EFFECTS OF TEMPERATURE AND SALINITY ON PRODUCTION AND HATCHING OF DORMANT EGGS OF *ACARTIA CALIFORNIENSIS* (COPEPODA) IN AN OREGON ESTUARY

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ABSTRACT

Experimental results indicate that induction of dormancy in *Acartia californiensis* eggs is temperature dependent and occurs below 15° C in two ways: 1) Cold adapted females spawn true resting eggs which exhibit major differences in hatching and survival rates from nondormant eggs under similar conditions. 2) Nondormant eggs spawned above 15° C may become dormant and have short-term viability at temperatures below 15° C. Salinity does not induce dormancy.

Hatching results of field-collected resting eggs at naturally occurring temperature-salinity combinations demonstrate that termination of dormancy is also primarily temperature dependent. Salinity, however, regulates rate and success of hatching. In addition, heavy naupliar mortality occurs following hatching at low salinities. Substantial hatching must occur in the field over much of the year. Since subsequent survival and population growth depends on the presence of favorable temperature and salinity conditions, nauplii which hatch during the low salinity winter and spring months in Yaquina Bay must be lost. This phenomenon is viewed as a "leaky" population diapause.

Resting eggs were also demonstrated for *Epilabidocera longipedata* and *Eurytemora affinis*, an occurrence previously undescribed in the literature.

Resting eggs have been known to be a common adaptation in freshwater zooplankton since the turn of the century (see reviews in Hutchinson 1967 and Elgmork 1967). The existence of a comparable resting egg phase in the life cycle of marine neritic species was postulated for many years to explain the seasonal disappearance of coastal species from the water column (e.g., Fish and Johnson 1937; Barlow 1955; Conover 1956). Preliminary evidence of marine calanoid resting eggs was first reported by Sazhina (1968) for the species *Pontella mediterranea* and *Centropages ponticus*.

Zillioux and Gonzalez (1972) conclusively demonstrated with laboratory and field evidence that the seasonal disappearance of *Acartia tonsa*, a common coastal species, coincides with the production of overwintering eggs as water temperatures fall below 14.5° C. Subsequent research has shown that egg dormancy is an important adaptation in many boreal and temperate neritic calanoids, including both summer-fall species (e.g., *Tortanus forcipatus*, Kasahara, Onbé, and Kamigaki 1975; *Labidocera aestiva*, Grice and Gibson 1975) and winter-spring species (e.g., *A. clausi*, Uye and Fleminger 1976; *C. abdominalis*, Pertzova 1974). Egg dormancy probably enables most coastal species to survive periods during which conditions in the water are unfavorable.

Environmental factors such as temperature or photoperiod usually govern the induction of dormancy in arthropods (Lees 1955). Both the adult and/or the egg may be responsive to adverse environmental changes. Egg dormancy may result from a physiological response of the female to a changing milieu which modifies the eggs. Conversely, dormancy may develop as a response of the egg to changes in conditions as it sinks through the water column into the bottom sediments. There is evidence for both mechanisms in marine copepods. Zillioux and Gonzalez (1972) demonstrated that the production of resting eggs by *A. tonsa* is a response of the female to low temperatures. However, Uye and Fleminger (1976) examined egg development of four *Acartia* species (including *A. tonsa*) from southern California waters at various temperature and salinity combinations and concluded that dormancy is primarily a response of the egg to the milieu. Their results led them to hypothesize that exposure of the eggs to abnormal salinities may be necessary to induce dormancy in at least two of the species of *Acartia*. Once buried in the sediments,

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egg dormancy is maintained by low oxygen levels (Kasahara, Onbé, and Kamigaki 1975; Uye and Fleminger 1976). Light is also required to break dormancy in at least one species, *A. clausi* (Landry 1975a; Uye and Fleminger 1976).

Further work is required to fully demonstrate the factors regulating dormancy in coastal calanoids. A localized summer-fall population of *A. californiensis* in Yaquina Bay, Oreg., affords an excellent opportunity to examine this phenomenon, since the entire winter-spring period is passed in the resting egg stage. A field research program provided data for the correlation of population dynamics with temperature and salinity. Laboratory experiments were carried out to determine the relative importance of temperature and salinity in the formation and subsequent hatching of dormant eggs of *A. californiensis*. Analysis of the data provides additional information on the role of the female versus that of the egg in development of dormancy.

Acartia californiensis, a newly described species (Trinast 1976), is useful for comparative studies in egg dormancy since it displays close affinities to *A. tonsa* in both physiological and morphological features. Earlier studies in Yaquina Bay (Zimmerman 1972; Frolander et al. 1973; Johnson and Miller 1974; Miller et al. 1977) identified the species as *A. tonsa* in the belief that it represented a smaller, ecophenotypic variant of the larger, offshore, *A. tonsa* present in the northerly Davidson Current during the winter months. Furthermore, the unidentified "*Acartia* sp. I" discussed by Uye and Fleminger (1976) has been recently identified as *A. californiensis* Trinast by A. Fleminger,[2] thus permitting comparison of egg dormancy in northern and southern populations.

METHODS

The seasonal population cycle of *A. californiensis* in Yaquina Bay was determined by the collection of plankton samples twice weekly at Stations 21, 29, 39, 45, and 57 (Figure 1). Sampling was done from June to November in 1972-74 with a Clark-Bumpus sampler (mouth diameter 12.5 cm) fitted with a 112 μm mesh net which quantita-

[2]Abraham Fleminger, Scripps Institution of Oceanography, University of California, La Jolla, CA 92037, pers. commun. June 1978.

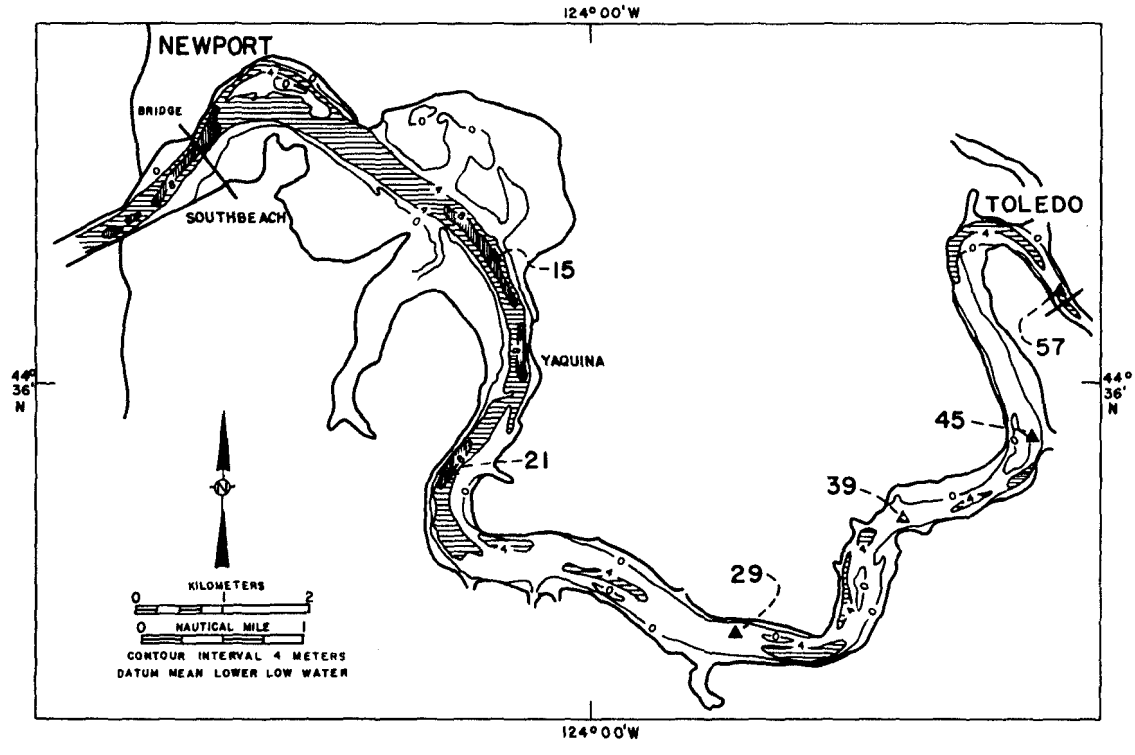

FIGURE 1.—Sampling stations for *Acartia californiensis* in Yaquina Bay estuary, Oreg.

tively retained all copepodite stages. Tows were oblique in a stepwise pattern from just above the bottom to near surface at midchannel. A calibrated propeller flowmeter in the mouth of the net was used to estimate the quantity of water filtered. Volumes filtered were typically 5-6 m^3. Temperature measurements and salinity samples were taken at the surface and just above the bottom at each station. Salinities were later determined by inductive salinometer in the laboratory.

RESTING EGG PRODUCTION

October Experiment

Adult *A. californiensis* were collected at Station 39 (Figure 1) on 9 October 1975, using a 239 μm mesh net towed slowly at 1-2 m depth. Surface temperature and salinity were 14.9° C and 26.8‰, respectively. Laboratory cultures were established the same day by sorting 50 female and 25 male adults into each of eight 1,400 ml beakers containing 1,200 ml of Millipore[3]-filtered water (25‰). Water temperature increased to room temperature (16.8° C±0.3°) during this time. Upon completion of sorting, all newly spawned eggs were removed by screening and discarded. Replicate cultures were then transferred to water baths and maintained at 21°, 17°, 13°, and 9° C (±0.1°). Continuous overhead lighting (low level) was used throughout the experiment.

