A Response-Surface Approach to the Combined Effects of Temperature and Salinity on the Larval Development of *Adula californiensis* (Pelecypoda:Mytilidae). II. Long-Term Larval Survival and Growth in Relation to Respiration

R. G. Lough and J. J. Gonor

School of Oceanography and Marine Science Center, Oregon State University; Corvallis, Oregon, USA

Abstract

Larvae of the bivalve mollusc *Adula californiensis* (Phillippi, 1847) were reared for 3 days, from fertilization to veliger stage, at optimum conditions (15 °C, 32.2 ‰ S), and then transferred to experimental temperatures and salinities for 22 more days to determine the effects of these factors on survival and growth. For larvae surviving to 25 days, maximum survival was estimated, by response-surface techniques, to occur at temperatures below 10 °C and at salinities above 25 ‰. A comparison of 60 % survival response contours for 3, 15 and 25-day old larvae indicated a progressive shift in temperature and salinity tolerance with age of larvae. The older larvae became more tolerant to reduced salinity, but less tolerant to high temperatures. Growth of the larvae over 25 days of culture was slight, and relatively independent of temperature and salinity conditions found in the environment. Oxygen consumption of 3-day old veliger larvae measured at various combinations of temperature and salinity generally increased from 7° to 18 °C, and then sharply decreased from 18° to 21 °C. A plateau of oxygen consumption from 9° to 15 °C at 32.9 ‰ S indicated that the larvae are adapted to oceanic rather than estuarine conditions. A comparison of 25-day larval survival, mean length, and growth, with oxygen consumption of 3-day old veliger larvae indicated that high temperatures (15 °C, and above) coupled with reduced salinities (26.1 ‰, and below) were unfavorable for prolonged larval life. Because of the lack of larval adaptations to estuarine conditions, larva survival and, hence, successful recruitment of this species within Yaquina Bay (Oregon, USA) depends upon the essentially oceanic conditions found only during the summer in the lower part of the Bay.

Introduction

When soft rock suitable for boring is present, adult populations of the mytilid bivalve *Adula californiensis* (Phillippi, 1847) are found under a wide variety of environmental conditions both inside and outside the Yaquina Bay estuary on the Oregon coast (USA). Both marine and estuarine populations are reproductively active. We have attempted to determine if this essentially marine species has larval adaptations to estuarine conditions, or if recruitment to the estuarine population is dependent on a tidally transported supply of late larvae originating from adult populations outside the estuary. Larval adaptations to estuarine conditions have been investigated by means of laboratory studies on the effect of temperature and salinity on larval developmental rate, survival, and growth to 15 days (Lough and Gonor, 1971, 1973). This paper reports on the effect of temperature and salinity on survival and growth of larvae to 25 days, and on the relation between larval survival and the effects of temperature and salinity on respiration.

Early studies on the respiration of molluscan larvae by Spark (1929, 1936) and Thorson (1936) were concerned with the role of temperature in metabolic adaptation. The first comprehensive study of respiration in pelagic molluscan larvae was made by Zeuthen (1947), who used Cartesian diver techniques to measure respiratory rates at different activity levels of species representing several classes of molluscs. Recent investigators have attempted to relate larval metabolism to the ecology of the animal. The rate of oxygen consumption was used as an index of physiological condition for *Teredo bartschii* (Clapp) larvae by Lane *et al.* (1954). Jørgensen (1952) calculated high growth efficiencies based on oxygen uptake and growth rates for two different stages of *Mytilus edulis* and for veligers of two marine gastropods. Walne (1965) estimated the percent of food used in growth and respiration by *Ostrea edulis* larvae, and found that respiration studies only indicate about half or less of the total food requirement. Millar and Scott (1967) correlated oxygen consumption with the decrease in protein, carbohydrate, and lipid content of starved *O. edulis* larvae.

Both resistance adaptation and capacity adaptation of tropical and temperate-zone fiddler-crab (*Uca*) larvae have been studied by Vernberg and Vernberg (1964) and Vernberg and Costlow (1966). However, they were only concerned with the influence of one environmental factor, temperature, upon larval metabolism. Within an estuarine system, the interaction of several factors would seem to be important in modifying larval metabolism, and thereby be important in determining the distribution of the species. The only previous study concerning the interaction of several factors upon invertebrate larval metabolism appears to be that of Engel and Angelovic (1968) on the in-

fluence of temperature and salinity upon the respiration of brine shrimp nauplii.

Methods

The procedures used to obtain and rear larvae from adult *Adula californiensis* collected within the estuary, as well as the statistical procedures used to analyze the data, have been described previously (Lough and Gonor, 1971, 1973). After 3 days in culture, larvae settle to the bottom of the culture vessels and remain attached there unless disturbed. Settled larvae did not undergo morphological metamorphosis; larvae 25 days old continued to swim when agitated, had very high survival rates, and continued to feed. Because we were unable to determine if these settled larvae represented a free-living juvenile phase normally occurring in nature, we have termed them late larvae in this paper rather than juveniles.

Survival and Growth of Late Larvae

An experiment was designed to determine if the later larval stages of *Adula californiensis* would be able to better tolerate a range of conditions once they had passed through early development under optimal conditions. Larvae were cultured from fertilization in 200 ml of 32.2‰ S seawater at 15 °C, at a concentration of 100 larvae/ml. Equal volumes of eggs from 5 adults were pooled for the experiment. After 72 h development, aliquots of these larvae were transferred to one of 4 experimental salinities, and distributed among 4 different temperatures.

Salinities of 13.3, 19.9, 25.9 and 32.7‰ and temperatures of 7°, 11°, 15° and 20 °C were used to produce 16 different environmental combinations. All cultures were grown in duplicate. After the third day of development, the cultures were fed algal concentrations varying between 980 to 1,620 cells/ml seawater in the culture flasks. Before each culture was transferred to the experimental conditions, and after 22 days in the experimental temperature-salinity conditions, a 5 ml sample was removed and preserved for counting and measuring.

The percentage of larval survival at 25 days was calculated for each temperature-salinity combination, using the corresponding 3-day count as 100% survival. The percent survival for the duplicate cultures was averaged. The growth increment was calculated by subtracting the mean length of 45 three-day old larvae grown at 33.2‰ S and 15 °C from the mean length of 15 larvae from each experimental condition.

Larval Respiration

The possibility that the effects of temperature and salinity on survival and growth might also be reflected in respiration of the early veliger larvae was investi-

gated. An experiment on respiratory rate was designed to match the 25-day survival-growth experiment. The experiment was of a 4 × 6 factorial design, employing 4 salinity levels of 20% decreasing dilution and 6 temperature levels from 7° to 20 °C.

