than *P. simpsoni*, with relatively less massive chelae than the latter. Statistically, there is a significant ($P = 0.05$) difference between the two crabs (independent of size) in length and width of carapace, length and depth of body, length of merus and ischium of the third maxilliped, and length and height of the major palm.

Habitat

There is a relatively effective isolation of the two mud crabs by habitat. *Panopeus simpsoni* occurs intertidally or subtidally in association with the American oyster, but not usually in the marsh bank environment. *Panopeus obesus* occurs in marsh banks, but not subtidally. Both species inhabit intertidal rubble areas.

Feeding

Though food type for both species is similar, *P. obesus* is much more aggressive in capturing and consuming prey.

These findings reinforce the conclusion of Williams (1983) that *P. obesus* and *P. simpsoni* are specifically distinct.

Acknowledgments

We thank R. W. Heard, J. F. Fitzpatrick, T. S. Hopkins, and G. Crozier for assistance and guidance to the senior author. W. Martz, J. Martin, G. Goeke, and D. Anderson helped in field sampling, and Faye P. Reames gave technical assistance. J. L. Russo gave statistical advice. B. B. Collette, D. L. Felder, R. B. Manning, B. Sullivan, and anonymous reviewers offered critical comments on the manuscript.

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EFFECT OF TEMPERATURE ON RATE OF EMBRYONIC DEVELOPMENT OF WALLEYE POLLOCK, *THERAGRA CHALCOGRAMMA*

Recent studies by the Northwest and Alaska Fisheries Center Auke Bay Laboratory of the National Marine Fisheries Service, Auke Bay, Alaska, have focused on causes underlying mortality of eggs and larvae of walleye pollock, *Theragra chalcogramma*, a species of considerable economic importance in Alaskan waters. One aspect of these studies is to predict age of walleye pollock embryos in samples from surveys at sea. Knowledge of age of embryos is necessary for estimating peak spawning time and daily production of eggs, and for predicting abundance and distribution of spawning fish. Because length of the incubation period is dependent on temperature of the water mass in which the eggs are developing (Hamai et al. 1971), embryo age (hours since fertilization) can be estimated provided water temperature is known.

In this study, we determined the relation between temperature and rate of development of walleye pollock embryos at constant incubation temperatures and at fluctuating temperatures (simulated). We then derived equations and a contour plot for estimating the age of an embryo (time from fertilization, in hours) at a given incubation temperature and stage of development. We also derived an equa-

tion to estimate hours to midpoint of the hatching interval.

Methods

On 4 April 1981, adult walleye pollock were trawled in Stephens Passage, southeastern Alaska (lat. 58°17'N, long. 134°42'W). One sexually mature female and one adult male were kept alive until spawned artifically in the laboratory, about 3 h later. The eggs were removed and fertilized according to the "dry" method (Kinne 1977).

Embryos were incubated about 4 h at 6°C to ensure that only viable eggs were used in the experiment. At the end of the 4-h period, each egg was examined visually before being transferred to an incubator. The incubators were then placed in water baths of the various experimental temperatures. By the time of the first observation, about 4 h later, water temperature in the incubators had reached experimental temperatures. Six groups of about 200 embryos each were incubated separately in identical incubators. Two of the groups were incubated at 6°C to provide an estimate of residual error. The other groups were incubated at 2°, 5°, 8°, or 11°C. These incubation temperatures fall within the range of incubation temperatures walleye pollock embryos usually encounter at sea.

The incubators were 3.5 l cylindrical containers made of black ABS (acrylonitrile-butadiene-styrene) plastic. Each incubator was filled with 2,500 ml of seawater (salinity 32.5‰) and covered with a clear Plexiglas[1] cover 3.2 mm thick. Seawater was not changed during the experiment. The incubators were kept in thermostatically controlled water baths at temperatures within ±0.2°C of the treatment temperature. Photoperiod was 12-h illumination and 12-h darkness. Illumination at the cover of each container was 170 lux (15.8 fc) from 60-W Soft White incandescent bulbs.