Adults were fed daily with a mixture of *Pseudoisochrysis* sp., *Isochrysis galbana*, and *Thalassiosira fluviatilis* at a concentration of approximately 200,000-250,000 cells • ml^{-1} Phytoplankton cultures were maintained in log-phase at 16.8° C. Viability of the algal species over the temperature range of 9°-21° C was not determined but assumed to be unimportant in the experimental design since *A. californiensis* adults were provided excess food daily. In addition, adult mortality was moderately low (estimated at <20%) during the acclimation and spawning period with similar losses observed in all cultures. Thus, selection of adults in response to temperature or food during the acclimation period was not likely a factor in influencing the type of egg spawned.

After the third day of adult acclimation, accumulated eggs were removed by gentle screening. The eggs were used in a preliminary experiment on hatching success which differed from the main experiment in that fewer hatching temperatures were tested for eggs produced at each acclimation temperature.

The main experiment was established with eggs collected on the eighth day of adult acclimation. Maximum egg age ranged from 5 days at 9° C to 1 day at 21° C because of differences in egg development rate as a function of temperature. Eggs from replicate cultures at a given acclimation temperature were mixed together in a Petri dish for sorting by pipette. Water temperature was maintained as close as possible to acclimation temperature during sorting. Depending on the number of eggs available, 10-15 replicate batches of 50 eggs each were placed in small 6 ml Petri dishes (1 cm depth, 3.5 cm in diameter) containing 4-5 ml of Millipore-filtered water (25‰). Dish bottoms were marked in a grid for ease in counting at 25× with a dissecting scope. Salinity changes caused by evaporation were prevented by floating the small dishes in transparent, tightly capped 100 ml beakers filled with 80 ml of water (also 25‰). Two and usually three or more replicate dishes of 50 eggs were then placed in each of five water baths at 21°, 17°, 13°, 9°, and 5° C, respectively. This procedure was repeated for eggs produced at all four acclimation temperatures (21°, 17°, 13°, 9° C), resulting in an experimental design (Table 1) using over 2,500 eggs.

Hatching success was determined daily by making separate counts of eggs remaining and hatched nauplii. Nauplii were captured and removed daily with a pipette. Every 5th day, water was changed by pipette with minimum disturbance to the remaining eggs. Unhatched eggs were maintained at the experimental temperature well past the time expected for normal hatching (Table 2) and then transferred to a favorable temperature (21° C) to determine subsequent hatching success.

November Experiment

Animals collected on 4 November 1975 at Station 39 (12° C, 21‰) were used to repeat the experiment at the prevailing field temperature and salinity conditions. By this time, the field population of *A. californiensis* was greatly reduced and egg production was assumed to be primarily resting eggs. Females were kept at 12° C and 21‰ during transfer to the laboratory and subsequent culturing. Eggs were collected on the fourth day of

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

TABLE 1.—Production of resting eggs and summer eggs by female *Acartia californiensis* collected for the October experiment, 9 October 1975. Hatching results at each temperature-salinity combination expressed as a mean percentage for two and usually three replicates of 50 eggs each.

Cumulative time (days)	Event		Conditions																					
0	Collection		14.9° C, 26.8‰; Station 39, Yaquina Bay, Oreg.																					
0-1	Preliminary acclimation		14.9° C, 25‰; (temperature increasing to 16.8° C)																					
	Spawning:				21° C				17° C						13° C						9° C			
5-9	Main experiment				25‰				25‰						25‰						25‰			
	Adjusted time	Hatching temp (° C)	21°	17°	13°	9°	5°	21°	17°	13°	9°	5°	21°	17°	13°	9°	5°	21°	17°	13°	9°	5°		
9	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
10	1		92	84	70	5	3	98	93	39	8	2	12	7	1	1	0	30	1	0	0	0		
11	2		96	100	79	12	6	99	99	63	17	2	18	11	3	1	0	42	25	0	0	0		
12	3	Results in cumulative	97		89	23	8	99	100	66	25	4	42	15	4	1	0	53	27	2	0	0		
13	4	percentage hatched	97		90	26	8	99		67	28	5	65	21	4	1	0	65	29	2	0	0		
14	5		97		90	32	9	99		67	31	5	73	32	4	1	0	72	31	3	0	0		
17	8				90	34	9			71	34	5	91	54	4	1	0	82	47	3	0	0		
20	11				90	35	9			71	35	5	97	76	4	1	0	89	64	3	0	0		
24	15					35	9				35	5	97	92		1	0	89	79		0	0		
29	20						9					5	97	99			0	89	87			0		
129	120						9					5					0					0		
		Warming (21° C) on day			11	15	120			11	15	120			11	15	120			11	15	120		
		Subsequent hatch (%)			0	39	0			19	61	0			91	96	30			76	81	71		
		Final hatch (%)	97	100	90	74	9	99	100	90	96	5	97	99	95	97	30	89	87	79	81	71		

spawning at 12° C and sorted into replicate batches of 50 eggs each. Hatching rate and success were determined at 21°, 17°, 15°, 13°, 9°, and 5° C in 25‰ water. Other procedures were as described above.

TABLE 2.—Nondormant egg development time for *Acartia tonsa* and *A. clausi* as a function of temperature, and time that unhatched eggs of *A. californiensis* were initially held at the same experimental hatching temperature (October experiment).

	Egg duration (days)		Incubation time (days)	
			A. californiensis	
Temp (° C)	*A. tonsa*[1]	*A. clausi*[2]	21°-17° C[3]	13°-9° C
21	1.3	1.2	5	20
17	1.9	1.5	3	20
13	3.1	2.2	11	11
9	8.5	3.8	15	15
5	45.1		120	120

[1]Data based on prediction of Bělehrádek function (Zillioux and Gonzalez 1972).
[2]Data from experimental observations of Landry (1975b).
[3]Temperatures at which *A. californiensis* eggs were spawned.

HATCHING OF RESTING EGGS COLLECTED IN THE FIELD

A series of preliminary experiments (J. K. Johnson, unpubl. data) had demonstrated that resting eggs of *A. californiensis* and *A. clausi* occur in similar numbers in the surface sediments in the vicinity of Station 39 (Figure 1). Unfortunately, the resting eggs of the two species are not distinguishable from each other on the basis of diameter, shape, or color. Therefore, the following hatching experiments include resting eggs of both species at unknown ratios. Much information and insight were gained in spite of this serious experimental limitation.

Salinity Experiment

Resting eggs were obtained by collecting mud with an Eckman-Birge grab 500 m upstream of Station 39 on 7 February 1976 during low tide. The upper 1-2 cm of sediments were saved for later screening. Temperature and salinity values near the bottom were 9.8° C and 4.1‰. An accompanying series of plankton tows from Station 21 to Station 45 verified that no copepodite stages of *A. californiensis* were present in the upper estuary, as expected from earlier field work. *Acartia clausi* was present in low numbers only at Stations 21 and 29 ($<50 \cdot m^{-3}$). It does occur at Station 39 during January to April but only at extremely low densities (ca. $5\text{-}20 \cdot m^{-3}$) during periods of high tides (Zimmerman 1972). Thus, few recently spawned eggs were likely to be present in the sediments collected at Station 39.

Sediment samples were maintained at field temperature (10° C) during transport to the

laboratory and storage in the dark for 1 mo. Resting eggs were removed (10 March 1976) by first sieving the sediments through 119 μm and 64 μm mesh nylon screens. This size fraction was diluted with water (5‰), stirred well, and allowed to settle. Approximately 5-6 ml of the surface sediments were then slowly introduced by pipette onto the surface of 8-10 ml of a 2.8 M aqueous sucrose solution (suggested by Brewer 1964) in each of several 15 ml centrifuge tubes. Following centrifugation at 1,000-2,000 rpm for 1-2 min, resting eggs of *Acartia* spp. and other species (including calanoids, harpacticoids, and rotifers) were removed by pipette from the water-sucrose interface. These eggs were nearly free of detritus. A thorough rinse with Millipore-filtered water (5‰) on a 64 μm mesh screen removed the sucrose solution. No evidence of egg distortion or crushing was found to result from the high osmotic gradient. In contrast, eggs centrifuged from surface sediments collected in September collapsed under similar conditions.

Following rinsing, *Acartia* spp. eggs were sorted from extraneous material and then mixed in a Petri dish in 5‰ water. Approximately 2,000-2,500 eggs were collected in 3-4 h of work. Most eggs had a clear, outer layer which surrounded a dark inner mass, similar to that reported for resting eggs of *Labidocera aestiva* (Grice and Gibson 1975). A moderate number of eggs (ca. 10-20%), lacking the clear layer, had ended diapause and progressed to various stages of embryogenesis. These latter eggs were most likely undergoing development in the uppermost sediment layer when collected in February. Only those eggs with a clear outer layer were used for the experiment.

Thirty-five resting eggs were sorted by pipette into each of 43 small (6 ml) Petri dishes. The accompanying water at 5‰ (ca. 1 ml) was removed by pipette. Water of 11 salinities, ranging from 0‰ (glass distilled) to 23.5‰, was then added to batches of three or more of these dishes. Three complete rinses of appropriate salinity were added and removed before the final addition. The Petri dishes with eggs were maintained at 17° C (±0.1°) in covered beakers as described above. Continuous overhead lighting was provided. Water was replaced every 5th day.

