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Relationship of egg production of *Calanus pacificus* to seasonal changes in phytoplankton availability in Puget Sound, Washington[1]

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Abstract

Two methods of estimating daily, in situ egg production rate for *Calanus pacificus*, a planktonic marine copepod that releases its eggs directly into the sea, were developed. The model for both methods separates egg production rate into daily frequency of spawning (% day^{-1}) and clutch size (eggs female^{-1}). One method involves direct observations of spawning frequency and clutch size in a random sample of females held in controlled conditions for a 12–24-h period immediately after capture. The second method involves a visual assessment of the ovaries of live or preserved females and uses the observation that egg laying in *Calanus* occurs at night to predict daily rates of spawning. The methods were used to examine the relationship between egg production rate and phytoplankton availability in two areas of Puget Sound with different seasonal patterns of phytoplankton abundance and size composition. Egg production rate was hyperbolically related to phytoplankton concentration. The results show the potential for factors influencing phytoplankton standing stock to influence the timing and magnitude of recruitment into *Calanus* populations.

The life history of copepods in the genus *Calanus* appears to be well suited for effective utilization of the seasonal phytoplankton production in temperate oceans. Late preadult stages spend the winter in diapause, thus enduring the scarcity of food resources. In most species the overwintering stages molt into adults in late winter or early spring and are ready to take advantage of the vernal increase in algae. The spring increase of *Calanus* and its correlation with the spring bloom of phytoplankton are well documented (Heinrich 1962; Williams and Lindley 1980).

Spawning by *Calanus* is not necessarily restricted to spring, however; depending on location, females of many species can be abundant and able to spawn well into autumn. Although not always distinct, one–four breeding periods between March and October have generally been observed (Marshall and Orr 1955). Broods have been identified by pulses in densities of eggs, nauplii, or early copepodite stages. Because they rely on abundance estimates only, studies of *Calanus* populations do not permit quantitative evaluation of the mechanisms determining the considerable variability in the timing and magnitude of the recruitment pulses. It is important to understand the factors involved, as the success of the later recruitment periods may determine the magnitude of the overwintering seed stock for spawning the following spring.

The abundance of females and the mean egg production rate per female together determine the timing and magnitude of spawning. Some investigators have emphasized the importance of the former. McLaren (1978) reanalyzed the seasonal pattern of *Calanus finmarchicus* population abundance in Loch Striven observed by Marshall et al. (1934) and concluded that each generation developed and spawned the succeeding generation primarily according to the temperature regime; the implication is that food resources were not limiting egg production or at least played a relatively minor role. Other workers (e.g. Ruud 1929; Ussing 1938; Marshall and Orr 1955) believed that successive broods of *Calanus* depend on outbursts of phytoplankton growth. Experimental studies of the relationship between algal food concentration and growth rates (Vidal 1980) and egg production rates (Runge 1984) of *Calanus pacificus* suggest that this species exploits the recurrent spring and summer blooms of the temperate ocean,

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but relationships between phytoplankton availability and egg production rate of natural populations have not been examined. How do females of the spring and summer generations respond to the availability of food resources at the time they mature? To produce eggs, do they require high phytoplankton standing stocks, or can they utilize alternative food resources (Lebour 1922; Marshall 1924; Corner et al. 1974; Landry 1980) or perhaps rely on lipids accumulated during development (Gatten et al. 1980)?

Here I investigate the extent to which egg production in natural populations of *C. pacificus* in Puget Sound is dependent on the availability of phytoplankton. I examine seasonal fluctuations of egg production rate in two fjord basins of Puget Sound, Dabob Bay and the main basin, which differ hydrodynamically and consequently have different seasonal patterns of phytoplankton availability.

Dabob Bay and the main basin are situated within 40 km of each other (Fig. 1). Dabob Bay (17 × 5 × 0.2 km) is characterized by low tidal excursions (<0.2 km: Kollmeyer 1965). The seasonal pattern of phytoplankton growth (Shuman 1978) fits the classic temperate model of phytoplankton growth (spring diatom bloom, summer transition, fall diatom bloom) described by Heinrich (1962). The main basin (60 × 10 × 0.3 km), on the other hand, is characterized by vigorous tidal mixing (Ebbesmeyer and Barnes 1980), so that nutrients rarely limit phytoplankton growth (Winter et al. 1975). Periods of partial stabilization of the water column and high incident solar radiation bring on diatom blooms—frequently intense—and sustain relatively high interim levels of phytoplankton abundance throughout summer (Campbell et al. 1977). Female *C. pacificus* are actively feeding and reproducing in both areas from about mid-March until mid-October.

Calanus spawns fertilized eggs directly into the water, so egg production cannot be measured by the egg ratio method (Edmondson 1960). To estimate egg production rates, I observed clutch size and frequency of spawning in a sample of females for 12–24 h immediately after capture (cf. Dagg 1978). I also used a second method which combined laboratory observations of clutch size (Runge 1984) with estimates of the proportion of the female population carrying mature oocytes. For both methods, I verified and made use of observations that egg laying in *Calanus* is frequently on a diel cycle (Harding et al. 1951; Marshall and Orr 1952; Mullin 1968). A substantial portion of this paper is devoted to the development of these techniques.

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Methods

The sampling program was conducted between 1977 and 1980. In Dabob Bay, samples were collected at one or more of five stations several kilometers apart on the deep, central portion of the bay (Fig. 1) and analyzed aboard ship. Cruises lasted 1–4 days. In the main basin, samples were always taken at or near station 1 (Fig. 1), a midchannel location used in previous studies (e.g. Winter et al. 1975), and usually analyzed in the laboratory (transit time, ~2 h). In 1979 water samples from Dabob Bay were also collected from a seaplane and flown back to Seattle (transit time, ~1 h) for phytoplankton analysis.