[1]Reference to trade name does not imply endorsement by the National Marine Fisheries Service, NOAA.

Embryos were removed from the containers at about 4-h intervals for the first day, then at least daily thereafter. The embryos were preserved in Gilson's fixative and later classified according to a seven-stage classification system, based on easily recognized developmental features (Table 1). For quantitative purposes, we used whole integers for midpoint of stages (Ferraro 1980). The start and end of each stage is quantified by adding or subtracting 0.5 to the stage number. We also recorded the time when larvae were first observed in culture vessels and the time when all embryos had hatched.

TABLE 1.—Stages of embryo development used for walleye pollock.

Stage I:	Fertilized egg without germ disc.
Stage II:	From germ disc through 32-cell stage.
Stage III:	From 64-cell stage to completion of blastoderm.
Stage IV:	From germ ring to germ ring enveloping egg, but before completion of epiboly.
Stage V:	From completion of epiboly to embryo extending at least three-fourths way around yolk and caudal region not directed off axis of embryo.
Stage VI:	From caudal region slightly off axis of embryo to markedly off axis, and tip of tail just reaching embryo head.
Stage VII:	Tip of tail extending beyond embryo head to hatching.

Statistical Analyses

Because the embryos were incubated for 4 h at 6°C before they were placed in the incubation containers, the data were corrected (Table 2) for the delay in attaining experimental temperatures using Ferraro's (1980) method. In our experiment, the correction factor was the ratio of development time to Stage VII for embryos incubated at 2°, 5°, 8°, and 11°C relative to development time to Stage VII at 6°C (see Table 2 for derivations and Table 3 for corrected midpoint and duration of each stage).

We developed a general predictive equation with temperature-dependent coefficients to estimate the age of a walleye pollock embryo, given a stage of development and incubation temperature over the range of 2°-11°C. For each experimental temperature, the midpoint age (in hours) for each developmental stage was plotted against the stage (Fig. 1),

TABLE 2.—Derivation of correction factors to adjust development data for differences between experimental and pre-experimental temperatures (°C) in walleye pollock. See Table 1 for description of stages.

Item	2°	5°	6°	8°	11°
Hours (midpoint) to Stage VII (unadjusted data).	514	322	278	187	153
Ratio of hours to Stage VII relative to Stage VII at 6°C.	1.85	1.16	1.00	0.67	0.55
Hours from fertilization to transfer.	2.75	3.75	2.25	3.25	2.25
Expected age (in hours) at time of transfer to culture vessels. Line 2 × line 3.	5.09	4.48	2.25	2.18	1.24
Correction factor to unadjusted data. Line 4-2.25 h.	+2.84	+2.23	0	−0.07	−1.01

TABLE 3.—Midpoint (h) and duration (h) of stage of walleye pollock embryos for Stages I-VII at experimental temperatures 2°, 5°, 6°, 8°, and 11°C. See Table 1 for description of stages.

	2°C		5°C		6°C		8°C		11°C	
Stage	Midpoint (h)	Duration (h)	Midpoint (h)	Duration (h)	Midpoint (h)	Duration (h)	Midpoint (h)	Duration (h)	Midpoint (h)	Duration (h)
I	2	4	<1	1	<1	<1	<1	<1	<1	<1
II	12	16	8	14	8	14	5	8	4	6
III	58	76	38	44	35	40	25	30	22	28
IV	130	70	82	44	80	50	55	30	45	20
V	240	150	145	80	133	56	91	42	69	38
VI	382	136	230	90	205	90	149	72	111	46
VII	508	116	317	84	277	54	208	46	155	40