Egg and naupliar counts were usually made daily. There were some 2-day intervals. Naupliar mortality between observations was also recorded. Salinity was increased in some replicates at various time intervals to determine viability of remaining dormant eggs. Hatched nauplii were captured and reared to copepodite stages (17° C, 20‰) for positive species identification because of uncertainty in distinguishing between *A. californiensis* and *A. clausi* nauplii.

Salinity and Temperature Experiment

The effect of salinity on hatching of resting eggs was later evaluated at three more temperatures (15°, 12.5°, 10° C) to obtain hatching rates at temperature-salinity combinations normally present in the upper estuary during later fall and early spring months. Resting eggs were obtained by 2.8 M sucrose centrifugation of sediments collected at Station 39 on 18 February 1978. Water conditions were 9.4° C and 7.6‰. Transport to the laboratory, storage (2 days), screening, centrifuging, and sorting were all done at 10° C. Two or three replicates of 35 resting eggs (clear-layer type) were prepared at 5‰, 10‰, 15‰, and 25‰ for each temperature. Other details were as described in the salinity experiment.

Additional beakers were established at all temperature-salinity combinations to determine the approximate ratio of *A. californiensis* to *A. clausi* resting eggs by rearing hatched nauplii to copepodites. These beakers contained the unsorted mixture of resting eggs of *Acartia* spp. and other accompanying species plus detritus which collected at the water-sucrose interface during centrifugation. The number of eggs in each beaker was variable, ranging between 50 and 250. Hatched nauplii were removed daily and transferred to new beakers at the next higher salinity (+5‰) and temperature (+2.5° C). Salinity and temperature were increased every 5th day to maxima of 15° C and 25‰. This was done to improve survival while avoiding abrupt change. Ratios were determined when most individuals had molted into the copepodite stages. Hatched nauplii from the Petri dishes were also reared when practical to the copepodite stages.

RESULTS

Field Population Cycle

The twice-weekly sampling program was adequate to follow the seasonal cycle and distribution of *A. californiensis* in upper Yaquina Bay. Only field data for 1972 are presented, since the population had similar cycles in 1973 and 1974.

The population range extended from Station 21 to Station 57 (Figure 1) during the months of maximum abundance (August, September). However, few adults or copepodites were ever present at these two boundary stations. The bulk of the population was restricted to the region from the vicinity of Station 29 to Station 45 with an approximate population center at Station 39. Location of the population center was not static at Station 39 because of the diurnal tides. It was occasionally found during sampling cruises downstream at Station 29 or upstream at Station 45.

The maximum density of *A. californiensis* observed on a given sampling day was considered to be the center of the population, regardless of location in the upper estuary. A plot of maximum density values for adults and copepodites I-V on successive sampling dates represents the seasonal cycle of the population (Figure 2). The persistent alternation between peak abundance of adults and copepodites is evidence that successive generations remain distinct. The fact that successive density estimates at the population center show such a refined feature creates confidence that the approach is valid.

Physical factors such as temperature and salinity can be correlated with population growth and decline on the premise that optimal conditions at a given time coincide with maximum spatial population abundance. Thus, given the population concentration from Station 29 to Station 45, mean temperature and salinity data (surface and bottom) at Stations 29 and 45 (Figure 3) can be considered to represent the lower and upper range of the most favorable physical conditions possible for growth of *A. californiensis* in the estuary at any given time. The means closely reflect the entire water column (4-6 m depth) as the upper estuary is well-mixed (Type D; Burt and McAlister 1959) during June to October with a maximum vertical gradient of 1°-2° C and 1-3‰. The fortnightly periodicity seen in the data is an effect of the progression of tidal stage upon twice-weekly sampling conducted during the fixed hours from 0900 to 1400. A sharp decline in salinity during late November and December (Figures 3, 4) occurred as a result of the beginning of the winter rains and resulting heavy runoff. Salinity in the upper estuary remained extremely low (Figure 4) until the following spring when rainfall and runoff decreased, and the salt wedge intruded up the bay.

The appearance in early June of *A. californiensis* copepodites occurred when water temperatures were between 15° and 18° C and salinities were 10-20‰ (Figure 3). Abundance gradually increased throughout June and much of July. A population explosion began in late July and continued throughout August, a period when water temperature and salinity were 17°-22° C and 20-31‰. The subsequent population crash in early

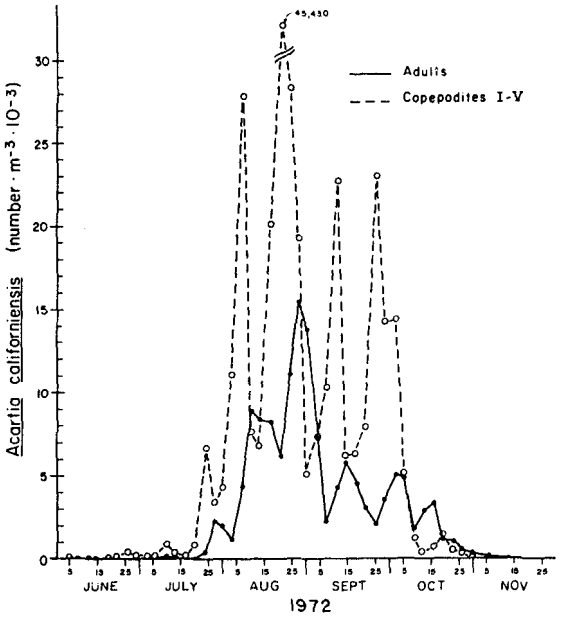

FIGURE 2.—Seasonal population cycle of *Acartia californiensis* in Yaquina Bay (1972) based on maximum abundance of adults and copepodites (I-V) observed on successive sampling days.

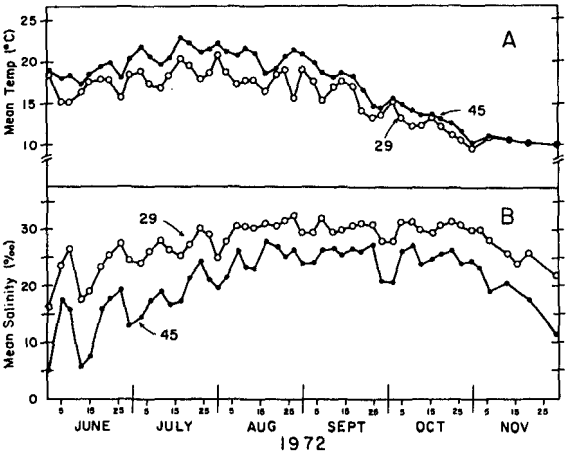

FIGURE 3.—Temperature (A) and salinity (B) profiles at Stations 29 and 45 during June to November 1972. Points represent means of surface and bottom values. Envelopes correspond to general range of most favorable physical conditions for *Acartia californiensis* population growth at a given time.

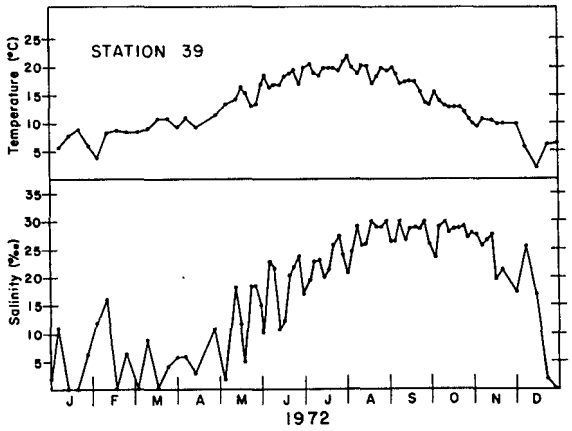

FIGURE 4.—Annual profile of bottom temperature (° C) and salinity (‰) at Station 39 in 1972. Values represent general range of temperature-salinity experienced by resting eggs in surface layer of sediments. December-May values from unpublished data of H. F. Frolander (School of Oceanography, Oregon State Univ., Corvallis, OR 97331).

September (19°-22° C, 25-30‰) was followed by a gradual decline to complete absence in late November. Production of nondormant eggs remained important throughout September, as evidenced by the large numbers of copepodites present in the water column. However, copepodite recruitment nearly ceased by the first week of October, indicating that most reproduction was likely in the form of resting eggs. Some nondormant eggs were still produced, however, since a small pulse of copepodites was seen in the last half of October. Mean temperature had dropped to 13°-15° C at the end of September when recruitment began to fail. The population was gone by December at a field temperature of 9°-10° C. Salinity remained high, relatively stable, and presumably in a favorable range (25-30‰) during the September-October decline and disappearance. Salinity began to drop only in November when *A. californiensis* was essentially absent from the water column.

Resting Egg Production

October Experiment (Preliminary)

Eggs collected on the third day of adult acclimation to 21°, 17°, 13°, or 9° C had essentially similar hatching rates and cumulative hatching success at a given temperature to eggs collected on the eighth day. The similarity in results indicates that *A. californiensis* can shift from production of nondormant eggs to production of resting eggs in only 1-2 days in response to a significant lowering of water temperature. The absence of significant changes in egg hatching time and viability with increasing adult acclimation time demonstrates that the eggs produced were not adversely affected by the rapid change in water temperature (2-4 h) at the beginning of the acclimation period. The only effect observed was an initially lower fecundity in those females which experienced the largest temperature changes (16.8° C to 21° C or 9° C). In these latter two cultures, fecundity increased with acclimation time.