Egg production rates—Zooplankton was collected with a 571-μm mesh net towed obliquely from about 200 m to the surface at speeds of about 1.8 km h^{-1}. The catch was immediately diluted into 1-gallon (4-liter) jars, and *C. pacificus* females were sorted under a dissecting microscope into Petri dishes until enough had been obtained for egg production measurements. A por-

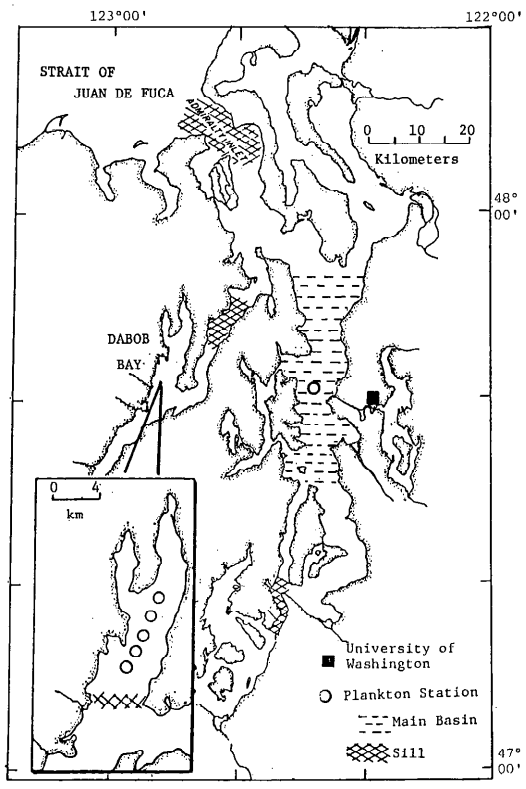

Fig. 1. Map of Puget Sound showing location of study sites and stations.

tion of the catch was usually preserved in 4% formaldehyde with BHT added (*see* English and Heron 1976).

For direct observations of egg production, females (usually between 30 and 100) were placed individually into plastic dishes (15-ml capacity) containing unfiltered seawater from a depth of 12–15 m, covered with plastic lids and fit into trays designed to hold them securely. The trays were kept in a small refrigerator or cold room at ambient temperature (±1.5°C), which had a seasonal range of 8°–14°C in Dabob Bay. The dishes were searched for eggs over the next 12–24 h. Eggs recently released (those with four or fewer cleavages) were counted to obtain an estimate of clutch size; older clutches were not included because of the potential for bias due to cannibalism (Runge 1981). Egg production rates were calculated as in Table 1.

For predictions of daily egg production rate, females from the initial sort were anesthetized with MS-222 (*m*-amino benzoic acid ethyl ester, methane sulfonate salt: Cal Biochem) and the state of maturity of the oocytes within the ovary and oviducts of each copepod was evaluated as shown in Table 2. The categories were designed to distinguish previtellogenic from vitellogenic oocytes in live females and are similar to Marshall and Orr's (1952) states of maturity. If the category could not be clearly determined, the state of maturity was tabulated under the number between the two major categories (state 2, 4, or 6). The proportion of the population in states 5–7 was used to predict the frequency of spawning events. The number of eggs per spawning event was estimated from an equation relating clutch size to mean body size of females, based on observations of females feeding continuously at superabundant food concentrations (Runge 1984). Although food concentration can influence clutch size (Runge 1984), the effect is small in relation to changes in clutch production rate. For example, the mean clutch size of females 2.61 mm long ranged from about 33 eggs at low production rates to 46 eggs at maximal rates in laboratory experiments. In a population spawning at a frequency of 10% per day, use of the maximum rather than the

Table 1. Determination of in situ egg production rate (E: eggs female^{-1} d^{-1}) of *Calanus pacificus* by two methods.

Direct observation:		$E = F_{do} \times C_f$
where	F_{do}	is the fraction of females spawning, by observation of females placed separately into Petri dishes (1 day^{-1}), and
	C_f	is the mean clutch size from same observations (eggs female^{-1}).
Prediction:		$E = F_{ev} \times C_r$
where	F_{ev}	is the fraction of females spawning, by evaluation of the maturity of ova in the oviducal diverticula (1 day^{-1}), and
	C_r	is the clutch size obtained from regression on prosome length (laboratory data), using mean prosome length of field population.

corrected clutch size in the calculation gives a value of 5 rather than 3 eggs female^{-1} d^{-1}. I chose to ignore the food effect in determining egg production rate by this method (Table 1).

Prosome lengths of females were measured under a dissecting microscope. Female dry weights were either calculated from the regression ln W = 2.5(ln L) + 2.99 (Runge 1980) or determined directly by rinsing live specimens in distilled water, drying in an oven at 60°C, and weighing on a Cahn electrobalance.

Phytoplankton availability—The upper 30 m of the water column was sampled with water bottles at 5–12 irregularly spaced intervals. In Puget Sound, most of the phytoplankton standing stock is above this depth; on average, pigment concentrations at 30 m are about 5% of the maximum in Dabob Bay and 16% of the maximum in the main basin. The number of separate bottle casts used in the determination of phytoplankton availability during a sampling period ranged from 1 to 11. In 1979, phytoplankton estimates were based on 1–3 profiles. At least one sample was routinely fractionated with 5-μm (nominal pore size; 7-μm effective pore size) or 10-μm Nitex screens, and often with a 73-μm screen as well (Runge and Ohman 1982).

Chlorophyll *a* was extracted in 90% acetone and measured by the fluorometric method with corrections for pheopigments (Holm-Hansen et al. 1965). The procedure for extracting pigments varied depending on the availability of equipment. Before April 1979, samples were usually sonicated, but on some occasions ground with a tissue grinder; these techniques give the same results (Shuman 1978). A sonicator was not available after April 1979, and samples were allowed to extract for 24–36 h in the dark at 5°C. This technique was chosen because grinding was too awkward for extracting pigments from Nitex screens, which do not break up in acetone. The drawback to the cold extraction technique is that it may underextract, particularly where Chlorophyceae or Cyanophyceae predominate (Holm-Hansen and Riemann 1978). When cold extraction was used, results were regularly compared with supplementary samples extracted by grinding; on five out of seven dates, values from the two techniques were within 15% of each other. In two instances, values obtained by cold extraction were lower by 27 and 52%. These occurred in summer in Dabob Bay when small flagellates predominate (Shuman 1978). I did not correct for this; the use of cold extraction may have resulted in underestimates of phytoplankton standing stock during this period.

Two comprehensive measures were determined from the data. Mean phytoplankton density was estimated by averaging integrated Chl *a* concentrations (mg m^{-2}) calculated by summing, over all sample intervals in the upper 30 m, the product of the average concentration between two successive sample depths and the distance between these depths. Maximum concentration in the water column was assumed to be equal to the mean maximum value of Chl *a* in profiles taken during a sampling period.

Table 2. Criteria for distinguishing four major states of oogenesis in *Calanus pacificus*, based on characteristics of oocytes in oviducal diverticula and oviducts of living females (dorsal view) at 12× magnification. Terminology from Marshall and Orr's (1952) classification given in parentheses to show approximate correspondence.