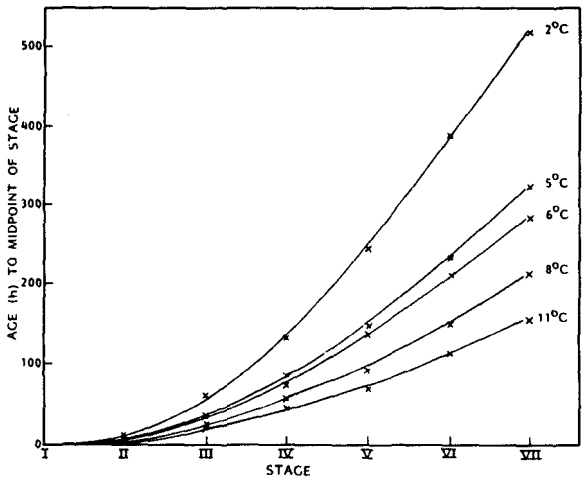

FIGURE 1.—Age-stage relation of walleye pollock embryos at 2°, 5°, 6°, 8°, and 11°C. Solid lines are regression lines of embryo age on developmental stage with experimental data adjusted for pre-experimental time and temperature; x's represent experimental data.

TABLE 4.—Regression equations for estimating age (in hours) of walleye pollock embryos at constant temperatures of 2°, 5°, 6°, 8°, and 11°C, where $\hat{Y}$ = predicted age and X = Stages I-VII. See Table 1 for description of stages.

Temperature (°C)	Regression equations
2	$\hat{y} = 50.0 - 73.4365X + 28.8571X^2 - 1.2778X^3$
5	$\hat{y} = 16.2857 - 27.5436X + 12.3512X^2 - 0.3194X^3$
6	$\hat{y} = 14.5714 - 25.9524X + 12.3036X^2 - 0.4583X^3$
8	$\hat{y} = 6.0286 - 12.3960X + 6.3286X^2 - 0.06111X^3$
11	$\hat{y} = 0.6000 - 5.0111X + 3.9119X^2 - 0.0056X^3$

TABLE 5.—The temperature-dependent coefficients, $a_0 \ldots a_3$, estimated from the least-square fits of the regression coefficients in Table 4. T = mean incubation temperature (°C). See Table 1 for description of stages.

$$\hat{a}_0 = 75.867 - 14.8769\,T + 0.7368\,T^2$$
$$\hat{a}_1 = -108.5209 + 20.0783\,T - 0.9770\,T^2$$
$$\hat{a}_2 = 41.4383 - 7.1494\,T + 0.3414\,T^2$$
$$\hat{a}_3 = -1.9642 + 0.3958\,T - 0.0199\,T^2$$

and the data were fitted to a third-degree polynomial model using stage as the predictor variable (Table 4). The regression equations in Table 4 were then used to derive the temperature-dependent coefficients, $a_0(T), \ldots, a_3(T)$, (Table 5), for the generalized predictive equation for embryo age,

$$\hat{y} = a_0(T) + a_1(T)X + a_2\,(T)X^2 + a_3(T)X^3 \quad (1)$$

where $\hat{y}$ = age (in hours), X = stage, and T = water temperature (°C). Equations for estimating the temperature coefficients are least square fits of the regression coefficients in Table 4 to a second degree polynomial model of temperature (Table 5). Over the temperature range of 2°-11°C, the standard error (obtained by comparing predicted with observed values) was normally distributed and equaled 3.03 h.

The temperature-dependent coefficients in our predictive model represent the rate (units: hours/stage) of embryogenesis in walleye pollock and are only meaningful when used in context with the embryo-staging classification in Table 1.

To verify that the curves in Figure 1 were different for each temperature, we statistically compared the curves for embryonic development at the two closest temperatures, 5° and 6°C. Cumulative measurements of development are dependent and violate assumptions underlying usual statistical comparisons among treatment groups. To avoid this problem of dependency, we transformed the development curves of embryonic development into time increments between midpoints of adjacent stages. Time increments are less correlated than cumulative measurements yet contain the same information (Box 1950). The time increments were then used as the dependent variable in the analysis of variance. The analysis of variance showed a significant temperature effect ($P < 0.01$) and significant interaction ($P < 0.05$), which indicates a real difference in development rates at 5° and 6°C.