October Experiment (Main)

Hatching successes of eggs spawned over the range of 9°-21° C give evidence that the type of egg spawned is a function of ambient temperature (Figures 5, 6). Experimental conditions and results are also summarized in Table 1. Females which spawned at 21° and 17° C (typical midsummer temperatures at Stations 29-45) produced nondormant summer eggs that developed normally at 21° and 17° C (Figure 5A, B). Development time was <36 h with nearly 100% of the eggs hatching. Lower hatching temperatures (13°, 9°, 5° C), however, were found to arrest development of summer eggs which then entered dormancy. The incidence of dormancy increased with decreasing hatching temperature: eggs spawned at 17° C had a total hatching success of 71% at 13° C compared with 35% at 9° C (Figure 5C, D) and 5% at 5° C (Figure 6). Thus dormancy in summer eggs is a response to low, unfavorable temperatures and may occur independently of parental influence.

More than adequate time (Table 2) was allowed in these experiments for "normal" hatching, given the prediction from a Bělehrádek function for *A. tonsa* (Zillioux and Gonzalez 1972) and the observed development rates for *A. clausi* (Landry 1975b). A subsequent increase in water temperature to 21° C broke the dormancy of the summer eggs previously incubated at 13° and 9° C (Figure 5C, D). Hatching resumed within a few hours at a rate similar to that observed earlier at 17° and 21° C (Figure 5A, B).

Mortality of summer eggs spawned at 17° C was low during the 11- and 15-day "holding" periods at 13° and 9° C (Figure 5C, D), evidenced by a final hatching success of 90-96% after increase to 21° C. However, dormant 21° C summer eggs experienced substantial mortality during the 15-day

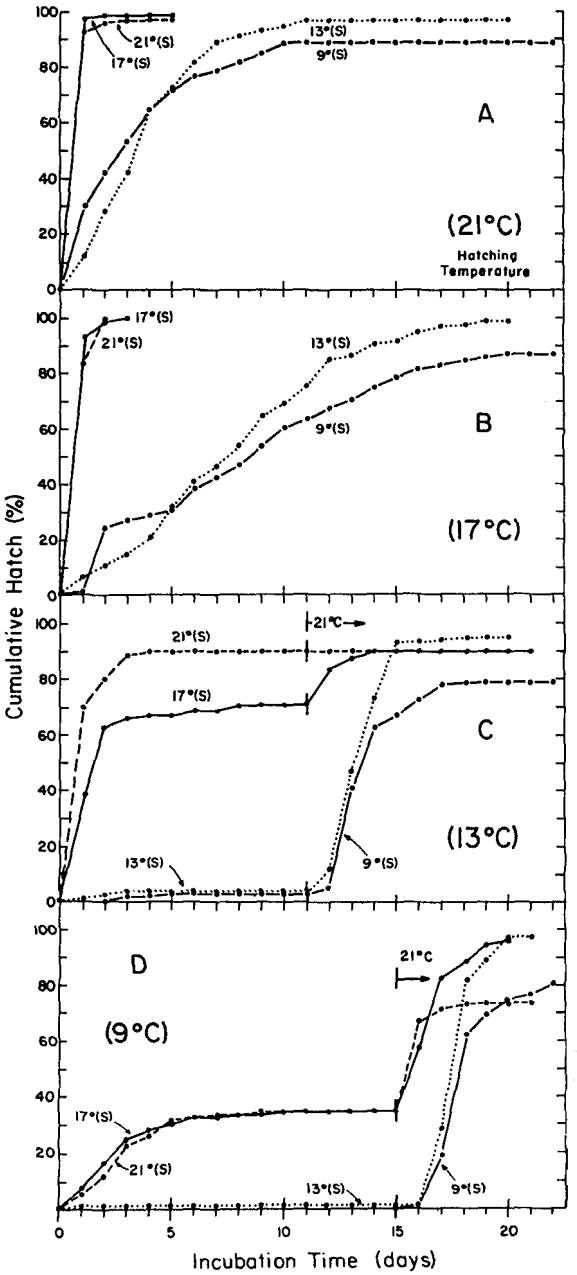

FIGURE 5.—Hatching success of *Acartia californiensis* eggs at: (A) 21° C, (B) 17° C, (C) 13° C, (D) 9° C in 25‰ salinity. Eggs spawned at different parental acclimation temperatures of 21°, 17°, 13°, and 9° C. Spawning temperatures are designated by (S). Hatching temperatures increased to 21° C on day 11 (C) and 15 (D).

holding period at 9° C (Figure 5D). Only 60% of the remaining eggs hatched following the temperature rise, resulting in a cumulative total of 74%.

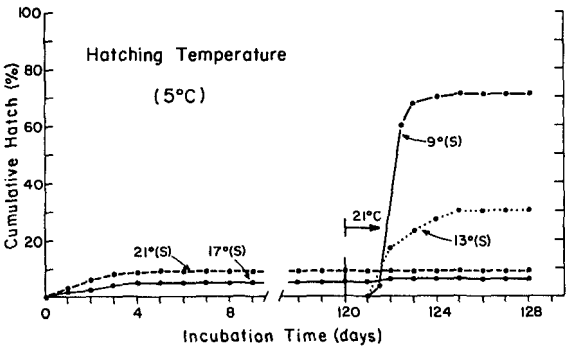

FIGURE 6.—Hatching success of *Acartia californiensis* eggs at 5° C in 25‰ salinity. Eggs spawned at 21°, 17°, 13°, and 9° C. Spawning temperature is designated by (S). Hatching temperature increased to 21° C on day 120.

Long-term survival of summer eggs was negligible at low temperature (120 days at 5° C). Only 1% of the 17° C spawned eggs subsequently hatched at 21° C. Many of the eggs remained normal in appearance, being greenish yellow, throughout the 120-day period. However, within 3-4 days at 21° C, nearly all dormant summer eggs had disintegrated. Most eggs probably died long before day 120, given the high mortality of 21° C spawned eggs after 15 days at 9° C (Figure 5D).

In comparison with dormant summer eggs, the eggs spawned at 13° and 9° C appear to be true resting eggs with an overwintering capacity. The final cumulative hatch of the two types of eggs was similar over the temperature range of 21°-9° C. However, there were major differences in hatching rates between eggs spawned at 9°-13° C and 17°-21° C at all five hatching temperatures tested (Figures 5, 6). For example, the 9°-13° C spawned eggs required 11 and 20 days at 21° and 17° C, respectively, to reach the final comparable hatch of 87-99%. This period was 10-20 times longer than that required by summer eggs.

While none of the 9°-13° C spawned eggs exhibited dormancy at 17° and 21° C, few hatched at the lower incubation temperatures. Only 3-4% hatched at 13° C in contrast to 70-90% for the summer eggs. Likewise only 0-1% at 9° C and 0% at 5° C hatched versus 35% and 5-10%, respectively, for the summer type (Figures 5D, 6).

Temperature was increased to 21° C on days 11, 15, and 120 for the 13°, 9°, and 5° C hatching treatments, respectively. Hatching resumed in all cases at a rate and with success similar to those of summer eggs. However, a 1-1.5 day delay occurred in each case before hatching resumed (Figures 5C,

D; 6). This delay, absent from the data on hatching of summer eggs following an identical temperature increase, is evidence of a difference in the character of dormancy in the two types of eggs.

Resting egg mortality was low during the 11- and 15-day incubation periods (Figures 5C, D). Approximately 96% and 80% final hatch occurred for eggs spawned at 13° and 9° C, respectively. The somewhat lower viability of the 9° C spawned eggs was also seen at hatching conditions of 17° and 21° C (Figure 5A, B). Survival remained high (71%) for 9° C spawned eggs following 120 days incubation at 5° C (Figure 6). In comparison, the 13° C spawned eggs had only 30% survival. This substantial difference in survival may not be important, as opposite results were found for the hatching success of resting eggs from the preliminary experiment under equivalent conditions (9° C spawn = 45%; 13° C spawn = 60% survival). The implication is that resting egg survival is about 50% after a 4-mo dormant period.

In most cases, hatching success at a given temperature was similar for eggs of a given type (summer or resting) spawned at different temperatures (Figures 5, 6). For example, 21° C spawned eggs displayed little difference in hatching time or success from 17° C spawned eggs at 21°, 17°, and 9° C. The discrepancy in summer egg hatching times and cumulative totals at 13° C was likely an experimental artifact since it was absent in the results of the preliminary experiment. Uye and Fleminger (1976) similarly reported finding no difference in hatching success at a given temperature for *A. clausi* eggs spawned at 17.5° and 13.5° C.

A notable exception to this pattern occurred for 13° and 9° C spawned eggs which were incubated at 21° and 17° C (Figure 5A, B). In both cases, the 9° C spawned eggs had a higher initial rate of hatching than 13° C spawned eggs. By day 5, this was reversed, and the rate for 13° C spawned eggs exceeded that for 9° C spawned eggs. It is possible that some of the 9° C spawned eggs had an enhanced metabolic rate relative to 13° C spawned eggs, caused by cold acclimation (suggested by Landry 1975b). Uye and Fleminger (1976) also found evidence that cold-acclimated eggs of *A. tonsa,* spawned at 6.5° C, tended to hatch more quickly at temperatures below 12.5° C than eggs spawned at 17.5° C.