State 1: (immature)	Development of oocytes within oviducal diverticula absent or rudimentary. Diverticula appear as lines extending from about the 2nd to the 6th prosomal segment.
State 3:	Dorsal channels of oviducal diverticula dilated to about half-maximum width, filled with previtellogenic oocytes, margins of oocytes indistinct. Diverticula appear like tubes of granular lipid.
State 5: (semiripe)	Well developed ova extending entire length of diverticula including anterior portion of cephalothorax; ova close to maximum size and cell margins distinct. Ova not readily visible in oviducts.
State 7: (ripe)	Rows of mature ova extending the length of diverticula and branching into the oviducts on either side of the body. Ova visible in oviducts. May be opaque or rose-colored. Release of eggs imminent.

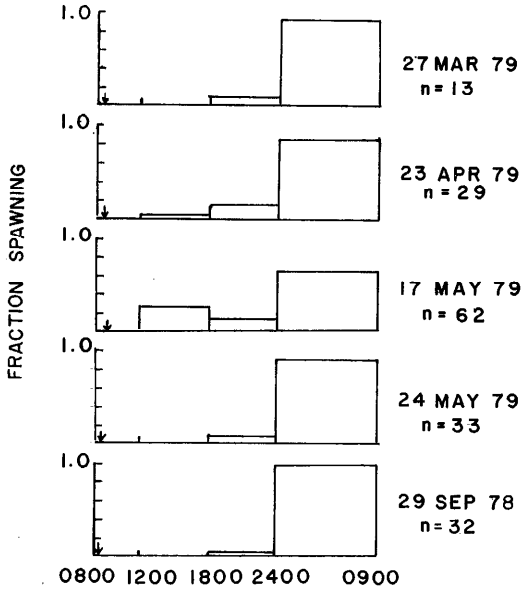

Fig. 2. Diel periodicity of spawning of *Calanus pacificus* in Puget Sound. Ordinate—fraction of total female sample releasing eggs in each time interval. Arrows show time of capture; *n* is number of spawning females. Other observations (Table 3) similar to ones shown for 23 April or 24 May.

Table 3. Specific rates of spawning (fraction spawning per day) in a random sample of females (number in parentheses) captured in the morning and held in standardized conditions for 24 h, and a sample of females captured in the evening of the same day and held for 12 h. Standard deviation (SD) estimated assuming a binomial distribution.

	Morning (24 h)	Evening (12 h)	SD
20 Apr 78	0.0(108)	0.06(112)	0.03
12 May 78	0.13(94)	0.25(133)	0.04
13 May 78	0.20(64)	0.18(38)	0.05
29 Sep 78	0.71(45)	0.78(28)	0.08
27 Mar 79	0.27(48)	0.40(73)	0.06
10 Apr 79	0.18(50)	0.08(39)	0.04
23 Apr 79	0.43(68)	0.36(44)	0.06
17 May 79	0.76(62)*	0.66(67)	0.06
24 May 79	0.48(69)	0.66(32)	0.07

* Includes daytime spawning.

Results

Frequency of spawning—To investigate the extent to which egg laying of *C. pacificus* in Puget Sound is on a diel cycle, I made 24-h observations nine times between March and September. Spawning was always synchronous; most of the females released eggs between 2400 and 0900 hours, and the rest almost always spawned between 1800 and 2400 (Fig. 2). On each occasion, I also performed the experiment for a 12-h period using females captured in the evening; spawning still occurred at night (Table 3), corroborating earlier evidence that stress of capture does not affect the timing of egg release (Harding et al. 1951).

Because of the diel pattern, I did not have to observe females for a full 24 h to determine the percentage releasing eggs each day. Except for certain conditions discussed below, overnight observations, from about sunset until about 0900, were sufficient.

The diel cycle in egg laying also facilitated use of the predictive method for determining the frequency of spawning in the population. Females were captured in the evening, usually within 2 h of sunset. Those classified in states 5 through 7 (Table 2) were assumed to be carrying ova that had completed oogenesis and were therefore ready for fertilization and release. In 15 of 19 cases, this technique predicted to within 10%, and usually better than 5%, the percentage of females observed to release a clutch of eggs overnight (Table 4). In three cases, the predictions underestimated the observed spawning by more than 10%. I attribute the underestimates to my inability to clearly establish the females as gravid. Although some oocytes in the oviducal diverticula of these females appeared well developed, cell margins of others were indistinct; I tabulated these females as state 4 and did not include them in the estimate of spawning fraction. This problem was acute on 17 September 1979 (Table 3); I rated 25% of the sample as obviously state 5, but could not decide whether another 60% should be so classified and could not make a reliable prediction.

Because rapid identification of gravid live females was not always easy, I attempted to better quantify this estimate by examining preserved animals. Although the opacity of the specimens precluded application of the criteria in Table 2, lipids in the maturing oocytes do appear as a dark band in the lateral view of a female. In April 1980, I compared direct observations of the frequency of spawning with the frequency dis-

Table 4. Comparison of predicted and observed rates of spawning (% day^{-1}). Predicted values from an assessment of females captured in the evening, done either on live specimens (criterion: states 5–7, Table 2) or on preserved specimens (criterion: width of oviducal diverticula >140 μm). Observed values from overnight observations of spawning in a sample of females from the same net haul.

	Prediction		Direct observation
	Live	Preserved	
19 Apr 78	9	—	8
12 May	25	—	22
24 Aug	39	51	60
27 Sep	68	72	78
16 Mar 79	1	0	0
27 Mar	34	36	40
11 Apr	21	—	13
23 Apr	29	—	36
17 May	68	—	66 (night) 76 (24 h)
24 May	68	71	66
6 Jun	31	31	27
11 Jun	4	2	2
23 Jul	39	53	66
30 Jul	5	10	9
20 Aug	68	—	69
30 Aug	0	—	1
4 Sep	46	—	62
17 Sep	*	79	86
23 Apr 80	62	60	59

* Prediction not possible (*see text*).