To further substantiate that the generalized predictive Equation (1) is valid for estimating age of walleye pollock embryos at any stage over the temperature range of 2°-11°C, we regressed age (ln) as a function

of temperature for Stages II-VII. The slopes of the resulting straight lines (Fig. 2) were compared using a multivariate general linear hypothesis model (Morrison 1967), which regresses a vector of observations (development time of each stage) against temperature. The hypothesis of parallel slopes was not rejected ($P > 0.05$); therefore, the relation between age and temperature is probably independent of the stage of development.

To facilitate estimating age (time after fertilization, in hours) of walleye pollock embryos, we generated a contour plot (Fig. 3) from the generalized predictive Equation (1) over the temperature range of 2°-11°C for development Stages II-VII. For both contour plot and generalized predictive Equation (1), the estimates of age of walleye pollock embryos can be made more precise by refining the staging scheme (Table 1) into fractions of stage development. The qualitative estimates of stage fractions are quantified by adding or subtracting the proportion of stage development to the stage number.

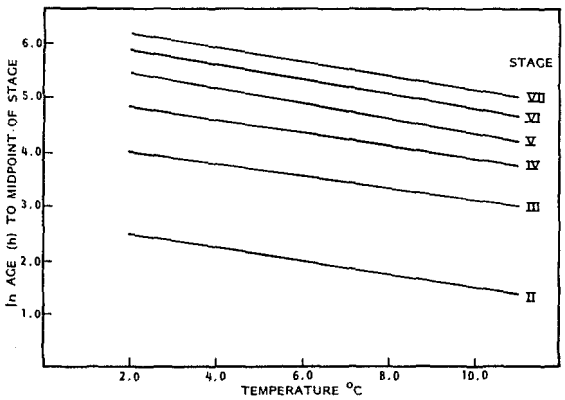

FIGURE 2.—Age-temperature relations of walleye pollock embryos, Stages II-VII.

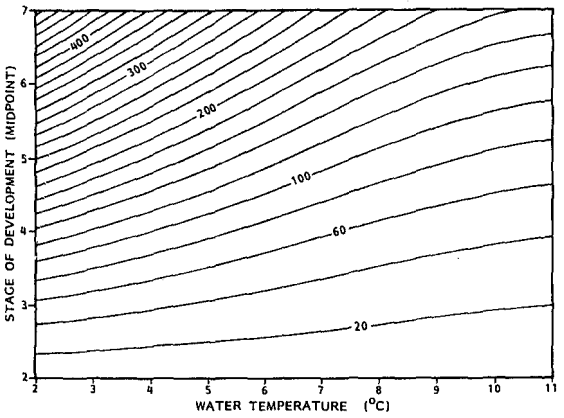

FIGURE 3.—Contours of predicted development time (h) of walleye pollock embryos as related to stage of development and water temperature (°C).

Similarity of the age-temperature relations among stages implies that embryo development is predictable regardless of whether water temperatures fluctuate or remain constant. We examined this implication mathematically by simulating stage of embryo development after 200 h given a mean temperature of 6°C. Examples of temperature variation used were 1) 100 h of embryo development at 4°C followed by 100 h at 8°C, 2) 100 h at 8°C followed by 100 h at 4°C, 3) 67 h at 2°C followed by 133 h at 8°C, and 4) 133 h at 8°C followed by 67 h at 2°C. We compared the simulations with 200 h of embryo development at constant 6°C. For various fluctuating temperatures, the mean of the simulations predicting stage of development was 6.08 with a standard error of 0.05. The value 6.08 corresponds closely to the computed stage of development of 6.06 for embryos reared at a constant temperature of 6°C. The standard error of 0.05 transforms into a standard error of 3.5 h in terms of development time and is similar to the standard error of 3.03 h of the generalized predictive Equation (1). The similarity of the standard errors (in hours) shows that temperature fluctuations exert a negligible decrease in accuracy of the generalized predictive Equation (1) and that an estimate of embryo development time based on mean temperature has the same reliability as an estimate based on a constant temperature. It should be noted, however, that mean development times were simulated and that estimates of mean development time based on empirical data are needed to verify the implication of the age-temperature relations.