Larvae to be used for respiratory studies were cultured to the veliger stage in densities suitable for normal development, using 33.0 ± 1.0‰ filtered seawater at a temperature of 15 °C. The larvae were derived from eggs pooled from at least 5 adult individuals. After 72 h development, the veligers were concentrated with a nylon screen and transferred to flasks of seawater at one of the 4 experimental salinities of 13.0, 19.8, 26.2 or 32.9‰. The maximum range for each salinity was ±0.5‰.

A Gilson differential respirometer with a shaking rate of 80 cycles/min was used for respiration determinations. Three or 4 ml of each larval suspension were transferred to a respirometry reaction flask containing 0.5 ml of 10% KOH in a side arm. After a 15 min equilibration period, respiration values were taken every hour for 5 h at one of the experimental temperatures of 7°, 9°, 12°, 15°, 18° or 21 °C. Each run consisted of 3 flasks of larval suspension of each experimental salinity at one of the experimental temperatures. Only one run was made at each temperature. All larvae were checked for viability before and after each run.

At the end of each respiration run, the larvae were preserved with formalin and later washed with distilled water to remove seawater. They were then dried at 68 °C to constant weight, and weighed on an analytical balance to the nearest 0.01 mg. An experimental weight error of ±22%, probably due to sea-water salt residue, was determined by weighing three samples of a known number of larvae. The results are expressed as µl oxygen consumed/h/10,000 veligers or 1.23 mg dry weight of larvae. All oxygen-consumption values were corrected to standard temperature and pressure, and the means of the replicates were determined for each temperature-salinity combination.

Results

Survival of Larvae after 22 Days at Different Temperatures and Salinities

Survival data for 25-day old *Adula californiensis* larvae after 22 days at experimental temperatures and salinities are presented in Table 1. Between the 12th and 14th day of the experiment, all the larvae died in 13.3‰ S at all temperatures and at 20 °C in all salinities. At 7°, 11° and 15 °C, survival decreased with decreasing salinities, but at a greater rate at the higher temperatures. There was little difference in percent survival except for larvae in salinities of 25.9 and 19.9‰ at 15 °C. There was approximately an 8% mortality (i.e., 92% survival) at optimum conditions (32.7‰ S, 11° and 15 °C) over the 22-day period.

Table 1. *Adula californiensis. Larval survival after 22 days in 16 temperature-salinity combinations. S, S² and T, T²: Linear and quadratic effects of salinity and temperature, respectively; DF: degrees of freedom*

Salinity (‰)	Percent surviving			
	7 °C	11 °C	15 °C	20 °C
32.7	92.7	93.6	92.3	0
25.9	96.7	86.8	5.0	0
19.9	86.4	79.7	24.8	0
13.3	0	0	0	0

Statistical analysis of survival

Regression step no.	Variable	R-square	t value (10 DF)	Significance level
1	T^2	0.405	0.77	Not significant
2	S	0.696	2.00	10%
3	$T \times S$	0.750	1.83	10%
4	S^2	0.772	1.01	Not significant
5	T	0.789	0.90	Not significant

Fig. 1. *Adula californiensis.* Response-surface estimation of percent survival of larvae after 22 days of development at 16 different temperature and salinity combinations

Statistical analysis of the survival data produced a polynomial equation explaining 78.9% of the variation:

$$Y_{\%\,survival} = -173.89 + 14.61\,(S) + 9.03\,(T) - 0.33\,(T \times S) - 0.15\,(S^2) - 0.26\,(T^2).$$

However, when computed together, none of the variables were shown to be highly significant (Table 1). The linear effect of salinity and the interaction effects of temperature and salinity were both only significant at the 10% level. The greater portion of the survival data (69.6%) could best be explained by the quadratic effects of temperature and the linear effects of salinity; both at the 1% level:

$$Y_{\%\,survival} = 7.87 + 3.26\,(S) - 0.21\,(T^2).$$

A response surface was estimated for 22-day larval survival (Fig. 1). Maximum survival of larvae reared to 25 days is predicted at 25 to 40‰ S and 0° to 10 °C. No survival is predicted above 20 °C or below 12‰ S.

The survival results at 22 days for larvae first reared for 72 h at optimal conditions and then transferred to the experimental conditions differed from those obtained at 15 days for larvae initially reared at the experimental conditions (Lough and Gonor, 1973). The significance of the differences between the fitted equations of the effect of temperature and salinity on survival at Days 15 and Days 22 in the two experiments was tested by the method given by Ostle (1963, p. 205). A compar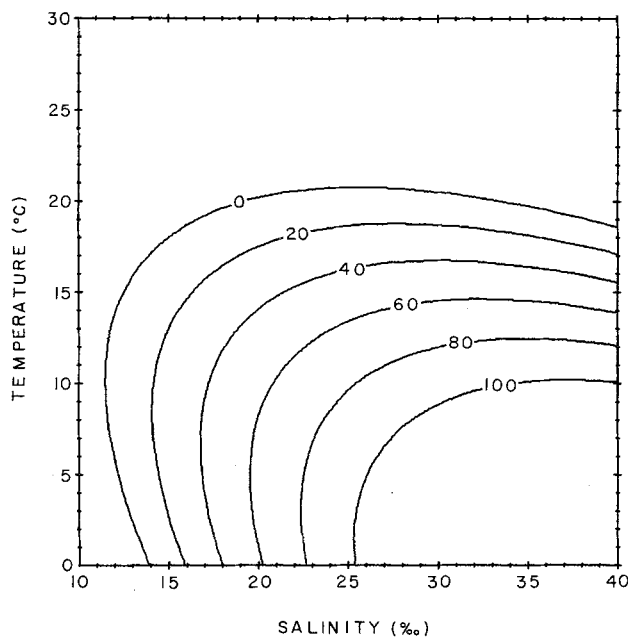ison of the polynomial equations by means of an analysis of variance is given in Table 2. This analysis indicates that the differences between response surfaces fitted to these two equations are highly statistically significant. Despite the early rearing at optimal conditions, increasing mortality effects of non-optimal conditions continue to become evident as late as Day 25 of rearing.

