Laboratory Development of *Euphausia pacifica* Larvae

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INTRODUCTION

Before one can make assumptions which allow for use of field samples to determine growth or mortality rates, it is important to establish the variability in developmental pathways and time to stage for your population of animals. This study serves to confirm the median time and range in time to stage for the development of the euphausiid, *Euphausia pacifica* off the coast of Oregon. This was accomplished through carefully monitored laboratory rearing of *E. pacifica* larvae from eggs through the juvenile stage. *E. pacifica* generally develops sequentially through 2 naupliar stages, a metanauplius stage, 3 calyptopis stages and 7 furcilia stages before becoming a juvenile. The variability in pathways is discussed in detail in the poster entitled "Variability in developmental pathways of individual larval *Euphausia pacifica*" by Tracy Shaw.

METHODS

We collected adult female *Euphausia pacifica* 25 miles offshore from Newport, Oregon (44°40'N). Gravid females were isolated in 1L jars filled with filtered seawater and allowed to spawn in the laboratory coldroom at 10.5°C. Animals were kept in the dark, except when the experiment was checked. Females were checked every few hours until they spawned, and eggs were checked similarly until they hatched. The development of four batches of eggs (A, 11, 42, 43) was monitored from hatching to juvenile. Developing animals were kept in small glass containers from hatching to FII and checked daily to determine stage of development. They were moved to clean water and fed to excess daily. They were fed a combination of *Isochrysis*, *Rhodomonas*, *Chaetoceros* and *Heterocapsa* cells.

When the animals molted to FIII they were isolated in individual 250 ml or 500 ml jars and checked daily for molts. Developmental progress was determined by staging the molts. All molts were recorded, even when developmental stage could not be determined. Animals were checked daily from FIII until they reached the juvenile (JUV) stage. By recording the time checked and knowing the time of hatching, we were able to keep track of time from hatching in fractions of days. Water was changed and animals were fed to excess twice per week from FIII to juvenile. Stages FIV and FV were considered one stage in this experiment, as they could not be distinguished by examining the molt.

For analysis of development time we created cumulative frequency curves by calculating the cumulative percent of animals that were in, or had passed through, a given stage every day (plotted in Figure 2). These percentages were then transformed with an arcsine transformation (units in degrees) so as to make it possible to fit a linear regression to the curve. Median times to stage were calculated using these regression equations to determine the time when 50% of the animals had reached a given stage.

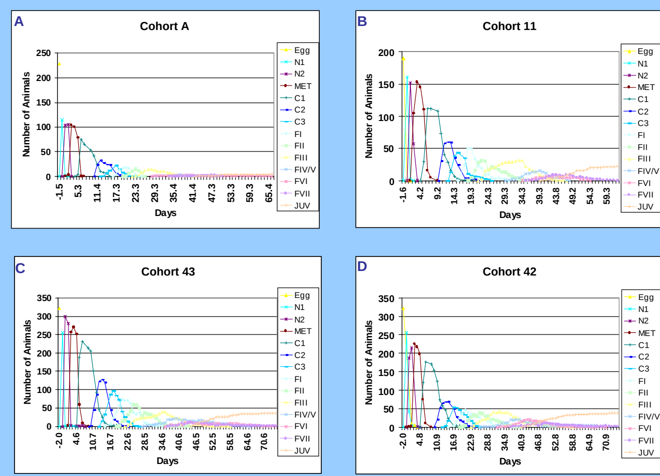

Figure 1 A-D. Developmental sequences for cohorts of *E. pacifica* larvae. A = cohort A, B = cohort 11, C = cohort 42 and D = cohort 43.

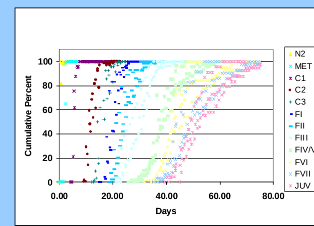

Figure 2. Cumulative frequency curves for E. pacifica larvae. These curves are based on the combined information from all Cohorts except A.

Median Development Time (days from hatching)				
Stage	8°C	**10.5°C**	**Range in stage**	12°C
Nauplius	0	**0.7**	**0.5-2.6**	0
Metanauplius	2.0	**2.3****	**0.8-7.3**	2.2
Calyptopis 1	8.0	**6.1****	**5.3-14.9**	4.5
Calyptopis 2	13.5	**13.7****	**9.6-20.9**	10.0
Calyptopis 3	18.5	**17.5****	**13.2-24.9**	14.0
Furcilia I	22.0	**20.9****	**16.7-31.0**	17.5
Furcilia II	31.0	**26.3****	**19.3-41.7**	23.0
Furcilia III	38.5	**32.1****	**22.3-52.8**	27.5
Furcilia IV/V	50.0	**43.0****	**28.9-61.8**	33.0
Furcilia VI	55.0	**50.6****	**32.3-67.6**	36.5
Furcilia VII	60.0	**55.1****	**35.4-72.3**	40.0
Juvenile	68.0	**58.0****	**40.4-....**	45.0

Table 1. Median development times for 3 temperatures.
Times in gray (8° and 12°C) are from Ross (1981). Times for 10.5° (in bold) are from this study. They were calculated from regression lines of the arcsine transformed data from a combination of cohorts 11, 42 and 43. Range in stage is the time when animals were first observed in a stage until most of them had passed on to the next stage. Upper limit of range was truncated when fewer than 4 animals remained in a stage. ** indicates p < 0.001 for the regression equations used to calculate the median times.