Long-term exposure to low temperature resulted in abnormal development for some resting eggs. This was only seen in 5-12% of the 9° C spawned eggs incubated at 9° or 5° C for 120 days. Abnormalities of the newly hatched NI nauplii ranged from mild to strong structural deformation. Some nauplii possessed an enlarged labrum or fused appendages (e.g., second antennae and mandible); some lacked appendages of one side of the body. One nauplius had two severe constrictions which divided the body into three lobes. Many of the abnormal nauplii were alive, though weak, at the time of observation. Uye and Fleminger (1976) also reported finding deformed NI nauplii and postulated that osmotic stress from abnormal salinities may have caused the deformations. In this case, however, deformation must have resulted from long exposure to low temperatures, since salinity was maintained at a favorable concentration of 25‰.

November Experiment

Different hatching results were obtained using eggs spawned by females acclimated at the November field temperature of 12° C. Hatching patterns (Figure 7) indicate that both nondormant and resting eggs were produced concurrently in the population. This is in contrast to production of resting eggs only in the 13° and 9° C treatments of females collected in October for the main experiment (Figures 5, 6). The evidence for the presence of both egg types is the two different hatching rates which occurred at summer temperatures (Figure 7). The initial hatching at 21° and 17° C occurred within the first day, similar to summer eggs at identical temperatures (Figure 5A, B).

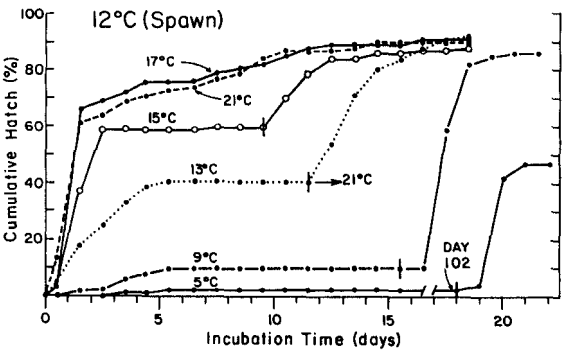

FIGURE 7.—Hatching success of *Acartia californiensis* eggs spawned by November-collected females at the field acclimation temperature of 12° C. Hatching temperatures varied from 21° C to 5° C; salinity was 25‰. Temperature increased to 21° C (denoted by vertical line) after variable periods of incubation below 17° C.

However, after reaching a total of 61-66% hatched, the rate decreased sharply with hatching continuing at a low, constant rate to a final total of 90-91% at day 16. The latter pattern resembles that found for resting eggs (13°, 9° C spawn) hatched at summer temperatures (Figure 5A, B). A comparable 56% hatch also occurred at 15° C within the first 2.5 days. However, development then ceased until temperature was increased to 21° C. Thus, at all three temperatures, approximately 60% of the eggs behaved as nondormant summer eggs, while the remaining 40% had characteristics of resting eggs.

Dormancy increased from 40% at 15° C to 98% at 5° C (Figure 7), presumably as a result of dormancy induced by low temperatures in otherwise nondormant eggs. This result is similar to that seen for summer eggs hatching at the lower temperatures (Figures 5C, D; 6).

Egg viability during short- and long-term dormancy was determined by increasing temperature to 21° C on days 9, 11, 15, and 102 for the 15°, 13°, 9°, and 5° C hatching treatments, respectively (Figure 7). Mortality was low during the 9-15 day incubation period at 15°, 13°, and 9° C with a final cumulative hatch of 85-91%. In contrast, only 45% of the dormant eggs incubated for 102 days at 5° C completed development into NI nauplii following temperature elevation. Probably few dormant summer-type eggs survived the long holding period at 5° C, given that about 40% of the eggs exhibited characteristics of resting eggs in 21°, 17°, and 15° C water. This conclusion is supported by the high mortality (99-100%) found for summer eggs incubated at 5° C for 120 days in the October experiment (Figure 6).

Hatching of Resting Eggs Collected in the Field

Salinity Experiment

The effect of salinity on hatching of resting eggs collected from field sediments was initially determined at 17° C, a temperature favorable for egg hatching (Figure 5) and population growth of *A. californiensis* (Figures 2, 3). Results presented in Figure 8 are the combined data for both *A. californiensis* and *A. clausi*, since their respective overwintering eggs could not be separated.

Hatching occurred at all salinities from 23.5‰ to 0‰ (Figure 8). Rates decreased only slightly with decreasing salinity from 23.5‰ (37%·day^{-1})

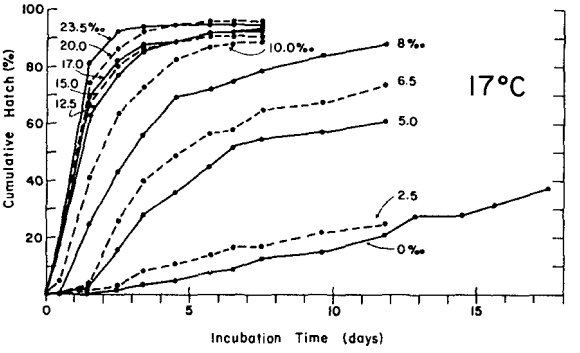

FIGURE 8.—Hatching success of field-collected resting eggs of *Acartia* spp. as a function of salinity (0-23.5‰) at 17° C.

to 12.5‰ (31%·day^{-1}; day 2.5). Final hatch in this salinity range was 91-96%. Initial hatching rates below 12.5‰ decreased markedly, ranging from 26%·day^{-1} at 10‰ to 1.8%·day^{-1} at 0‰. These latter rates, while reduced, were nevertheless substantial over a 2-wk span. Overall hatching success by day 12 was 21%, 61%, and 88% of the resting eggs at 0‰, 5‰, and 8‰, respectively. Furthermore, hatching was continuing, even in freshwater, as indicated by the slopes of the curves, when the experiment was ended. In comparison, resting eggs of *Tortanus forcipatus* do not hatch in freshwater when temperature is favorable (Kasahara, Onbé, and Kamigaki 1975).

The retarding effect of low salinity on hatching appeared to be limited to the actual process of naupliar escapement from the eggshell. Embryogenesis proceeded at similar rates (subjective observations) in all treatments (0-23.5‰), apparently independent of external salinity concentration. Developmental arrest, when present, normally occurred after the fully formed nauplius was visible inside the eggshell.

Exposure to low salinity (0-5‰) for varying periods during dormancy was not fatal for the majority of prehatch nauplii. In each case, high rates of successful hatching (Figure 9) quickly followed a salinity increase to 23.5‰. However, prehatch mortality of "holding" nauplii increased substantially as a function of salinity reduction when compared at equal time. This is seen in a final hatch of 79%, 71%, and 59%, following a salinity increase (day 12) to 23.5‰ for eggs previously incubated at 5‰, 2.5‰, and 0‰, respectively. Thus, an approximate 10% increase in mortality occurred for each 2.5‰ decrease in salinity below 5‰.

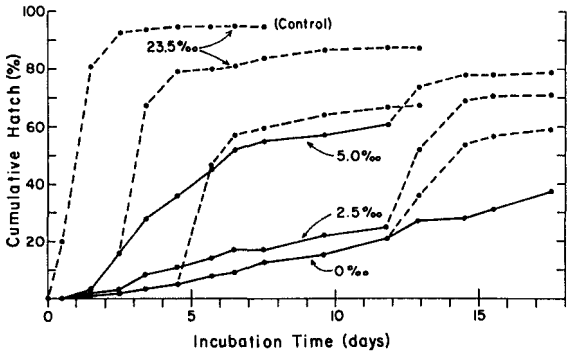

FIGURE 9.—Hatching success and viability ("holding" success) of *Acartia* spp. at 23.5‰ salinity (dashed line), following variable periods of exposure to low salinities (0-5‰) at 17° C. Initial hatching results at 23.5‰ used as a control.

Prehatch mortality also increased gradually with increasing time at low salinity (Figure 9). Total hatch in 23.5‰ after 2.5 and 12 days exposure to 5‰ was 87% and 79%, respectively. This corresponds to a loss of 8% viability within the first 2.5 days and 16% by day 12 as measured against the control hatch at 23.5‰. A comparable trend and loss rate occurred for 0‰ salinity-exposed eggs later hatching in 23.5‰. Losses in viability on days 4.5 and 12 were 27% and 36%, respectively.

The cause of death in prehatch nauplii is unknown, but presumably is related to exhaustion of energy reserves during the holding period. Mortality may also be partially caused by increased osmotic stress at progressively lower salinities. Eggs that died prior to hatching usually disintegrated with 2-3 days at 17° C.

The occurrence of naupliar mortality during the actual hatching process or within the following 24 h substantially increased at salinities below 12.5‰. This is shown by a comparison of cumulative hatching and subsequent naupliar mortality as a function of salinity on days 4.5 and 12 (Figure 10). Naupliar losses on day 4.5 increased from 1% at 12.5‰ to 73% at 5‰. Mortality was 100% at 2.5‰ and 0‰. Corresponding losses were 5-10% higher on day 12, indicating that survival following hatching decreased gradually with increasing time required to hatch.