Table 2. *Adula californiensis. Comparison, by analysis of variance, of the polynomial equations for 15 and 22-day survival. Hypothesis: no significant difference between 15 and 22-day survival polynomials. DF: Degrees of freedom; SSE: sum of squares error; MSE: mean square error*

Source	DF	SSE	MSE	F
Polynomial 1: 15-day survival	10	3119.85		
Polynomial 2: 22-day survival	10	6366.04		
Total: Polynomial 1 & 2	20	9485.89	474.29	
Polynomial 3: Combined 15 and 22-day survival	26	20829.35		
Difference: Between polynomial 3 and total	6	11343.46	1890.46	3.99[a]

[a] Significant at 1% level. $F_{(6,20,.99)} = 3.87$.

Growth of Larvae during 22 Days under Experimental Conditions

Mean length and growth increments of the veliger larvae after 22 days of rearing at experimental temperatures and salinities are given in Table 3. In general, there were only small differences in mean length and growth increments between all the temperature-salinity combinations where there was survival. At 15 °C and in salinities of 25.9 and 19.9 $^o/_{00}$, there was a greater mean length and growth increment than in the other experimental combinations. However, there was a greater range in size of individuals at the reduced salinities.

Statistical analysis of the 22-day mean-length data produced an equation explaining 76.4% of the variation:

$$Y_{\text{mean length}} = 35.82 + 7.43\ (S) - 2.21\ (T) - 0.12\ (T \times S) - 0.12\ (S^2) + 0.29\ (T^2) .$$

Table 3. *Adula californiensis. Size and growth increments for larvae surviving after 22 days. Abbreviations as in Table 1*

Mean length (μm) ± 1 standard deviation

Salinity (‰)	Temperature		
	7 °C	11 °C	15 °C
32.7	121.1 ± 3.4	120.5 ± 3.0	118.9 ± 3.5
25.9	121.7 ± 3.6	121.1 ± 3.4	137.2 ± 11.0
19.9	120.3 ± 4.7	119.2 ± 4.4	129.9 ± 13.3

Growth increment (μm)

	7 °C	11 °C	15 °C
32.7	10.9	10.3	8.7
25.9	11.5	10.9	27.0
19.9	10.1	9.0	19.7

Statistical analysis of growth increments

Regression step no.	Variable	R-square	t value (3 DF)	Significance level
1	T^2	0.327	1.33	Not significant
2	$T \times S$	0.458	1.26	Not significant
3	S	0.590	1.62	Not significant
4	S^2	0.751	1.43	Not significant
5	T	0.764	0.41	Not significant

No single variable was found to be highly significant (Table 3). The computer output of the variables, based on partial correlation coefficients, showed the quadratic effect of temperature to be the most important factor contributing to variation in the data.

A response surface was estimated for mean length of larvae surviving after 25 days of experimental conditions (Fig. 2). The skewed contours indicate a tem-
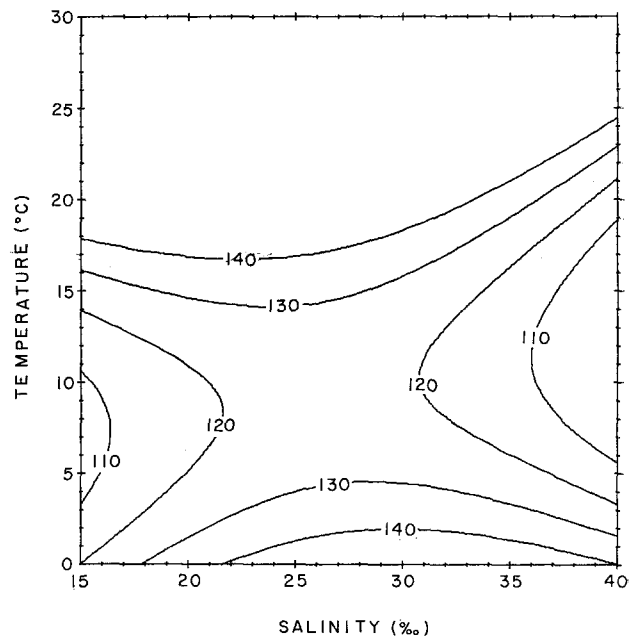

Fig. 2. *Adula californiensis*. Response-surface estimation of mean length (μm) of larvae after 22 days of development at 16 different temperature and salinity combinations
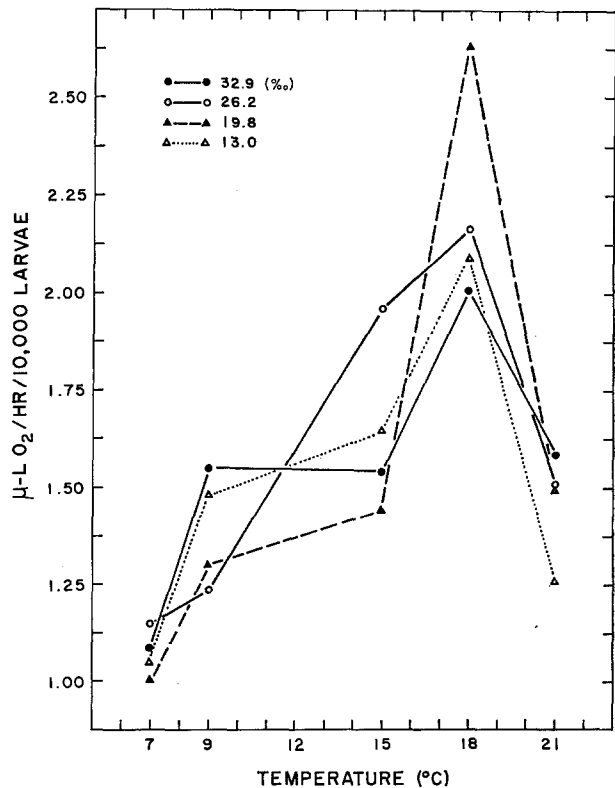

Fig. 3. *Adula californiensis*. Respiration of 72-h larvae at 24 different combinations of temperature and salinity. Respiration values are expressed as μl oxygen consumed/h/10,000 veliger larvae

perature-salinity interaction. The center of optimum mean length lies at about 10 °C and 26‰ S. The response-surface estimation of mean length cannot be considered entirely reliable, as only small differences in length were found in most of the temperature-salinity combinations.

Effects of Temperature and Salinity on Oxygen Consumption of 72-h Veliger Larvae

Data and graphs for oxygen consumption of the 72-h veliger larvae under experimental temperature-salinity combinations are presented in Table 4 and Figs. 3 and 4. The 12 °C oxygen-consumption values were not represented in the graphs and statistical analysis, because only one reading was taken after the first hour. In general, oxygen consumption increased with increasing temperatures from 7° to 18 °C, and decreased from 18° to 21 °C at all salinities (Fig. 3). At

32.9‰ S, however, there was little change in oxygen consumption between 9° and 15 °C. Q_{10} values for these data are given in Table 4.

