatty acid composition and content of diatoms, ratios of protein: carbohydrate, toxin effects associated with the presence of certain aldehydes, etc. and each of these factors have been reviewed succinctly in Paffenhofer et al. (2005) thus need not be restated here. Beyond the potential effects of the factors related to food quality, there are also response differences by the copepods themselves, for example, their susceptibility to toxins and detoxification capability (Wichard et al., 2008).

Another potential reason for the reduced EPR is that during the summer upwelling season in the northern California Current the blooms are often caused by long-chain diatom species easily extending beyond 1 mm in length, and those species are from the genera Chatoecerus, Thalassiosira, Leptocylindrus, Pseudo-nitzschia, Skeletonema, Guinardia, etc. We speculate that these species may be too large to be handled easily and thus would be less actively selected by copepods and is based on our observations that when blooms of large-cell diatoms were present, EPR were low (e.g. 7 June 2013). Studies by Peterson et al. (2002) and Vargas et al. (2007) both argued for low efficiency of primary production transfer through the classical diatom food chain in the productive coastal upwelling system and this may be partially related to inedible diatom species.

4.5. Potential recruitment rates (eggs day$^{-1}$ m$^{-2}$)

Ceballos and Alvarez-Marques (2006) may have been the first to coin the term “potential reproduction rate” (PRR) for the calculation of in situ female abundance $\times$ egg production rate. In our study, the highest PRR was found in 2013 whereas PRRs were very low throughout 2014 and as well in 2012 (Fig. 8). When shown as climatology, rates were highest in spring and lower during the summer upwelling season. Maximum rates were 174,977 eggs day$^{-1}$ m$^{-2}$ (the same units are used hereafter) with an average of 30,433. No recruitment was apparent after August of any year, because there were no females in the quantitative vertical towed net since most of them had entered diapause by that time (although in 2011 and 2013, there were enough females in the Bongo net tow in fall and winter to be used for the EPR incubations).

Niehoff et al. (1999) reported nearly the same result of PRR for Calanus finmarchicus from the North Atlantic at Webbership M – up to 200,000 but with an average of 34,187 pre-spring bloom and 34,855 during the bloom. Stenevik et al. (2007) working on the same species in the Norwegian Sea as part of the ESSAS study reported an average PRR of 146,926 before the spring bloom period and 220,742 during the spring bloom. Gislason and Astthorsson (2000) reported much lower PRRs, noting a maximum of ~16,000 for C. finmarchicus in oceanic waters but only ~1000 in shelf waters. Harris et al. (2000) reported a maximum of 180,000 for C. finmarchicus in the Norwegian Sea and an average of about 10,000 for C. helgolandicus from the English Channel (a coastal station at a similar water depth as Newport Line). Bonnet et al. (2005) compared data on C. helgolandicus from Stonehaven (North Sea, 50 m depth), English Channel (Plymouth, 55 m depth) and the Cantabrian Sea (N. Spain). Maximum values were 256,000 (Cantabrian Sea), 35,146 (English Channel) and 11,764 (Stonehaven). Ceballos and Alvarez-Marques (2006) reported maximum PRR of 56,140 for C. helgolandicus and 57,444 for Calanoides carinate in coastal waters off Spain. Jonasdottir and Koski (2011) reported similar maxima for Calanus helgolandicus in the North Sea (Dogger Bank) during summer with a range of 443–49,355 and for C. finmarchicus, 162–49,954.

Fig. 8. Interannual and seasonal changes of potential recruitment rate of Calanus marshallae during 2011–2014.

The PRR calculation may be considered as estimate of potential “recruitment rate”, but obviously do not account for egg mortality (Ohman et al., 2002; Peterson and KImmerer, 1994) nor for variable egg hatching or naupliar survival rates. However, the PRR calculation does illustrate the total number of egg produced by all females on a given day thus is an estimate of secondary production by the ‘population’, which is especially relevant because even if the females in an incubation produce high numbers of eggs, if there are few-to-no females in the ocean, then the data from incubations must be valued differently. In this regard, we have clearly shown that there was no recruitment after August in any of the four years of data reported here, a fact that adds value to the calculation of PRR.

4.6. Laboratory estimates of EPR compared to in situ results

In an extensive set of laboratory experiments, Peterson (1988) estimated the functional response of feeding rates, fecal pellet production rates and egg production rates by female C. marshallae fed a wide range of concentrations of T. weissflogii. The maximum EPR estimated from an in vitro model was only 23.5. This is similar to (for some cases) but mostly lower than values observed in this study during summer, and far less than rates observed in winter (e.g., an EPR of 53.4 in 2014 and four-year mean of 32.5) and spring (EPR of 44.7 in 2013 and four-year mean of 40.2). The reason for these large differences cannot be known but we are concerned that T. weissflogii, being an easily-grown ‘laboratory weed’, was not nutritionally adequate during those experiments despite every attempt to maintain cells in log-growth phase, as described in Peterson (1988). We suggest that some zooplanktologists may need to re-think past lab work which used easily cultured diatoms (‘weeds’) and re-do important rate measurements, especially if reported rates are less than rates known from in situ incubations. This is especially true for C. marshallae development time for individual life stages from N3 to adult – in the experiments reported by Peterson (1986), the CS stage required several weeks to mature; moreover, only females were produced in those experiments.
4.7. Limitation of our approach

We did not measure ingestion rates of the copepods in our study so strictly speaking, any statement about the importance of ciliates or diatoms should be stated as a hypothesis. Based on their herculean work on *C. pacificus* and *Pseudocalanus* in Dabob Bay (Leising et al., 2005; Pierson et al., 2005), a sobering discussion is provided by them on the problems and pitfalls associated with trying to relate data on phytoplankton species composition, *C. pacificus* ingestion and egg production rates to selective feeding, and inferences that one species or another affected EPR. Another extensive effort was shown by Jonasdottir et al. (2011) in a mesocosm study and again the problems with determining which factors control copepod egg production were discussed. However, the fact remains that not all factors related to food quality can be controlled in a laboratory experiment, making it nearly impossible to demonstrate mechanism(s) that account for the greatest cumulative amount of variance in EPR. This problem and potential solutions need not to be discussed here since many ideas and hypotheses are succinctly articulated in a workshop held in Naples in November 2002 (summarized in Paffenhofer et al., 2005). However, if there were ever a near-perfect experiment, the two studies cited above come to mind: the in situ study of *C. pacificus* from Dabob Bay and the mesocosm study in Norway.

5. Conclusions

We have shown that EPRs were highest in spring and winter and lowest during the summer upwelling season. Significant functional relationships were shown between EPR and Chl a, but only when the dates were parsed into winter–spring and summer. High egg production in winter and spring were attributed to a greater abundance of ciliates and likely dinoflagellates at that time. Variations in EPR during summer remain enigmatic in that on some occasions, extremely low rates were measured whereas at other times, extremely high rates were measured; based on Chl a concentrations, EPR should not have been food limited. We suggested that during certain mono-specific diatom blooms, EPRs were depressed whereas when diatom species richness was high during some blooms in summer, EPRs were high. Since we did not conduct copepod feeding experiments on natural particle assemblages, we do not know if ciliates continue to contribute as an important component of copepod diets in summer, but clearly, diatom blooms are important to sustain high EPRs although they do not always result in high EPRs. Finally, Vargas et al. (2006) remarked “that the classical marine food web model does not apply to some coastal upwelling systems”. Further, Calbet (2008) reminds us that the “strength of the mesozooplankton–microzooplankton link is traditionally overlooked in plankton studies”. We could not agree more.

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