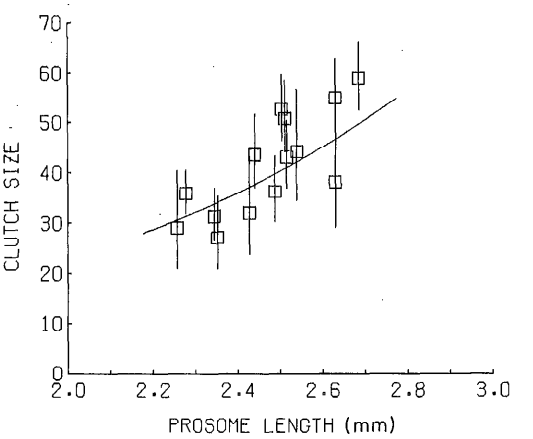

Fig. 3. Mean clutch size from direct observations (squares with 95% C.I.) arranged according to mean prosome length of females, and regression line from laboratory data [Runge 1984: ln (clutch size) = 0.87 + 1.13 (prosome length)].

tribution of the width of these dark bands in a random sample of females and found that *Calanus* bearing oocytes >140 μm were most likely to spawn. This is not unreasonable, as fertilized eggs are 155–165 μm in diameter. Analysis of preserved samples from earlier dates (Table 4) shows that this technique is a reliable alternative to immediate analysis of live samples.

Clutch size—Clutch sizes were first logarithmically transformed, as this transformation normalizes frequency distributions that otherwise may be skewed (Runge 1984). Mean clutch sizes of wild populations were significantly different (1-way ANOVA; $P < 0.01$) between sampling dates, ranging from 24 to 54 eggs per clutch. In Fig. 3, clutch sizes are arranged according to mean female body size, which varied seasonally (Fig. 4). Also shown in Fig. 3 is the regression of clutch size on body size determined from laboratory observations (Runge 1984) of females feeding continuously in superabundant food. The regression line falls within the 95% C.I. for 10 of the 14 direct observations of clutch size. In the remaining four cases, clutch sizes of field populations were higher by 10–27%.

Comparison of estimates of egg production rate—On 17 occasions (including the 14 observations in Fig. 3 plus three instances when spawning was negligible), I was able to calculate egg production rates by both methods (Table 1). A comparision (Fig. 5) indicates that egg production rates were measured accurately. Some of the variability between techniques can be eliminated by evaluating preserved rather than live females, as described above. The highest rate was observed on 17 May 1979 when some of the females spawned during daylight hours (Fig. 2); the daytime spawning was included in the direct observation but was of course missed in the evening assessment of the population. There may be an interaction between food availability, diel periodicity of spawning, and clutch size that causes the predictive technique to underestimate values derived from direct observations at rates above about 35 eggs female^{-1} d^{-1} (*see below*).

Seasonal variation in egg production rates—In 1979, I monitored seasonal variation in egg production rate in the two ba-

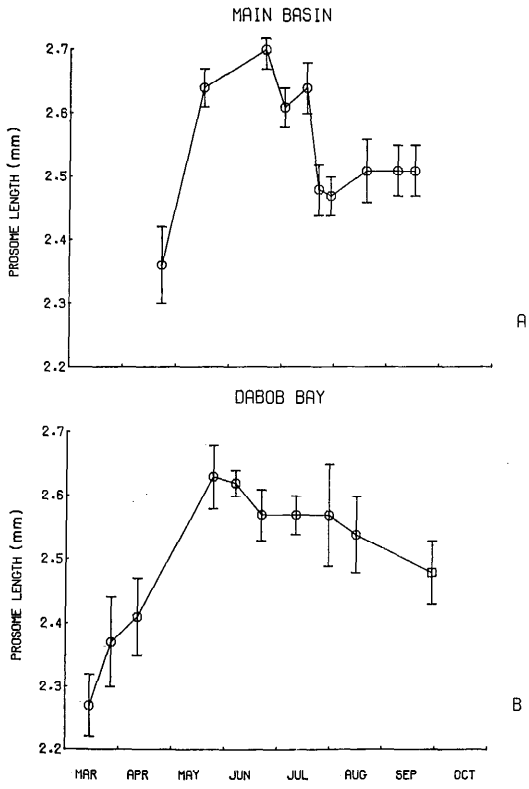

Fig. 4. Seasonal variation in prosome length of female *Calanus pacificus* in 1979. Means and 95% C.I.

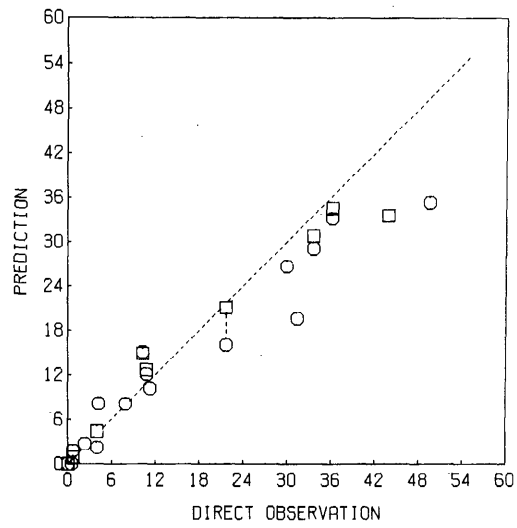

Fig. 5. Comparison of in situ egg production rates measured by two methods (Table 1), using data shown in Table 4 and Fig. 3. Rates derived from evaluation of live females (○) connected to those derived from preserved specimens (□) by a vertical line when both evaluations done on the same sample. Diagonal line shows the ideal one-to-one correspondence.

sins, particularly during the summer (Fig. 6). The data include observations used in Fig. 5. At both locations, the highest rates (Dabob Bay: 38 eggs female^{-1} d^{-1}; main basin: 48) were in May, with rates approaching these springtime levels in September. In the intervening months, the reproductive patterns at the two locations diverged considerably. In the main basin, *Calanus* produced eggs at variable rates in July, but maintained rates of 25–35 eggs female^{-1} d^{-1} throughout August and well into September. In Dabob Bay, on the other hand, egg production rate declined in June and remained low until the fall increase. On five of the six sampling dates during this period, egg production rates were <5 eggs female^{-1} d^{-1}. On the other sampling date (8–9 August), when a preserved zooplankton sample was taken but no phytoplankton observation made, the rate was <10 eggs female^{-1} d^{-1}. There may be somewhat more fluctuation than indicated in July, as measurements of egg production rates did not coincide with dates (*shown below*) when phytoplankton >7 μm was available in the subsurface maximum.

The observed field rates were consistently lower than maximum laboratory rates. Under laboratory conditions, females produce eggs at a rate close to 17% of their body carbon per day at 12°C, with a Q_{10} of about 1.65 (Runge 1984). The solid lines at the top of the graphs in Fig. 6 show the range of potential maximum rates based on seasonal changes in body size (Fig. 4) and temperature (Figs. 7 and 8) in both basins.