The relationship of larval respiration to salinity is not clear. In 26.2 and 19.8‰ S at temperatures of 7°, 15°, and 18 °C, there was an increase in oxygen consumption above that at 32.9‰ S. The greatest increase occurred at 15 °C (24.7%) and the least increase at 7 °C (6.5%), in 26.2‰ S. In the lowest salinity of 13.0‰, the oxygen consumption was below that at 32.9‰ and 7° or 15 °C; however, a 31.7% increase occurred at 18 °C. At 9° and 21 °C, there was a general decrease in oxygen consumption with decreasing salinities.

Regression coefficients were determined for the linear and quadratic effects of temperature (T, T^2), the linear and quadratic effects of salinity (S, S^2), the interacting effects of temperature and salinity ($T \times S$), the linear and quadratic effects of oxygen consumption

Table 4. *Adula californiensis*. Respiration of 72-h larvae under 24 temperature-salinity combinations. Each mean is followed by standard error and number of determinations in parenthesis. t, t^2: linear and quadratic effects of oxygen consumption by time; other abbreviations as in Table 1

Temperature	Salinity			
	13.0‰	19.8‰	26.2‰	32.9‰
7 °C	1.01 ± 0.09 (15)	1.10 ± 0.10 (15)	1.15 ± 0.10 (15)	1.08 ± 0.11 (15)
9 °C	1.30 ± 0.05 (10)	1.43 ± 0.10 (15)	1.24 ± 0.06 (15)	1.55 ± 0.06 (15)
12 °C	1.38 ± 0.20 (3)	2.02 ± 0.67 (3)	1.27 ± 0.02 (3)	1.58 ± 0.18 (3)
15 °C	1.44 ± 0.15 (15)	1.65 ± 0.16 (15)	1.92 ± 0.15 (15)	1.54 ± 0.13 (15)
18 °C	2.63 ± 0.33 (15)	2.09 ± 0.26 (10)	2.17 ± 0.24 (10)	2.01 ± 0.19 (10)
21 °C	1.50 ± 0.18 (15)	1.26 ± 0.16 (15)	1.51 ± 0.17 (10)	1.58 ± 0.14 (15)

Q_{10} values of respiration for 72-h larvae

	13.0‰	19.8‰	26.2‰	32.9‰
7°— 9 °C	3.15	3.71	1.46	6.10
9°—15 °C	1.38	1.27	2.08	+
15°—18 °C	7.98	2.20	1.51	2.43
18°—21 °C	+	+	+	+

Statistical analysis of respiration for 72-h larvae

Regression step no.	Variable	R-square	t value (9 DF)	Significance level
1	T	0.045	4.86	1%
2	T^2	0.277	5.70	1%
3	S^2	0.282	0.47	Not significant
4	$T \times S$	0.286	0.62	Not significant
5	$T \times t$	0.289	3.07	2%
6	t	0.354	1.52	Not significant
7	t^2	0.356	0.43	Not significant
8	$S \times t$	0.356	0.22	Not significant
9	S	0.356	0.08	Not significant

+: Oxygen consumption decreased.

by time (t, t^2), the effects of temperature by time $(T \times t)$, and the effects of salinity by time $(S \times t)$.

The polynomial expression produced for larval oxygen consumption explained only 35.6% of the data variation:

$$Y_{oxygen\ consumption} = -0.7212 - 0.0054\ (S) + 0.4920\ (T)$$
$$-0.4342\ (t) - 0.001\ (T \times S) - 0.0013\ (S \times t)$$
$$+0.251\ (T \times t) + 0.0158\ (t^2).$$

The linear and quadratic effects of temperature at the 1% level were the most significant factors affecting oxygen consumption of the larvae (Table 4). Simple correlation coefficients used by the computer to order the variables in importance for additive regression verified the importance of these factors. Temperature by time $(T \times t)$ was significant at the 2% level. All other variables contributed little to explaining variation in the data.

Response surfaces were generated for laval oxygen consumption based on 20 temperature and salinity combinations. Only the 1st and 5th hour readings are shown, since there were only minor differences in the intervening readings (Fig. 4A, B).

The 1st hour response surface (Fig. 4A) shows a ridge of high oxygen consumption (2.0 to 2.5 µl/h) through the salinity range, between temperatures of about 10° and 18 °C. Oxygen consumption on either side of the ridge decreases rapidly. Above 33‰ S, a predicted ellipse of maximum oxygen consumption (2.5 µl/h) lies between temperatures of 9.5° and 16.5 °C.

The oxygen-consumption contours are approximately the same at the end of the 5th hour reading (Fig. 4B), except that the maximum consumption (2.5 µl/h) now includes salinities greater than 26‰ at temperatures between 8° and 16.5 °C.

Discussion

Effects of Temperature and Salinity on Larval Survival

A significant difference was demonstrated between 15 and 22-day predicted survival. This appears to result from progressive changes in tolerance during development. To facilitate comparisons between larvae 3, 15 and 25 days old, for each of these ages only the predicted 60% survival contours are indicated in Fig. 5. A progressive reduction in tolerance to higher temperatures and an increase in tolerance to reduced salinity is apparent. After the first 3 days, 60% survival is predicted to occur near 23 °C at salinities of 25 to 30‰, while after 15 days no survival is predicted under these conditions (Lough and Gonor, 1973). At 25 days, survival falls off rapidly with increasing temperatures at all salinities, reaching 0% at 20 °C. At temperatures below 15 °C, 60% survival is predicted at salinities where earlier survival was less than 40% of maximum.