We further substantiated that the mean of fluctuating temperatures could be used to estimate development time by comparing results from our generalized predictive equation with the development time observed by Hamai et al. (1971). Hamai et al. collected walleye pollock adults near Hokkaido, Japan, and reared the embryos from these fish at three different temperature ranges: 7.8°-14.5°C, 5.1°-10.6°C, and 0.0°-6.7°C. Only the temperature range 5.1°-10.6°C ($\bar{x} = 6.6$°C) and stage at completion of epiboly (our late Stage IV) were comparable with our data. We determined time to completion of epiboly over the temperature range 5.1°-10.6°C from their figure 3 (100 h). In our experiment, predicted development time to completion of epiboly at 6.6°C was similar (92 h) to time for completion of epiboly observed by Hamai et al.

The only other study on embryonic development of walleye pollock embryos comparable with ours is

Yusa (1954). Yusa described the development of walleye pollock embryos incubated at 6°-7°C. We classified Yusa's developmental data according to the stages of our Table 1 and calculated age (in hours) at 6.5°C to the midpoints of Stages II-VII. Rates of development of walleye pollock embryos were similar (Table 6) for both studies.

Our study was not designed to determine hatching time of individual walleye pollock embryos. Although we recorded the presence of larvae in culture vessels, we did not monitor distribution of hatching times.

A preliminary estimate of hours to hatching, however, can be derived using the midpoint age of the observed hatching interval, y, and the empirically derived Equation (1):

$$\ln \hat{y} = \frac{1}{a + bT} \qquad (2)$$

where least square estimates of a and b are $\hat{a} = 0.15012$, $\hat{b} = 0.00431$, and T = water temperature (°C). The estimated hours to midpoint of hatching using Equation (2) are similar to the observed midpoint ages at hatching (Table 7).

Conclusions

In general, walleye pollock embryos developed more rapidly at higher temperatures, as indicated by shorter time intervals between stages at higher temperatures (Fig. 1). Rates of embryonic development at the temperatures used in our study were significantly different from each other; however, the rates were similarly related to temperature regardless of stage of development (Fig. 3).

The age (in hours from fertilization) of a walleye pollock embryo at any stage of development (Table 1) can be estimated from the mean incubation temperature by 1) determining the temperature-dependent coefficients in Table 5 and then 2) solving the generalized predictive Equation (1). Together, these equations describe the relationship between age, stage of development, and temperature for walleye pollock at easily identifiable stages (Table 1) for temperatures within the range 2°-11°C. Simulated temperature fluctuations had no measurable effect on the accuracy of the generalized predictive equation; therefore, an estimate of the age of an embryo based on mean temperature apparently has the same reliability as an estimate based on a constant temperature. Although not as accurate as the equations, the contour plot (Fig. 3) can also be used to approximate the age (time from fertilization, in hours) of an embryo given a mean incubation temperature and stage of embryonic development. At 6.5°C, rates of development of walleye pollock embryos from Alaskan and Japanese waters are similar.

TABLE 6.—Age (time from fertilization, in hours) of walleye pollock embryos at 6.5°C to midpoints of development for Stages II-VII. Data from Yusa (1954) and this study.

	Age (h) at midpoint of stage					
Source	II	III	IV	V	VI	VII
Yusa 1954	7	20	60	133	213	267
This study	8	32	73	126	193	268
Difference (h)	−1	−12	−13	+7	+20	−1

TABLE 7.—Predicted and observed hours to hatching of walleye pollock embryos at temperatures of 2°, 5°, 6°, 8°, and 11°C.

	2°	5°	6°	8°	11°
Predicted hours	544	338	294	225	158
Observed hours	555	333	285	232	158
Hours difference	+11	−5	−9	+7	0

Acknowledgment

We thank Ken Krieger, Auke Bay Biological Laboratory, Auke Bay, Alaska, for constructing and maintaining the laboratory facilities used in this study.

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