Observation of hatching success in salinities below 8‰ revealed that many nauplii died during the hatching process or immediately thereafter. At 5‰, many of the dead nauplii were found only partially freed from the cracked-open eggshell. More typically, the nauplius was found lying next to the empty eggshell, indicating immediate death followed hatching. Some nauplii successfully hatched, but never unrolled. Others escaped the outer egg membrane, but died while still inside the osmotically swollen inner membrane. Eggs which had obviously cracked open prior to full development, and exhibiting an extrusion of cellular debris, were also occasionally seen at 0‰ and 2.5‰.

The specific effects of salinity on resting eggs of *A. californiensis* cannot be separated from those of *A. clausi* in the results above. However, supportive evidence indicates that eggs of both *Acartia* species hatched at substantial rates at all salinities from 0‰ to 23.5‰. For instance, 41% of the copepodites reared from viable nauplii during the course of the experiment were *A. californiensis*. While there is no information on possible differential mortality during culturing, an estimate of 41% is probably low for the percentage of *A. californiensis* resting eggs in the experiment. Initial rearing conditions actually favored *A. clausi*, a species that is much more euryhaline in Yaquina Bay. Thus, even when considering the lowest hatching rate at 0‰ salinity on day 17 (Figure 7), it is clear that the 37% hatch (100% mortality) had to include at least some *A. californiensis* eggs. Approximately another 40-50% of the holding eggs at 0‰ were no longer viable. Therefore, one can conclude that prolonged exposure of *A. californiensis* resting eggs to extremely low salinities at 17° C will result in death for the majority, whether it be from prehatch or posthatch salinity stresses.

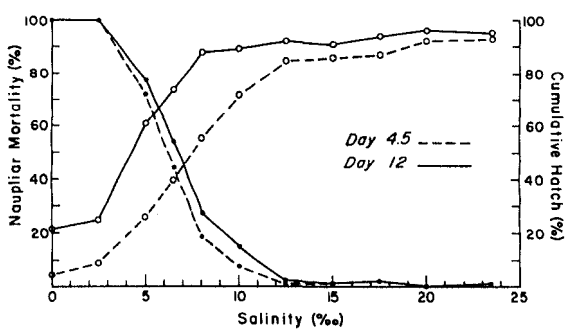

FIGURE 10.—Comparison of cumulative hatch of field-collected resting eggs of *Acartia* spp. (open circles) and subsequent mortality within first 24 h (solid circles) at salinities from 0‰ to 23.5‰. Results on day 4.5 (dashed line) and 12 (solid line) based on 3 replicates of 35 eggs at each salinity.

Salinity and Temperature Experiment

The effect of salinity on hatching of resting eggs was reexamined (Figure 11) at lower temperatures (15°, 12.5°, 10° C) which are marginal to unfavorable for growth of the *A. californiensis* population (Figures 2, 3). The basic intent was to determine, if possible, the lowest termperature-salinity combination(s) which could break diapause of the overwintering eggs.

General hatching patterns as a function of salinity (Figure 11) were similar to those observed at 17° C (Figure 8) in that hatching rates decreased with decreasing salinity at all three temperatures.

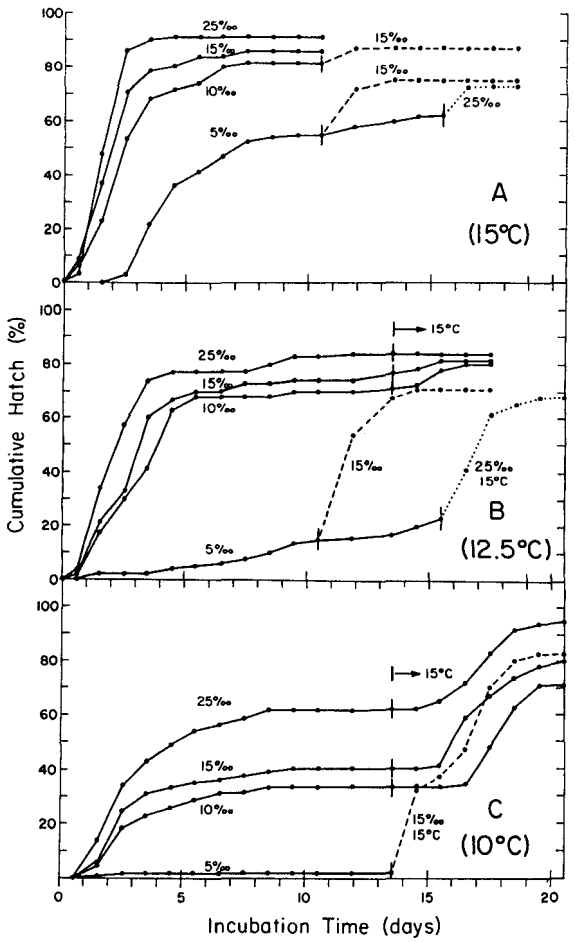

FIGURE 11.—Hatching rate and cumulative success of field-collected resting eggs of *Acartia* spp. in various salinities at: (A) 15° C, (B) 12.5° C, and (C) 10° C. Lower temperatures and salinities increased to 15° C and 15‰, respectively, after variable periods of incubation to determine short-term "holding" success at suboptimal conditions.

Lower temperatures similarly reduced hatching rates and final hatches at a given salinity. However, the interactive effect of temperature with salinity increased nonlinearly as temperature decreased (Figure 11). As a result, the percentage of resting eggs remaining in a state of dormancy became progressively larger with decreasing temperature.

Few *Acartia* spp. resting eggs incubated at 15° or 12.5° C (Figure 11A, B) failed to terminate diapause and begin development. As observed at 17° C, embryogenesis progressed to the final prehatch stage, apparently independent of external salinity. Subsequent holding time (pseudodormancy) depended on the interaction of salinity and temperature. For example, hatching rates at 25‰, 15‰, and 10‰, while progressively reduced, were still relatively high with very little dormancy evident. The majority of eggs hatched within 3-4 days. Final hatches were high and in a narrow range from 91-81% at 15° C to 84-71% at 12.5° C. However, some prehatch holding did occur at these higher salinities since an increase in salinity (10‰ to 15‰; Figure 11A) or temperature (12.5° to 15° C; Figure 11B) resulted in an additional 5-9% hatch.

Prehatch holding at 5‰ was increasingly more important with decreasing temperature (Figure 11A, B). At 15° C, the 5‰ hatching rate was greatly reduced, but was still substantial and similar to that seen at 17° C (Figure 8). Over 50% hatch was attained by day 7, with hatching continuing to 62% on day 15. Viability of the remaining eggs (38%) was low since a salinity increase to 25‰ only yielded an additional 11% hatch. In comparison, prehatch holding in 5‰ at 12.5° C (Figure 11B) was pronounced since the hatching rate was reduced to only 1-2% • day^{-1}. However, the holding dormancy was only a temporary condition since hatching continued throughout the 15-day exposure to 5‰. Viability was reduced but still substantial as seen in a final hatch of 68% in 25‰. Thus, hatching in 5‰ water at 12.5° C or 15° C would have probably continued until all eggs either hatched or died from exhaustion of energy reserves during the holding state.

Hatching results at 10° C (Figure 11C) were considerably different from results at 12.5° C and 15° C in that dormancy was a major factor at all salinities. For example, final hatches (prior to changes) at 25‰, 15‰, and 10‰ were 61%, 40%, and 33%, respectively. This represented a 23-38% increase in dormancy from that at 12.5° C. Persis-

tence of dormancy at 5‰ was nearly complete with only 2% hatch achieved.

The nature of continued dormancy at 10° C (Figure 11) was unusual in that both diapause and prehatch holding cooccurred. Dormant eggs remaining on day 13 at 25‰, 15‰, and 10‰ appeared to still be in diapause since no apparent change in coloration or internal structure occurred during incubation at 10° C. A subsequent 1-3 day delay in hatching after temperature was increased to 15° C is also indicative that the eggs were still in diapause (as seen in the October temperature experiments; Figure 5C, D). Copepodites reared from nauplii hatched at 15° C in the 25‰, 15‰, and 10‰ treatments after day 13 were found to be mainly *A. californiensis* (87%; $n = 23$). As a result, most of the eggs remaining in diapause at 10° C over the 25-10‰ range were probably those of *A. californiensis*.

In contrast to results at 25-10‰ (Figure 11C), diapause did not persist in eggs of either *A. californiensis* or *A. clausi* exposed to 5‰ at 10° C. The reason for this difference is not known. While only 2% of the eggs had hatched, nearly all remaining eggs had broken diapause and were in the final prehatch holding state many days before the temperature increase on day 13. The difference in the nature of dormancy is reflected by the rapid hatching rate with no delay period following the temperature increase. In spite of high viability seen after day 13, the termination of diapause and the failure to hatch at 5‰ and 10° C demonstrate that egg survival of both *Acartia* species was short-term and limited by available energy reserves.

Posthatch naupliar mortality within the first 24 h as a function of salinity at 15°, 12.5°, and 10° C was very similar to that shown for 17° C (Figure 10). The mortality range in 15‰, 10‰, and 5‰ water, for example, was 2-7%, 12-22%, and 80-100%, respectively, as compared with 2%, 15%, and 78% at 17° C. Typically, the percent survival decreased slightly at a given salinity as temperature decreased. Therefore, at salinities >10‰, survival was high with mortality primarily increasing with decreasing temperature. Below 10‰, the NI nauplii experienced increasingly heavy mortality primarily as a function of decreasing salinity.