The increasing tolerance to a wider range of salinity from early to late larval stages while temperature tolerance decreases is consistent with the ability of adult populations to live in places in the estuary where
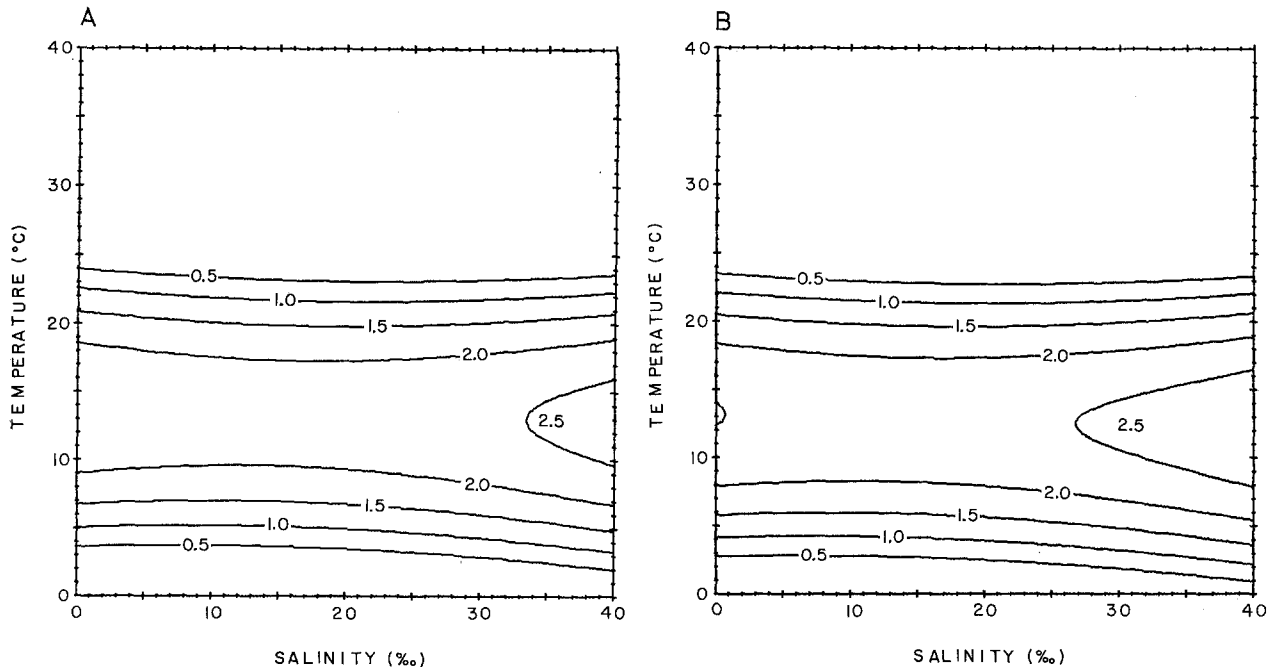

Fig. 4. *Adula californiensis*. Response-surface estimation of (A) 1st hour, (B) 5th hour respiration values of larvae at 24 different combinations of temperature and salinity. Respiration values are expressed as µl oxygen consumed/h/10,000 veliger larvae
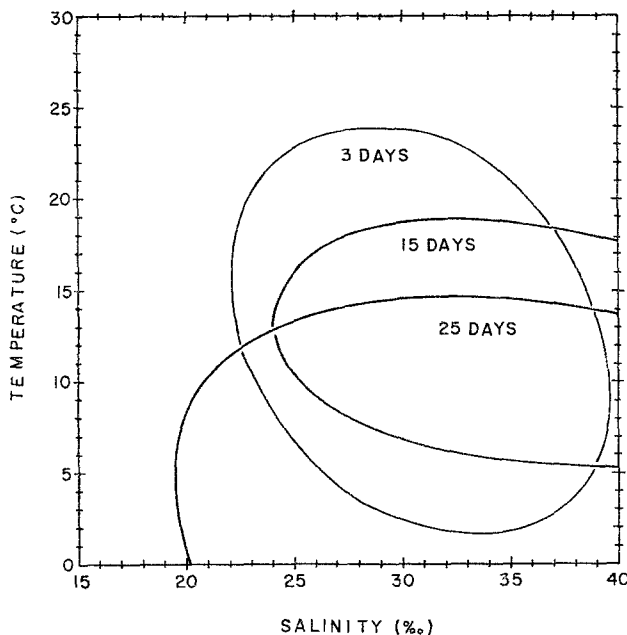
Fig. 5. *Adula californiensis*. Comparison of 60 % survival contours for 3, 15, and 25-day old larvae reared at 16 different temperature and salinity combinations

salinity varies seasonally over a great range, but where temperatures do not vary much beyond the oceanic range (Lough and Gonor, 1971, 1973).

Effects of Temperature and Salinity on Larval Growth

Adula californiensis larvae reared for 22 days in the various experimental conditions showed only small differences in mean lengths; the same result was obtained for 3 and 15-day old larvae (Lough and Gonor, 1973). The larvae did not grow significantly during the course of the experiment. Most bivalve larvae have been reported to at least double in size during the pelagic phase before metamorphosis, and this growth is affected by temperature, salinity, and food availability. Scheltema (1965) concluded that salinity affects larval growth less than any other factor in the environment, while the species and concentration of food usually affect growth more than any other factor. *A. californiensis* larvae were observed to have ingested *Isochrysis galbana* at all temperature and salinity combinations where the larvae developed normally past the veliger stage. There were no harmful effects noted with any of the concentrations of algae used in the experiments. The concentrations of *I. galbana* used were considered more than adequate, since food was never completely consumed before the next feeding. *A. californiensis* larvae are able to survive for a considerable period of time without feeding; more than 10 days at optimum conditions.

Minimal growth of *Adula californiensis* veliger larvae and the failure to metamorphose over 25 days of culture suggests two possibilities: (1) the culture conditions were inadequate, i.e., proper food was not provided for normal growth; or (2) culture conditions were adequate, and the larvae do not normally remain in the pelagic phase for a long time, but begin settling as soon as the veliger stage is reached. Perhaps the larvae delay any measurable growth until metamorphosis. The structural reorganization upon metamorphosis would allow them to filter and assimilate a greater quantity of food.

Effects of Temperature and Salinity on Larval Respiration

The density of larvae and their level of activity are two important biological factors that affect oxygen consumption. At high densities of larvae, oxygen consumption is depressed (Walne, 1966; Millar and Scott, 1967). The latter workers found that, as the density of *Ostrea edulis* larvae increased from about 25 to 250 larvae/ml, the oxygen consumption decreased from 5.4 to 0.2 μl O_2/h/1000 larvae, respectively. The 0.2 μl O_2/h/1000 larvae (= 2.0 μl O_2/h/10,000 larvae) at 250 larvae/ml is comparable to oxygen consumption values obtained for *Adula californiensis* larvae at 8 to 16 times this density of larvae. Because of this relatively low rate, it was necessary to use such high densities of *A. californiensis* larvae for the oxygen-consumption values to register on the respirometer. The oxygen-consumption rate did not appear to be greatly altered when the density of larvae was doubled from 16,000 to 30,000 larvae/ml in the reaction flasks.