Seasonal variation in phytoplankton availability—In the main basin, phytoplankton samples were taken weekly from mid-June until early September 1979, less frequently before and after (Fig. 9). Probably phytoplankton densities were high before sampling began, as conditions of high light intensity and relatively low tidal excursions coincided early in April. In an intense diatom bloom in the middle of May, all of the pigment was retained in the >73-μm fraction; phytoplankton concentrations

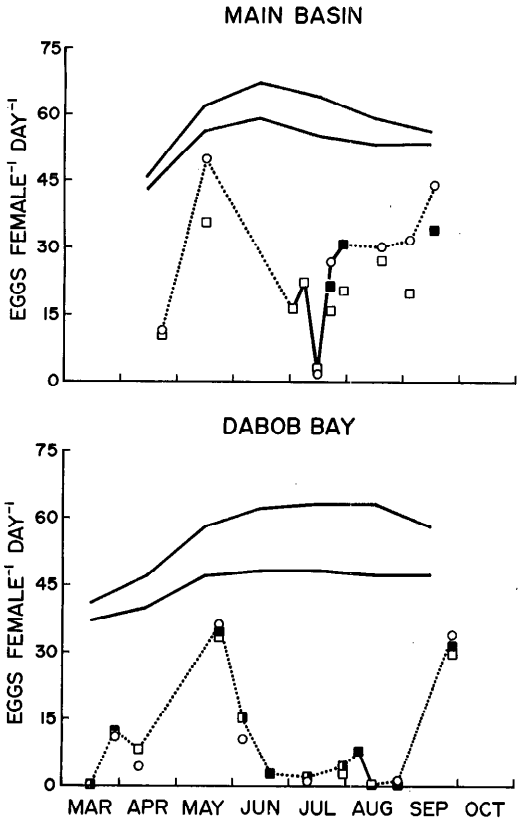

Fig. 6. Seasonal variation of egg production rate (eggs female^{-1} d^{-1}) of *C. pacificus* in the main basin and Dabob Bay. Last point in September in Dabob Bay refers to 1978; all other data from 1979. Direct observations of spawning and clutch size—○; predicted egg production rate (preserved specimens)—■; predicted (live specimens)—□. Sampling dates are connected with dotted line when more than 10 days apart. Solid lines above data points—range of maximum potential egg production rates based on laboratory experiments, using vertical temperature range and mean body size at each sampling date.

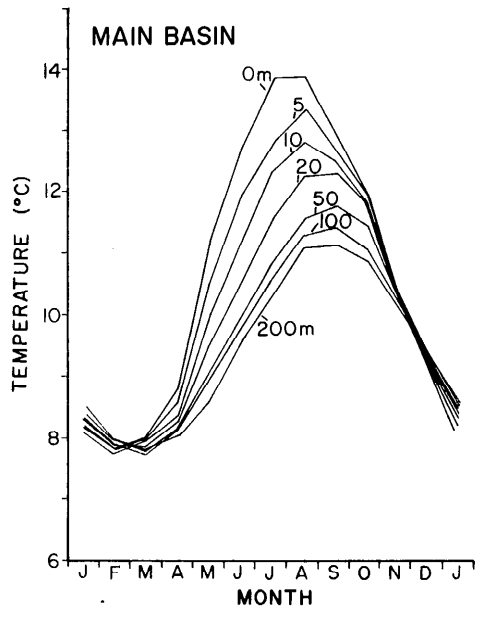

Fig. 7. Seasonal variation in mean temperature of the main basin at selected depths, computed from data taken since 1932 (from Helseth et al. 1980).

then declined sharply and remained relatively low until mid-July. By the end of July, however, all of the chlorophyll was again in the >73-μm fraction and concentrations reached 200 mg m^{-2}. In August and through the last sampling in mid-September, most of the chlorophyll was in the fraction between 7 and 73 μm and, except for one date in August, concentrations were >100 mg m^{-2} in the water column and >5 μg liter^{-1} in the chlorophyll maximum.

In Dabob Bay, samples were taken regularly between March and September. Phytoplankton standing stock first increased in the second week of April (Fig. 10). Maximum chlorophyll concentrations at this time were about 6 μg liter^{-1}, 60% of it in the >73-μm size category. Highest springtime standing stocks were observed in May, when chlorophyll concentrations reached 200 mg m^{-2}, about half of it between 7 and 73 μm. Relatively high phytoplankton densities persisted well into June, although for the most part in a pronounced subsurface maximum. The summer pattern that developed in the absence of strong tidal mixing in Dabob Bay contrasts markedly with the pattern in the main basin. The transition to a well stratified system in which phytoplankton <7 μm predominate began in early July in 1979. Pigments were usually concentrated in a narrow subsurface chlorophyll maximum in the vicinity of the thermocline. Size composition shifted to larger phytoplankton in the chlorophyll maximum on 27 June and 19 July, but this phenomenon was ephemeral (Runge 1981), and in August samples taken in and above the chlorophyll maximum did not show much phytoplank-

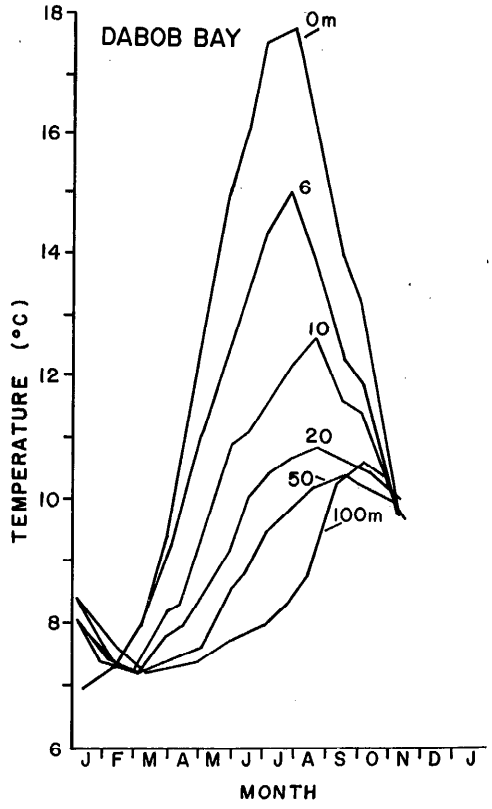

Fig. 8. Seasonal variation in mean temperature of Dabob Bay in 1979. Data from bathythermographs, a portable, battery-operated CTD recorder, or from the U.S. Navy (Inter-Ocean STD recorder).