RESULTS

Figure 1:
- Survival from egg to juvenile was 12% for cohorts 11, 42 and 43. It was 4% for cohort A
  - Egg hatching was variable: cohort A = 50%, 11 = 85%, 42 = 80%, 43 = 93%
  - There was relatively little mortality from FIII to juvenile.
- It is evident from the long tails on some of these curves that some animals linger for extended periods of time in certain stages.
- Cohort A was not included in further analysis due to high mortality.

Figure 3:
- Stage duration is longest by far for C1 and FIII.
  - Duration for FIV/V is inflated due to the combination of 2 stages, though not all individuals pass through both stages.

Figure 4:
- Juvenile development curves are not significantly different for cohorts 42 and 43
- Animals in cohort 11 reach their median development time as juveniles significantly faster than those in cohorts 42 and 43 due to a higher slope.

Table 1:
- Median times to stage for all furcilia and most calyptopis are in range with those calculated by Ross (1981).
- A given population of larvae, hatched on the same day can vary by nearly a month in the time to develop to juveniles, thus making cohort analysis difficult.

Table 2:
- Regression equations are not significant for N2 or metanauplius, and are significant for C1 only in cohort 11.
  - All other equations shown are highly significant ($p < 0.001$).
- All cohorts develop at a similar rate up until the FI stage.
- Slopes in cohorts 42 and 43 drop and stay fairly constant after FIII, this does not occur for cohort 11.
- Median time to juvenile is 7-8 days faster in cohort 11 than in 42 or 43.

	Cohort 11			Cohort 42			Cohort 43		
Stage	**Regression Equation**	**r^2**	**Median Developmental Time (days)**	**Regression Equation**	**r^2**	**Median Developmental Time (days)**	**Regression Equation**	**r^2**	**Median Developmental Time (days)**
Nauplius 2	Y=40.77x + 9.69	0.868	0.9	Y=30.28x + 14.03	0.874	1.0	Y=12.32x + 46.14	0.376	---
Metanauplius	Y=43.75x – 53.93	0.995	2.3	Y=29.01x – 17.16	0.632	2.1	Y=14.89x + 6.70	0.639	2.6
Calyptopis 1	Y=26.56x – 116.36	0.956**	6.1	Y=21.93x – 96.67	0.891	6.5	Y=20.99x - 79.6	0.841	5.9
Calyptopis 2	Y=6.90x – 45.26	0.84**	13.1	Y=8.23x – 75.61	0.953**	14.7	Y=8.74x – 72.24	0.926**	13.4
Calyptopis 3	Y=9.01x – 117.67	0.936**	18.1	Y=8.62x – 114.84	0.993**	18.5	Y=9.73x – 123.1	0.971**	17.3
Furcilia I	Y=7.87x – 108.45	0.875**	19.5	Y=8.56x – 142.07	0.967**	21.9	Y=8.59x – 137.74	0.979**	21.3
Furcilia II	Y=6.86x – 120.29	0.933**	24.1	Y=5.83x – 113.3	0.980**	27.2	Y=5.32x – 99.39	0.963**	27.1
Furcilia III	Y=5.86x – 125.31	0.978**	29.1	Y=5.31x – 126.27	0.992**	32.3	Y=4.18x – 95.1	0.969**	33.5
Furcilia IV/V	Y=5.21x – 160.35	0.958**	39.4	Y=2.86x – 81.14	0.962**	44.1	Y=3.17x – 93.41	0.972**	43.7
Furcilia VI	Y=5.97x – 215.0	0.91**	43.6	Y=2.29x – 71.14	0.910**	50.7	Y=2.78x – 99.86	0.987**	52.1
Furcilia VII	Y=4.61x – 172.66	0.960**	47.2	Y=2.58x – 96.76	0.970**	55.0	Y=2.24x – 84.02	0.990**	57.6
Juvenile	Y=4.14x – 170.01	0.980**	51.9	Y=2.32x – 91.81	0.979**	59.0	Y=2.38x – 99.26	0.981**	60.6

Table 2. Regression equations used for determining median time to stage for development of cohorts 11, 42 and 43. ** indicates p < 0.001

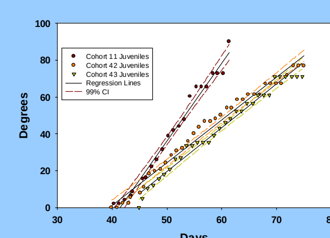

Figure 4. Cumulative frequency curves (arcsine transformed) for juveniles of 3 cohorts. Curves have been fit with a regression equation and 99% confidence intervals. 90° = 100%, 45° = 50%

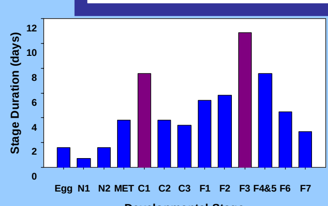

Figure 3. Stage durations for combined cohorts of *E. pacifica* larvae, not including cohort A. Stage duration is defined as time between median development times of subsequent stages. Magenta bars represent "bottleneck stages."

CONCLUSIONS

•Our median times to stage are generally within the range of other studies of *E. pacifica* development times.
•There is a very broad range in the length of time that it takes for animals in the same cohort to reach any given stage.
•Calyptopis 1 and Furcilia III appear to be the bottlenecks in the development of *E. pacifica* larvae.
•Of the 3 cohorts which survived with good numbers to the juvenile stage, one (cohort 11) reached this stage significantly faster than the other two.

FUTURE DIRECTIONS

- Re-do these experiments, checking every 4 hours to improve data for stages N1-C1.
- Analyze regressions using ANCOVA in order to determine which slopes differ significantly.
- Carry out the same experiments for *Thysanoessa spinifera* larvae.

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