The rate of oxygen consumption for 72-h veliger larvae at 15 °C and 32.9‰ S is 1.54 μl O_2/h/10,000 larvae or 0.00074 ml O_2/48 h/100 larvae. Full-salinity seawater at 15 °C and normal pressure has an oxygen content of 5.79 ml/l or 0.0579 ml/ml (Harvey, 1966). Assuming that there was no gaseous exchange with the atmosphere, the consumption data suggests that larvae reared under culture conditions of full-salinity seawater, 15 °C, and at densities of approximately 100 larvae/ml would only use about 14% of the available oxygen every 48 h, the time interval between water changes. The oxygen content of all the other experimental temperature and salinity combinations would also be above 5 ml/O_2/l. The oxygen content of the cultures can, therefore, be said not to be a limiting factor. Similarly, since the calculated oxygen content was not found to be limiting, values obtained in the respiration experiments would appear to be reasonable.

Oxygen consumption in poikilotherms generally increases with increasing temperature up to some critical level, and then decreases sharply (Kinne, 1964; Nicol, 1967). Oxygen consumption for *Adula californiensis* larvae increases from 7° to 18 °C and declines sharply between 18° to 21 °C. A high Q_{10} of 6.1 oc-

curred between 7° and 9 °C for *A. californiensis* larvae in 32.9‰ S. There was little change in oxygen consumption between 9° and 15 °C at oceanic salinity (32.9‰). A Q_{10} of 2.43 occurred between 15° and 18 °C at 32.9‰ S. From this information, it seems that the larvae have enzymatic systems which enable them to compensate their metabolic rate between 9° and 15 °C, but not at the extremes of the temperature range. The larvae are metabolically well adapted to oceanic rather than estuarine temperatures.

In the lower salinities, the interpretation of the Q_{10} values becomes difficult. Salinities beyond the tolerated range always result ultimately in reduced rates of metabolism (Kinne, 1964). Stenohaline species which suffer from osmotic damage whenever the salinity deviates significantly from the normal usually have a lower metabolism in both sub- and supra-normal salinities. In general, at lower salinities, *Adula californiensis* larvae do not appear to compensate their metabolism to any consistent degree at any temperature.
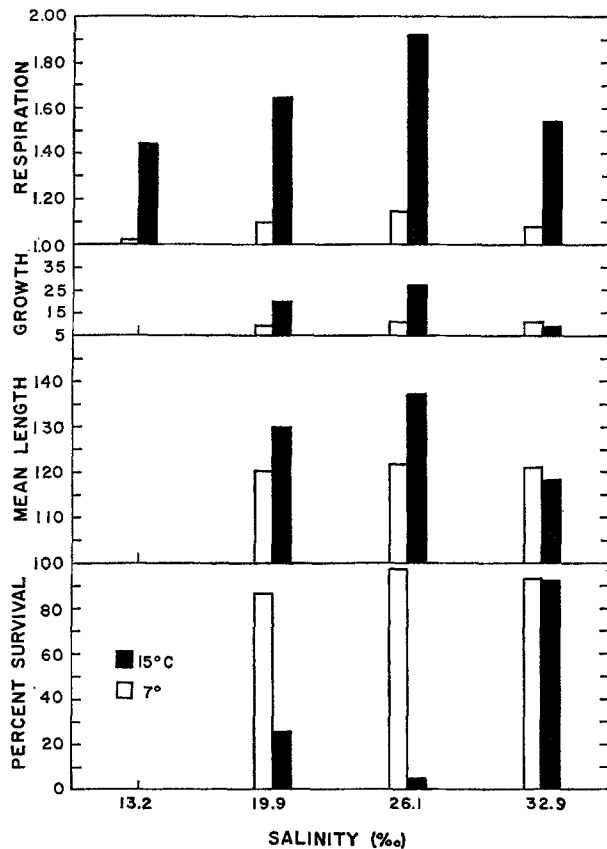

Fig. 6. *Adula californiensis*. Comparison of percent survival, mean length, and growth of larvae after 25 days of rearing, with respiration of 72-h veliger larvae. Mean lengths and growth units are given in microns, and respiration values are expressed as μl oxygen consumed/h/10,000 veliger larvae

Relationship of Respiration to Survival and Growth

Since temperature and salinity both affect metabolism, it is expected they would also affect larval survival and growth. A comparison of survival, mean length, growth, and respiration at 7° and 15 °C, in 4 experimental salinities, is made in Fig. 6.

The greater mean length and growth increments at 19.9 and 26.1‰ S correspond to the increased respiratory rates at the same salinities. Small differences in survival, mean length, and growth at 7 °C, 19.9 and 26.1‰ S are reflected in small differences of respiration values under the same conditions. At 15 °C, the relationship of survival to respiration is exactly the opposite. The decrease in survival at 15 °C and 26‰ S below that of 19.9 and 32.9‰ S is significantly greater than the experimental error. Both duplicate cultures showed this trend. The slight increase in survival at 7 °C, 26.1‰ S over 32.8‰ S cannot be considered highly significant. Where the respiratory rate dropped below that at 32.9‰ S (i.e., in 13.2‰ S) there was no survival at the end of 25 days.

The respiratory increase at 19.9 and 26.1‰ S may be attributed to the observed increase in swimming activity. Growth also increased slightly in diluted seawater, especially at the higher temperatures. Surprisingly, those conditions that resulted in the highest respiratory rate and greatest growth proved to have the highest mortality. The conditions of high temperature (15 °C, and greater) and reduced salinity (26.1‰, and below) probably represent a stress situation.

Ecological Implications of Results

The results of this study permit inferences concerning the survival, growth, and metabolic response of *Adula californiensis* larvae to temperature and salinity conditions found in Yaquina Bay. During the summer months along the Oregon coast, upwelled cold, high-salinity water is carried several miles into estuary entrances with the tidal influx. According to Burt and McAlister (1959), Yaquina Bay has a moderately large salinity difference between bottom and surface water during late winter to spring (type B or partly mixed estuary: Pritchard, 1955), but has small differences between bottom and surface during summer and fall (type D or well-mixed estuary). Bottom temperature and salinity curves for locations within the estuary show minimal variation around the entrance and maximal variation upstream (Lough and Gonor, 1973).

Spawning of *Adula californiensis* in Yaquina Bay has been observed from June through October. Mass spawning occurred on the incoming tide, after the adults had been exposed at low tide to heat and light from the sun.