Additional information on hatching behavior and fraction of *A. californiensis* resting eggs were obtained by a comparison of the proportions of copepodites reared from nauplii which initially hatched from unsorted egg mixtures at each temperature-salinity combination (Table 3). Hatching at the most optimal of the given experimental conditions for both species (15° C, 25-15‰) resulted in copepodite proportions of 51% and 62% *A. californiensis*. A similar percentage (54%) was observed at 12.5° C and 25‰. As these estimates are based on independent rearing treatments, it is reasonable to conclude that roughly 55% of the field resting eggs were those of *A. californiensis*. This result corresponds very well with other estimates of percent abundance determined in earlier unpublished experiments.

Copepodites of *A. californiensis* were absent in the cultures which initially hatched in 10‰ and 5‰ at both 15° and 12.5° C (Table 3). As nearly all resting eggs were previously found to terminate diapause at these lower salinities (Figure 11A, B), the absence of *A. californiensis* is the result of mortality during either prehatch holding or subsequent naupliar stages. This is verified, in part, by the increasing mortality of NI nauplii (*Acartia* spp.) below 12.5‰ (Figure 10). Furthermore, hatching rate differences over the range of 25-10‰ were small as shown in Figure 11A, B. For example, at 15° C, a total hatch of 86% and 81% was observed at 15‰ and 10‰, respectively. Yet, the proportion of *A. californiensis* copepodites was 62% at 15‰ and 0% at 10‰ (Table 3). Similarly at 12.5° C, only 6% survived to copepodites when hatched at 15‰ as compared with 54% at 25‰. It must be reemphasized that the temperature and salinity values referred to here and in Table 3 are hatching conditions only. Rearing was under more favorable conditions (see Methods). Therefore, as

TABLE 3.—Proportion of *Acartia californiensis* and *A. clausi* copepodites that survived following hatching at 12 temperature-salinity combinations. Temperature and salinity levels for rearing to copepodite stages were gradually increased to 15° C and 25‰ to increase posthatch survival (see text for further details).

	10° C			12.5° C			15° C		
Salinity (‰)	*n*	*A. clausi* (%)	*A. calif.* (%)	*n*	*A. clausi* (%)	*A. calif.* (%)	*n*	*A. clausi* (%)	*A. calif.* (%)
5	0	0	0	4	100	0	0	0	0
10	16	100	0	24	100	0	119	100	0
15	15	100	0	71	94	6	40	38	62
25	235	100	0	28	46	54	183	49	51

diapause was ended at all salinities at 12.5°-15° C, it is evident that individuals hatching from resting eggs of *A. californiensis* exposed to salinities below 15‰ experienced early death, even when temperature and salinity were increased to more favorable levels following hatching. As a result, these data can be used to define minimal hatching conditions for growth to maturity and can be correlated with the fall disappearance and summer repopulation of *A. californiensis* in Yaquina Bay.

Acartia californiensis copepodites were absent at all salinities at 10° C (Table 3). Absence over the range of 25-10‰ was probably the result of continued diapause of the resting eggs of *A. californiensis*, as previously demonstrated (Figure 11C). Some of the latter eggs may have hatched at 25‰, given the likelihood of a 62% hatch (Figure 11C) and an estimated resting egg ratio of 55% (Table 3). However, egg hatching at 15‰ and 10‰ (40-33%; Figure 11C) can be completely attributed to *A. clausi* since it composed ca. 45% of the resting eggs.

An unrelated but important observation concerns the hatching of a few *Epilabidocera longipedata* Sato (= *E. amphitrites* McMurrick) 10° C, 25‰) and *Eurytemora affinis* (Poppe) (10°-15° C, 5-25‰) from the unsorted resting egg mixtures used to obtain *Acartia* spp. ratios. Identification was made at the late copepodite stages. Neither species has previously been reported as possessing a resting egg stage. Both species are absent at Station 39 in Yaquina Bay during the winter months, insuring that the eggs were in diapause when collected.

DISCUSSION

Environmental Conditions Resulting in Egg Dormancy

Many shallow-water neritic and estuarine calanoid species with multivoltine life cycles are now known to survive long periods of adverse environmental conditions by facultative production of resting eggs. Field observations and laboratory results have demonstrated that this is true for *A. californiensis* in Yaquina Bay. After 4 or 5 successive generations with substantial recruitment (July-September 1972; Figure 2), the planktonic population declined rapidly and was gone by mid-November. The failure of recruitment in early October coincided with a decline in temperature below 15° C. Salinity remained relatively high and stable at 25-30‰, during the population disappearance, implying temperature dependence for the production of resting eggs (Figures 3, 4).

Experimental results confirmed the hypothesis that diapause in *A. californiensis* eggs is essentially a response of the spawning females to low temperatures, similar to that shown for *A. tonsa* (Zillioux and Gonzalez 1972), *Tortanus forcipatus* (Kasahara, Onbé, and Kamigaki 1975) and possibly *Pontella meadi* (Grice and Gibson 1977). The shift from summer egg to resting egg production occurred between 15° and 13° C (Figure 5), a temperature range comparable to that observed in the field. Salinity was not a factor, since it was maintained at a constant and favorable level (25‰) in all treatments. Food quantity and quality were excluded as factors by daily providing the adults with an ample ration consisting of three prey species. Photoperiod is known to influence induction of diapause in some cladocerans (cf.: *Daphnia magna*, Bunner and Halcrow 1977; *D. pulex*, Stross and Hill 1965). However, it was eliminated as a possible extrinsic factor by the use of continuous lighting.

The production of overwintering eggs was most likely initiated by changes, possibly hormonal, within the female in response to the extrinsic stimulus of adverse temperature. Extensive research on the physiology of insects has confirmed the regulation of diapause by hormones (e.g., Lees 1955; Sláma et al. 1974). Moreover, Carlisle and Pittman (1961) found differences in forebrain neurosecretions between summer and overwintering "dormant" CV copepodites of *Calanus finmarchicus* that resembled diapause in insects. Watson and Smallman (1971) similarly reported significant changes in small tissue patches in the head region of the cyclopoid copepod, *Diacylops navus*, that correlated with induction and cessation of diapause. Since dormant egg production can be rapidly induced in *A. californiensis* by lowering water temperature, it is probable that the controlling physiology is somewhat different.

The temperature-dependent maternal role in the induction of dormancy in *A. californiensis* eggs is contrary to results reported by Uye and Fleminger (1976) for *A. californiensis* (= *Acartia* sp. I) in a southern California lagoon. They concluded that egg dormancy in *A. californiensis* must occur independently of maternal influence since 90-100% of the eggs hatched at all temperatures in the annual field range (10°-25° C). Expo-

sure to salinity extremes was suggested as a possible cause of induced dormancy. Their conclusion, however, was based upon the hatching behavior of *A. californiensis* eggs which were spawned at 17.5° C. On the basis of my observations (Figure 5), these latter eggs were most likely all summer eggs which exhibited increasing dormancy below 10° C. It is likely that female-induced dormancy would have been observed if a spawning temperature below 15° C had been used.

Summer eggs of *A. californiensis* possess the capacity for short-term facultative dormancy when exposed to temperature below 15° C (Figure 5). Hatching resumed within hours following temperature elevation above 15° C. This type of arrested development, temporarily induced by unfavorable external conditions and ended with the return of a favorable environment, represents a state of "quiescence" as the term is used by Andrewartha (1952), Lees (1955), and Wigglesworth (1972) for other arthropod groups.

Quiescence of nondormant eggs at low temperatures is probably a characteristic of most calanoid species which inhabit highly variable environments such as estuaries and lagoons (Uye and Kasahara 1978). For example, Uye and Fleminger (1976) found that *A. tonsa* eggs which were spawned at 17.5° C (a favorable temperature) exhibited dormancy only at 7.5° and 5° C, a result which they also demonstrated for *A. californiensis*. In both species, survival during dormancy at 7.5° and 5° C was of short duration, since no hatching occurred following a temperature elevation after 28-30 days. The lack of long-term viability is supporting evidence that the respective eggs were in a quiescent state and not true resting eggs.

In each species, the percentage of quiescent summer eggs increased as temperature decreased. However, quiescence occurred at significantly higher temperatures in eggs of *A. californiensis* from Yaquina Bay, shown by a 35% hatch at 10° C (Figure 5D), as compared with 100% for the southern California population (Uye and Fleminger 1976). Both sets of eggs were spawned at 17° or 17.5° C. The considerable difference in thermal induction of quiescence in summer eggs may represent a genetic gradient reflecting the latitudinal separation of the two populations. Selection for quiescence in this warmwater species is probably more important in Oregon estuaries because of lower water temperatures (2°-22° C range) and longer winters (Figure 4). Less of a selective advantage would exist in California estuarine and lagoonal waters with a narrower annual range of 10°-25° C (from Uye and Fleminger 1976). Furthermore, any genetic gradient caused by differential selective pressures would be reinforced by the localized confinement of populations within estuaries or lagoons, which must greatly reduce gene flow.

The adaptive value of quiescence may be greatest in temperate estuaries (e.g., Yaquina Bay) where eggs in the bottom sediments typically experience large variations in temperature over successive tidal cycles. However, since viability of *A. californiensis* eggs in the quiescent state at low temperatures is limited to 1-2 mo, as shown above (Figures 5, 6) and in figure 5F of Uye and Fleminger (1976), the importance of quiescence in overwintering must be considered negligible.