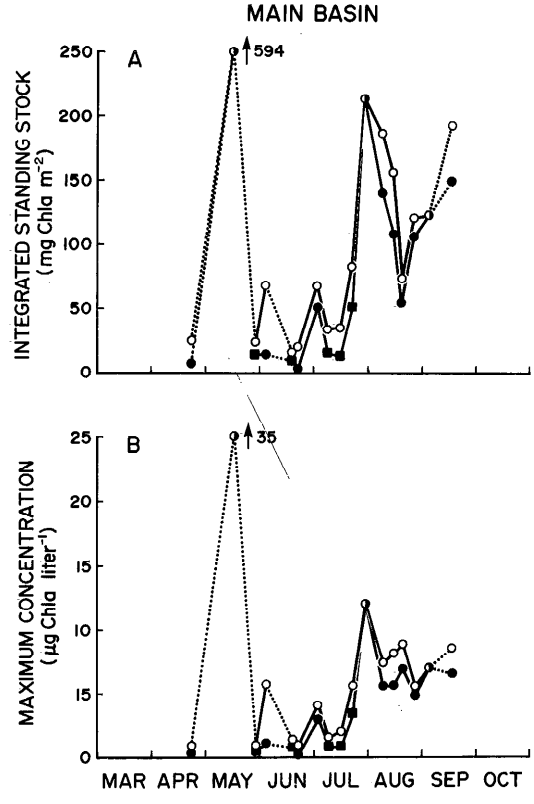

Fig. 9. Seasonal variation in estimates of integrated (A) and maximum (B) chlorophyll concentrations in the main basin in 1979. Open circles: Total concentration—○; >7 µm—●; >10 µm—■. Sampling dates are connected with dotted line when more than 10 days apart.

ton >7 µm. The summer system persisted until sometime in September, when a brief fall bloom showed standing stocks at springtime levels.

Data from Dabob Bay in 1977 and 1978 fit the general pattern of 1979, but the timing and persistence of the spring and summertime situations can vary considerably. In 1977, for example, summer characteristics were observed sooner: standing stocks were relatively low and most of the pigment passed through 5-µm screens in early June as well as July. In 1978, evidence from cruises (Runge 1981), sediment trap data (A. Copping pers. comm.), and plankton tows with a 73-µm mesh net (M. D. Ohman pers. comm.) indicate that the major diatom bloom did not occur until mid-June. The summer system, moreover, was disrupted weeks earlier in 1978 than in 1979; I observed relatively high levels of food (max Chl *a* concn >7 µm: 8 µg liter^{-1}) in the third week of August and in September as well (Fig. 10).

Relationship between phytoplankton availability and egg production rate—In both basins, the seasonal variation in egg production matched the seasonal pattern of phytoplankton availability. The highest rates of egg production corresponded with the high standing stocks of the spring and autumn blooms. Phytoplankton availability and egg production rates were consistently high during summer in the main basin but low in Dabob Bay. The correlation between the two variables is highly significant (Table 5).

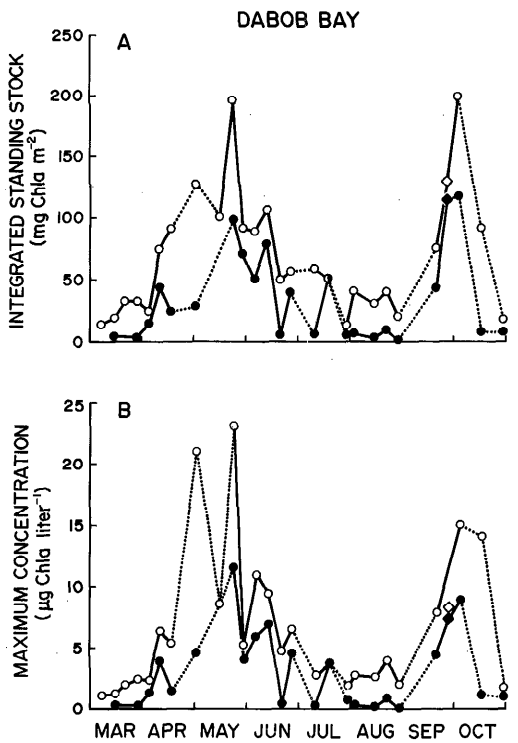

Fig. 10. As Fig. 9, but in Dabob Bay in 1979. Data from September 1978—◇, ◆.

I used data from 1977 and 1978, as well as from the 1979 seasonal study, to examine the functional relationship between egg production rate and both total and size-fractionated (5-µm screen) estimates of food availability (Figs. 11 and 12). When egg production was measured by more than one method, I used the direct observations or, if unavailable, the evaluation of preserved specimens, over the evaluation of live animals. In some cases size fractionations were not done and only the total estimates of standing stock were available. To eliminate variability due to seasonal changes in female body size, I converted egg production rates here to specific growth rates (µg C µg C^{-1} d^{-1}) using conversions for egg carbon (0.25 µm C egg^{-1}: Frost 1980) and body carbon (0.42 µg C μg^{-1} dry wt: Vidal 1980). The relationship for *C. pacificus* in Puget Sound is hyperbolic with respect to food concentration, whichever measure is used. Because parametric and nonparametric

Table 5. Spearman rank correlation coefficients (r_s) of egg production rate with estimates of phytoplankton availability (total—Chl *a* retained on GF filters). A subset ($n = 21$) of the phytoplankton samples was also size-fractionated with 5-µm Nitex screens. All correlations highly significant ($P < 0.001$).

	Integrated		Maximum	
	Total	5 µm	Total	5 µm
$n = 27$	0.86		0.72	
$n = 21$	0.89	0.90	0.79	0.86

(Tate and Clelland 1957) regression analyses gave similar results, I present only the parametric regressions (Figs. 11 and 12).

Discussion

In estimating egg production rates, I have assumed that oocytes are fertilized and released immediately on completion of vitellogenesis, subject to constraints imposed by the diel cycle. Marshall and Orr (1955) suggested that *C. finmarchicus* can defer release of mature oocytes until conditions are favorable for growth of the resulting nauplii, the stimulus to initiate spawning perhaps being the availability of food, and that egg laying in dishes when food conditions did not appear favorable was due to the stress of capture. Evidence for the deferral of egg laying is equivocal, however. Observations of increasing clutch size in *Calanus* in early spring, which Marshall and Orr (1952) argued reflects a gradual storage of mature oocytes, could instead be attributed to changes in food availability and female body size. Laboratory studies of the effect of food concentration on egg production rate in *C. finmarchicus* (Marshall and Orr 1952), *Calanus marshallae* (Peterson 1980), and in *C. pacificus* show that female *Calanus* produce clutches at low food concentrations. Moreover, if stress of capture stimulated egg release, it should have upset the diel spawning pattern in my study, which did not occur. I contend that the spawning behavior of freshly captured females mirrors the behavior of their counterparts in the sea.