Once spawning takes place and the eggs are fertilized, the developing larvae are either swept out to

sea or upstream. Studies by Burt and McAlister (1959) indicate that it is possible for materials introduced into Yaquina Bay at any point to be eventually distributed throughout the length of the estuary. Maximal survival for *Adula californiensis* during the first 3 days of development has been estimated by the surface-response method to be limited to temperatures ranging from 9° to 16 °C and salinities from 28 to 33‰ (Lough and Gonor, 1973). Those larvae swept out of the bay would be favored by the oceanic conditions prevailing outside. Within 2 to 4 tidal cycles, the larvae would have developed to the veliger stage (depending on water temperature) and be capable of feeding. These same larvae, under constant conditions of approximately 19‰ S, would not survive to 15 days. However, our experiments indicate that the majority of those larvae successfully reaching the veliger stage could tolerate constant salinity conditions as low as 19.9‰ up to 22 days in water of 7° to 11 °C. The effect of higher temperatures found upstream (15 °C and above), when combined with reduced salinity, would drastically increase mortality.

Larvae transported upstream during the months from June through October would probably be able to tolerate conditions found as far as 4.3 miles up the bay, where temperatures range from 12° to 18 °C and salinities from 25 to 32‰ during this period. The oceanic zooplankton population found in Yaquina Bay has been reported by Frolander (1964) to be limited in distribution to salinity conditions greater than 19.6‰. Salinities of 19.6‰ and greater correspond to the "life zone" area estimated for *Adula californiensis* larvae.

Older veliger larvae have enhanced survival and growth at a combination of low temperatures and a salinity of about 26‰, as indicated by the respiration results and correlated survival experiment. Perhaps by this time in development, the veligers have acquired the adult capacity to tolerate some dilution and, thereby, be somewhat adapted to estuarine conditions. Davis (1958) and Stickney (1964) have noted that the salinity tolerance of larvae of various bivalve species is determined in part by the salinity at which the parents were kept prior to spawning. For those larvae originating from bay-mouth populations, the low temperature and higher salinity of the bay entrance provide the most favorable environment for survival and growth. This does not take into consideration other factors such as suitable substrate, food, and predators.

Since there was little growth during 25 days of culture, and metamorphosis was not observed, perhaps the larvae normally do not have a long planktonic life but quickly settle out of the water column upon reaching the shelled stage, to assume a semi-pelagic life until metamorphosis. The very yolky eggs of *Adula californiensis* would place it in Thorson's classification as a species with a planktotrophic larvae but

a short pelagic life. For this larval type, feeding on the plankton is of secondary importance, while spreading of the larval stock is the main reproductive strategy for successful recruitment.

Many bivalve larvae are reported to be capable of searching for suitable substrate upon which to settle and metamorphose (Cole and Knight-Jones, 1949; Bayne, 1965; Walne, 1966; etc.). Experiments indicate that *Adula californiensis* larvae do not actively seek a suitable substrate prior to metamorphosis. The larvae may be able to crawl into small worm holes that permeate the surface of the mudstone, since young adults in the order of 2 to 3 mm in length were found burrowed into the surface to a depth of 3 or 4 cm. Only after metamorphosis would the larvae have some ability to select their location, as young adults of the small size mentioned above were observed to crawl rapidly with their foot when disturbed. Larger sized adults cannot crawl. This would enable the small juveniles to avoid direct competition with the adults.

Larvae of oyster species limited to estuarine conditions have the behavioral mechanism of vertical migrations with tide changes, which acts to retain the larvae within the estuary (Carriker, 1951; and other investigators). Since the larvae of *Adula californiensis* require essentially oceanic temperatures and salinities for optimal survival, it is doubtful that they have such a mechanism. The behavior experiment with vials of layered water of different salinity indicates that *A. californiensis* trochophores normally distribute themselves throughout a water column as long as salinities remain around oceanic (Lough and Gonor, 1971). In a water column of layered salinities, the larvae may be able to selectively determine their position. However, during the summer upwelling this behavior would not have much adaptive value, as the estuary is well-mixed. Preliminary studies of monthly gonad samples do not indicate a cessation in oogenesis at any time of the year, so it may be possible for spawning to occur during the winter months when the estuary is layered.

Most adult bivalves are able to withstand some salinity fluctuations by (1) closing their valves, (2) having tissues that are able to tolerate a high dilution. The larvae of *Adula californiensis* are highly sensitive to dilute salinity conditions in early development, but become progressively less sensitive later. If the larvae are able to spread and survive over an area as wide as the adults are found, then the larval form must either tolerate or avoid the extremes of salinity found at times in the adult habitat. Larvae completing early development to the more tolerant later stage near or outside the bay mouth may survive when brought into the estuarine portions of the bay by tidal flow, and settle where mudstone is available. It seems most likely that successful recruitment of *A. californiensis* within the bay would depend on extensive spawning during the

summer season, when there are optimum conditions for larval survival. Within the region of the bay with acceptable temperatures and salinities, the major limiting factor appears to be the availability of suitable substrate. Only two mudstone areas exist in the estuary, near the mouth and at Coquille Point, and both areas are heavily populated by the adults. Larvae settling out on the nearby tide flats without a suitable substrate would probably be preyed upon or buried under mud.

Summary

1. Larvae of *Adula californiensis* (Phillippi, 1847) were reared for a 3-day period (to veliger stage) at 15 °C, 32.2‰ S, and then transferred to 16 different combinations of temperature (7°, 11°, 15°, 20 °C) and salinity (13.3, 19.9, 25.9, 32.7‰) for 22 days, to determine the effects of these factors on late larval development.

2. After 22 days of rearing under experimental conditions, no larvae survived at 20 °C at any salinity nor at 13.3‰ S at any temperature. In general, survival decreased with decreasing salinity, but decreased at a greater rate at the higher temperatures.

3. Multiple-regression analysis showed the quadratic effect of temperature and the linear effect of salinity to be the more important factors affecting survival. A survival-response surface was estimated for larvae reared under the experimental conditions for 22 days. Maximum survival was estimated at 25 to 40‰ S and at temperatures below 10 °C.

4. A comparison of 60% survival contours for 3, 15, and 25-day old larvae indicated a progressive downward shift in temperature tolerance with increased age of larvae. The 25-day old larvae also showed an increase in tolerance to reduced salinity. It is postulated that the larvae slowly develop tolerances which enable the adults to survive and reproduce in the estuarine conditions of Yaquina Bay.

5. Larval growth was not significant over 25 days of rearing, and only small differences in mean lengths were measured for larvae reared under any of the experimental conditions. A multiple-regression analysis of mean length showed no single variable to be highly significant. Larval growth appears to be relatively independent of the normal range of temperatures and salinities found in the environment.