Perhaps the most important source of error in estimating food availability is my frequent reliance on a small number of bottle casts to represent phytoplankton stand-

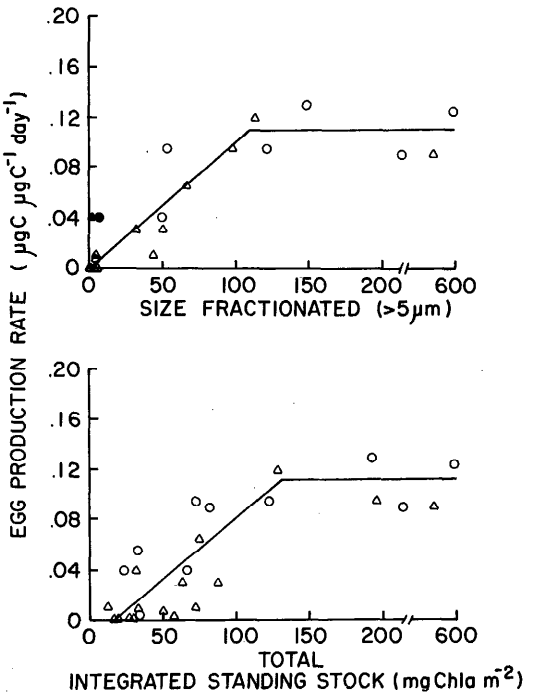

Fig. 11. Weight-specific egg production rate of *Calanus pacificus* in Puget Sound as a function of size-fractionated (5-µm Nitex screen) and total (GF filter) integrated phytoplankton standing stock. Main basin—O; Dabob Bay—△. Regression analyses: $E = 0.00102(SF) - 0.0025$ ($r^2 = 0.85$; SF < 110); $E = 0.00086(T) - 0.012$ ($r^2 = 0.58$; T < 140); $E = 0.11$ (SF ≥ 110; T ≥ 140), where E is egg production rate (µg C µg C^{-1} d^{-1}), SF is size-fractionated integrated standing stock (mg Chl *a* m^{-2}), and T is total integrated standing stock (mg Chl *a* m^{-2}). Two points (solid symbols) in SF graph not included in regression analysis.

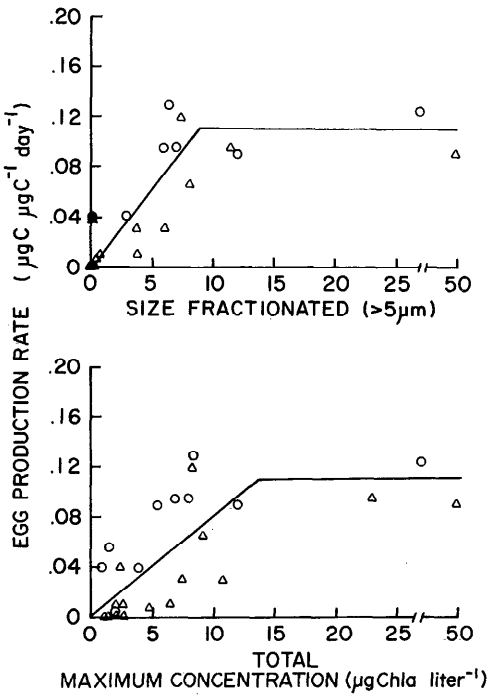

Fig. 12. Weight-specific egg production rate as a function of size-fractionated and total maximum chlorophyll concentration in the water column. Regression analyses: $E = 0.01265(SF) - 0.0032$ ($r^2 = 0.72$; SF < 9); $E = 0.008(T) + 0.0005$ ($r^2 = 0.42$; T < 14); $E = 0.11$ (SF ≥ 9; T ≥ 14), where E is egg production rate (µg C µg C^{-1} d^{-1}), SF is size-fractionated maximum concentration (µg Chl *a* $liter^{-1}$), and T is total maximum concentration (µg Chl *a* $liter^{-1}$). Symbols and explanations as in Fig. 11.

ing stock (Edmondson 1965). Egg production of *Calanus* on a given night conceivably represents a response to food conditions over the previous few days and over several kilometers of area. My analysis of variability of phytoplankton concentrations at this scale of space and time in Puget Sound indicates that estimates from a single bottle cast would be within a factor of two of the mean value at the 95% significance level (Runge 1981). Since the relationship between chlorophyll *a* and phytoplankton carbon and nitrogen concentration depends on environmental conditions, my use of chlorophyll *a* as the only measure of phytoplankton must also be regarded as an important source of error.

Despite the potential for error in the estimates of both food availability and egg production rate, the correlations between the two variables were strong. Phytoplankton standing stocks fluctuate by more than a factor of 10 during the course of a year; hence, even my potential twofold error in estimating food availability would not necessarily mask a relationship.

My results are consistent with the hypothesis that recruitment into *Calanus* populations requires relatively high phytoplankton standing stocks. In general, *Calanus* females could not sustain egg production when phytoplankton concentrations were low. However, there is a suggestion that they may, at times, supplement

their diet significantly with food from other sources. For example, I observed substantial egg production rates on two occasions (solid symbols in Figs. 11 and 12) when phytoplankton >7 μm was virtually nonexistent, both instances in early spring: in Dabob Bay in March (before the spring bloom) and in the main basin in April. The main basin situation may represent advection of surface water of low phytoplankton density over females deeper down. However, I found high concentrations of ciliated protozoans in water samples taken at the same time, and females may have been eating them.

The difference between Dabob Bay and the main basin in rates of *Calanus* egg production reveal the potential for the hydrodynamic regime to influence recruitment rate by modulating the availability of food resources. In the main basin of Puget Sound, the time of the onset of the spring bloom occurs reliably from year to year, and food is frequently available throughout summer. In Dabob Bay, on the other hand, the timing and duration of food resources appears to depend on wind events and is much less predictable; *Calanus* may not encounter high standing stocks until June and food is, in general, unavailable for much of the summer. The result for the *Calanus* population is that the input of new individuals (i.e. egg production) occurs less frequently and usually at a lower magnitude than in the main basin.

I have observed the relationship between the egg production rate of *Calanus* and phytoplankton concentration both in the natural environment, where the abundance and composition of food were variable in time, and in the laboratory, where food availability was kept constant. In Fig. 13, the regression lines representing the reproductive response of Puget Sound (Figs. 11 and 12) and laboratory (Runge 1984: fig. 5) populations are plotted together, with phytoplankton carbon concentration (μg C liter^{-1}) as the common abscissa. Only the size-fractionated data are shown; I will assume that phytoplankton passing through 5-μm Nitex screens could not be utilized by *Calanus* females on the basis of observations of

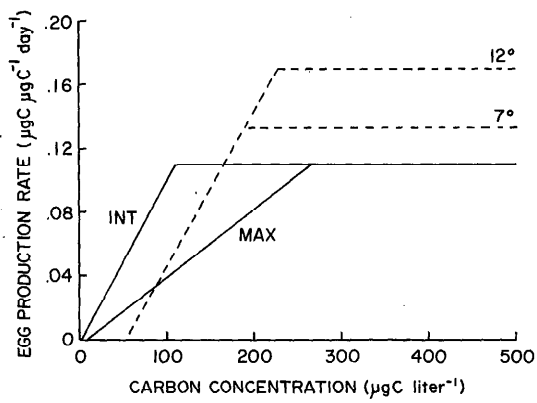

Fig. 13. Weight-specific egg production rate as a function of phytoplankton carbon concentration for laboratory (dashed lines) and Puget Sound (solid lines) populations. Laboratory regression (12°C) derived from data in Runge (1984): $E = 0.0011(P) - 0.073$ ($r^2 = 0.82$; $P < 220$); $E = 0.17$ ($P \geq 220$), where E is egg production rate (μg C μg C^{-1} d^{-1}) and P is phytoplankton concentration (μg C liter^{-1}). Maximum laboratory rates at 7°C ($E = 0.125$) also shown. Puget Sound regressions from Figs. 11 and 12; size-fractionated data only. Conversion factors given in text.

feeding by Frost (1972). There is considerable potential for error just in the conversion to carbon. Nevertheless, the approach is useful for examining the influence of natural feeding and migration cycles on the egg production of *Calanus*. In the discussion that follows, I explore hypotheses to explain the differences between the laboratory and field results.

In Puget Sound, the maximum rate at which *C. pacificus* produced eggs ranged between 9 and 13% (avg 11%) of its body carbon per day (Figs. 11 and 12). Weight-specific rates of females kept in optimal food conditions in the laboratory, on the other hand, were 17% d^{-1} at 12°C and 13% d^{-1} (extrapolated) at 7°C (Runge 1984). Temperatures at which eggs are produced in Puget Sound are not known precisely, but it is clear that maximal rates at sea were usually lower than laboratory-derived values. Since newly molted females may take up to a week before fully participating in the spawning process (Marshall and Orr 1952), laboratory measurements may more accurately reflect maximum egg production rates because females were acclimated to experimental food

densities for at least 5 days. This acclimation period would allow females immature at the time of capture to develop the capacity to spawn. I determined the proportion of females with undeveloped oviducal diverticula (state 1: Table 2) in preserved samples from the main basin in 1979. Presumably, this fraction would include all females not involved in spawning. The proportion varied between 5 and 15% of the female population over the course of the summer; exclusion of these females from the calculation of maximum egg production rate would raise the average value from 9–13% to between 11 and 15% per day. The importance of female age distribution to the proper interpretation of estimates of recruitment rates has been discussed elsewhere (Edmondson 1965; Taylor and Slatkin 1981).

Female *Calanus* in Puget Sound were migrating on a diel cycle (Runge 1981) and thus encountered phytoplankton for only part of each day. In the laboratory, females were continuously exposed to high food concentrations. For wild populations to attain maximum potential (i.e. laboratory) rates, females must be able to capture in 8–12 h what they can assimilate in 24 h under laboratory conditions. Previous investigations (McAllister 1971; Runge 1980) suggest that migrating *C. pacificus* can ingest in 8–10 h what continuous feeders consume in 24 h; whether egg production rates are also equivalent depends on the efficiency with which females can assimilate food gathered in pulses. This question can be answered by laboratory experiments (e.g. Dagg 1977).

There is in addition the possibility of a constraint on maximum egg production rates imposed by diel vertical migration. Laboratory observations (Runge 1984) indicate that, in nonlimiting food conditions and at temperatures >8°C, *C. pacificus* spawns more than one clutch in a 24-h period; at 15°C, which is reasonably close to the maximum temperature experienced by *Calanus* in Puget Sound, the minimum interval between spawning events is about 14 h. Since vertically migrating females stay in the surface layer for less than the minimum time to develop a second clutch of eggs and, presumably, eggs are released at or near the surface to enhance survival of nauplii (Marshall and Orr 1955), maximum rates would be limited to the number of eggs in a single clutch. Several alternatives questioning the assumptions of this argument can be put forward (Runge 1981). For example, when conditions are favorable for production of a second clutch, *Calanus* females may remain at or near the surface at least part of the day instead of migrating to deeper water. Females adapted to a diel cycle may pack more oocytes into the ovaries when food is superabundant than females acclimated to laboratory conditions and no longer constrained by the diel cycle; this may explain the somewhat higher clutches occasionally observed in field populations (Fig. 3). Females could be releasing eggs at depth. Any of these cases would mitigate the effects of diel vertical migration and would also result in underestimates of egg production by the predictive method at high food concentrations (e.g. 17 May: Fig. 2).

At limiting food concentrations, the regressions of egg production rate on mean concentration and on maximum concentration diverge (Fig. 13). This must be so, because the chlorophyll concentration was rarely uniform from the surface to 30 m. The two measures therefore describe different ways in which females may respond to the vertical structure of the phytoplankton. For example, the population of females could be distributed uniformly in the surface layer during feeding sessions (or, each female could range throughout the surface layer when feeding); in this case, the integrated concentration would be the more appropriate measure of food availability. On the other hand, females could search out and feed in the layer of maximum concentration. According to Fig. 13, the latter does not occur: use of maximum concentrations substantially underestimates laboratory rates. Alternative interpretations are also possible (Runge 1981). Experiments to determine the temperature dependence of egg production, the degree to which *Calanus* can utilize algae <5 μm for growth, and the extent to which egg production of *Calanus* feeding on an alternating cycle of food availability and deprivation deviates from egg

production of *Calanus* feeding continuously would focus our understanding of feeding and reproduction of *Calanus* in the sea.

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