6. Oxygen consumption was measured for the 72-h veliger larvae at salinity and temperature combinations of 13.0, 19.8, 26.2, 32.9‰ and 7°, 9°, 12°, 15°, 18°, 21 °C. The respiratory rate generally increased from 7° to 18 °C, and then sharply decreased from 18° to 21 °C. A time-series analysis showed that the linear and quadratic effects of temperature and temperature by time were the more significant factors affecting oxygen consumption. The small change in oxygen consumption from 9° to 15 °C at 32.9‰ S is believed to indicate that the larvae are well adapted to the normal range of oceanic conditions found off the Oregon coast.

7. A comparison of 25-day old larval survival, mean length, growth, and 72-h oxygen consumption at 7° and 15 °C, in 4 experimental salinities indicated that conditions which resulted in the highest respiratory rate and greatest growth were associated with the highest mortality. High temperatures (15 °C, and above) and reduced salinity (26.1‰, and below) are believed to be abnormal stress conditions under which the larvae are unable to maintain themselves over long periods.

8. Since the early development of *A. californiensis* requires near oceanic temperature and salinity conditions, the successful recruitment of this species to the bay adult-populations requires the production of a large number of gametes during the summer season when there are optimum conditions for larval survival. The planktotrophic larvae are believed to have a short pelagic life before settlement. Within the Yaquina Bay region, where acceptable temperatures and salinities occur, the major limiting factor appears to be the availability of a suitable substrate.

Literature Cited

Bayne, B. L.: Growth and the delay of metamorphosis of larvae of *Mytilus edulis* (L.). Ophelia 2, 1—47 (1965).

Burt, W. V. and W. B. McAlister: Recent studies in the hydrography of Oregon estuaries. Res. Briefs Fish Commn Ore. 7, 14—27 (1959).

Carriker, M. R.: Ecological observations on the distribution of oyster larvae in New Jersey estuaries. Ecol. Monogr. 21, 19—38 (1951).

Cole, H. A. and E. W. Knight-Jones: The setting behavior of the European flat oyster, *Ostrea edulis* L., and its influence on methods of cultivation and spat collection. Fishery Invest., Lond. (Ser. 2) 17, 1—39 (1949).

Davis, H. C.: Survival and growth of clam and oyster larvae at different salinities. Biol. Bull. mar. biol. Lab., Woods Hole 114, 296—307 (1958).

Engel, D. W. and J. W. Angelovic: The influence of salinity and temperature upon the respiration of brine shrimp nauplii. Comp. Biochem. Physiol. 26, 749—752 (1968).

Frolander, H. F.: Biological and chemical features of tidal estuaries. J. Wat. Pollut. Control Fed. 36, 1037—1048 (1964).

Harvey, H. W.: The chemistry and fertility of sea waters, 224 pp. Cambridge: Cambridge University Press 1966.

Jørgensen, C. B.: Efficiency of growth in *Mytilus edulis* and two gastropod veligers. Nature, Lond. 170, p. 714 (1952).

Kinne, O.: The effects of temperature and salinity on marine and brackish water animals. II. Salinity and temperature-salinity combinations. Oceanogr. mar. Biol. A. Rev. 2, 281—339 (1964).

Lane, C. E., J. G. Tierney and R. E. Hennacy: The respiration of normal larvae of *Teredo bartschi* Clapp. Biol. Bull. mar. biol. Lab., Woods Hole 106, 323—327 (1954).

Lough, R. G. and J. J. Gonor: Early embryonic stages of *Adula californiensis* (Pelecypoda:Mytilidae) and the effect of temperature and salinity on developmental rate. Mar. Biol. 8, 118—125 (1971).

— — A response-surface approach to the combined effects of temperature and salinity on the larval development of *Adula californiensis* (Pelecypoda:Mytilidae). I. Survival and growth of three and fifteen-day old larvae. Mar. Biol. **22**, 241—250 (1973).

Millar, R. H. and J. M. Scott: The larvae of the oyster *Ostrea edulis* during starvation. J. mar. biol. Ass. U.K. **47**, 475—484 (1967).

Nicol, J. A. C.: The biology of marine animals, 2nd ed. 699 pp. London: Pitman & Sons 1967.

Ostle, B.: Statistics in research; basic concepts and techniques for research workers, 2nd ed. 585 pp. Ames: Iowa State University Press 1963.

Pritchard, D. W.: Estuarine circulation patterns. Proc. Am. Soc. civ. Engrs **81**, 1—11 (1955).

Scheltema, R. S.: The relationship of salinity to larval survival and development in *Nassarius obsoletus* (Gastropoda). Biol. Bull. mar. biol. Lab., Woods Hole **129**, 340—354 (1965).

Spark, R.: Studies on the biology of the oyster (*Ostrea edulis*). VII. Some observations on the influence of temperature on pelagic larvae and their uptake of oxygen. Rep. Dan. biol. Stn **35**, 103—105 (1929).

— On the relation between metabolism and temperature in some marine lamellibranchs, and its zoogeographic significance. Biol. Meddr **13**, 1—27 (1936).

Stickney, A. P.: Salinity, temperature and food requirements of soft-shell clam larvae in laboratory culture. Ecology **45**, 283—291 (1964).

Thorson, G.: The larval development, growth, and metabolism of Arctic marine bottom invertebrates compared with those of other seas. Meddr Grønland **100**, 1—55 (1936).

Vernberg, F. J. and J. D. Costlow, Jr.: Studies on the physiological variation between tropical and temperate zone fiddler crabs of the genus *Uca*. IV. Oxygen consumption of larvae and young crabs reared in the laboratory. Physiol. Zoöl. **39**, 36—52 (1966).

— and W. B. Vernberg: Metabolic adaptations of animals from different latitudes. Helgoländer wiss. Meeresunters. **9**, 466—587 (1964).

Walne, P. R.: Observations on the influence of food supply and temperature on the feeding and growth of the larvae of *Ostrea edulis* L. Fishery Invest., Lond. (Ser. 2) **24**, 1—45 (1965).

— Experiments in the large-scale culture of the larvae of *Ostrea edulis* L. Fishery Invest., Lond. (Ser. 2) **25**, 1—53 (1966).

Zeuthen, E.: Body size and metabolic rate in the animal kingdom with special regard to the marine micro-fauna. C.r. Trav. Lab. Carlsberg (Sér. chim.) **26**, 17—161 (1947).

First author's address: Mr. R. G. Lough
                    Oregon State University
                    Marine Science Center
                    Marine Science Drive
                    Newport, Oregon 97365
                    USA