Summer and resting eggs of *A. californiensis* may cooccur in equal proportions in the cumulative spawn at temperatures below 15° C as shown in the November experiment with females which spawned at their field acclimation temperature of 12° C (Figure 7). Zillioux and Gonzalez (1972) reported similar spawning results for *A. tonsa* females at acclimation temperatures of 9°, 11.4°, and 14.5° C. In each case, approximately 50-60% of the eggs were nondormant and hatched. It is not known from these data if the same female can produce both egg types at once. It is likewise not known if a female producing only resting eggs at lower temperatures can switch back to summer egg production if temperature increased above 15° C. These questions will need to be resolved by observations on individual females.

There is an apparent discrepancy between the November and October observations. Females collected from 15° C water in October produced exclusively dormant eggs when rapidly cooled to 13° or 9° C. It is reasonable to have expected all of the November eggs spawned at the field acclimation temperature of 12° C to have also been dormant, given the October results. Perhaps there are effects upon the initiation of dormant egg production from both low temperature to which a female is fully acclimated and from sudden temperature reductions. Response to the latter stimulus would protect against more than usually moderate cooling rates in the fall. Given normal field conditions, however, there probably is considerable variation between individual females in the population in the threshold temperature which induces dormant egg production. As a result, cooccurrence of both egg types would be expected during a significant

portion of the fall population decline. Some field evidence of this is seen in the weak pulse of copepodites found in late October, indicating that substantial hatching did occur below 15° C earlier in the month (Figures 2, 3).

Termination of Diapause and Population Reappearance

Hatching of field-collected resting eggs of *Acartia* spp. at various temperature-salinity combinations indicates that termination of dormancy is essentially temperature dependent. However, hatching rates and survival are regulated by salinity. My conclusions with respect to *A. californiensis*, based on the often disparate observations, are:

1. Approximately half of the experimental resting eggs were *A. californiensis*.
2. Embryogenesis and hatching occurred at all salinities tested at 12.5°-15° C (5-25‰) and 17° C (0-23.5‰).
3. Diapause persisted at 10° C over the range of 10-25‰, while embryogenesis occurred at 5‰.
4. Hatching was retarded at low salinities, particularly below 10‰.
5. Developmental arrest in low salinity normally occurred at the last stage of embryogenesis. Viability of "holding" prehatch nauplii was limited to 1-2 mo, depending on temperature.
6. Mortality losses were increasingly severe below 10‰ for hatched nauplii and substantial for "holding" eggs.
7. No nauplii survived to reach the copepodite stages at salinities below 15‰ at 12.5° C or at any salinity at 10° C.

Exposure to low temperatures over a prolonged period (comparable to cold stratification for seeds of many temperate, deciduous plants) is unnecessary for the release of diapause in *A. californiensis* overwintering eggs. This is probably the normal condition for most *Acartia* species (see Zillioux and Gonzales 1972 and Uye and Fleminger 1976). It is not universal, however, as the resting eggs of *Pontella meadi*, a neritic species, require a chilling period before hatching can occur (Grice and Gibson 1977). A similar requirement is implied but not conclusively demonstrated for resting eggs of *P. mediterranea* and *Centropages ponticus* (Sazhina 1968). Overwintering eggs of some freshwater calanoids (e.g., *Diaptomus oregonensis*, Cooley 1971) are also known to require exposure to low temperatures prior to hatching.

While chilling is unessential for termination of diapause in *A. californiensis* resting eggs, some effects from chilling were observed. For example, all field-collected eggs were found to commence development at 12.5° C (Figure 11) while laboratory-spawned eggs terminated diapause only above 15° C (Figures 5, 6, 7). Thus, exposure to winter temperatures appears to lower the hatching threshold. The time required is unknown but may be quite short. Newly spawned resting eggs initially hatched at very low rates when incubated at 17° or 21° C (Figure 5). However, after 11 and 14 days of chilling, hatching rates approached those of summer eggs when placed in favorable temperatures.

The "holding" phenomenon induced by low salinities represents a second type of short-term quiescence in *A. californiensis* resting eggs. It differs from the temperature-induced quiescent state seen in summer eggs in that quiescence does not set in until the final stage of embryogenesis. In addition, salinity-induced quiescence is much weaker, since hatching continues at low levels. In these aspects, it closely resembles the dark inhibition of summer egg hatching of *A. clausi* (Landry 1975a). Development of *A. clausi* eggs in the dark proceeds to the prehatch naupliar stage before "holding" occurs. Viability of eggs in this darkness-induced quiescence is even shorter than that of *A. californiensis* in low salinity-induced quiescence. Uye and Fleminger (1976) and my own data (unpubl.) indicate it is 20-25 days.

On the basis of field collections in February, experimental results and the temperature and salinity cycles in the field, significant hatching (or embryogenesis followed by holding) of resting eggs must occur over much of the year. Low oxygen tension in sediments, while important in inhibiting hatching (Kasahara, Onbé, and Kamigaki 1975), is probably not a critical factor, since resting eggs in the bottom sediments are continually exposed to oxygenated surface layers by turbulence and erosion. Termination of diapause does not always coincide with the presence of favorable environmental conditions for naupliar growth. Those resting eggs which undergo development and then either enter quiescence or hatch during the winter-spring months, a period of very low salinities, must be soon lost. Such a process would partially account for the seasonal decline in resting egg numbers in the sediments with

increasing time as observed by Kasahara, Uye, and Onbé (1975). Successful repopulation is possible at any point in spring that daily mean salinity is in excess of 10‰, which usually occurs in early June. Production of resting eggs in the annual cycle of *A. californiensis* is thus viewed as a "leaky" population diapause that is consistent with the opportunistic nature of estuarine copepod species.

It is possible that the leaky character of the diapause is retained because of the occasional success of the early-hatching portion of the population in years with early termination of winter rains. In those years, this fraction of the population would be strongly favored by the end of the growing season because of its early start. In years with prolonged spring rains, the late-hatching fraction would be favored. Variations in the weather cycle, thus, may prevent development of absolute and restrictive requirements for termination of diapause.

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LITERATURE CITED

ANDREWARTHA, H. G.
1952. Diapause in relation to the ecology of insects. Biol. Rev. (Camb.) 27:50-107.

BARLOW, J. P.
1955. Physical and biological processes determining the distribution of zooplankton in a tidal estuary. Biol. Bull. (Woods Hole) 109:211-225.

BREWER, R. H.
1964. The phenology of *Diaptomus stagnalis* (Copepoda: Calanoida): The development and the hatching of the egg stage. Physiol. Zool. 37:1-20.

BUNNER, H. C., AND K. HALCROW.
1977. Experimental induction of the production of ephippia by *Daphnia magna* Straus (Cladocera). Crustaceana 32:77-86.

BURT, W. V., AND W. B. MCALISTER.
1959. Recent studies in the hydrography of Oregon estuaries. Res. Briefs Fish Comm. Oreg. 7(1):14-27.

CARLISLE, D. B., AND W. J. PITMAN.
1961. Diapause, neurosecretion and hormones in Copepoda. Nature (Lond.) 190:827-828.

CONOVER, R. J.
1956. Oceanography of Long Island Sound, 1952-1954. VI. Biology of *Acartia clausi* and *A. tonsa*. Bull. Bingham Oceanogr. Collect., Yale Univ. 15:156-233.

COOLEY, J. M.
1971. The effect of temperature on the development of resting eggs of *Diaptomus oregonensis* Lill; (Copepoda: Calanoida). Limnol. Oceanogr. 16:921-926.

ELGMORK, K.
1967. Ecological aspects of diapause in copepods. *In* Symposium on Crustacea, p. 947-954. Mar. Biol. Assoc. India, Symp. Ser. 2.

FISH, C. J., AND M. W. JOHNSON.
1937. The biology of the zooplankton population in the Bay of Fundy and Gulf of Maine with special reference to production and distribution. J. Biol. Board Can. 3:189-322.

FROLANDER, H. F., C. B. MILLER, M. J. FLYNN, S. C. MYERS, AND S. T. ZIMMERMAN.
1973. Seasonal cycles of abundance in zooplankton populations of Yaquina Bay, Oregon. Mar. Biol. (Berl.) 21:277-288.

GRICE, G. D., AND V. R. GIBSON.
1975. Occurrence, viability and significance of resting eggs of the calanoid copepod *Labidocera aestiva*. Mar. Biol. (Berl.) 31:335-337.
1977. Resting eggs in *Pontella meadi* (Copepoda: Calanoida). J. Fish. Res. Board Can. 34:410-412.

HUTCHINSON, G. E.
1967. A treatise of limnology. Vol. II. Introduction to lake biology and the limnoplankton. Wiley, N.Y., 1115 p.

JOHNSON, J. K., AND C. B. MILLER.
1974. Dynamics of isolated plankton populations in Yaquina Bay, Oregon. Proc. 3d Annu. Tech. Conf. Estuaries Pac. Northwest, p. 27-35. Oreg. State Univ. Eng. Exp. Stn. Circ. 46.

KASAHARA, S., T. ONBÉ, AND M. KAMIGAKI.
1975. Calanoid copepod eggs in sea-bottom muds. III. Effects of temperature, salinity and other factors on the hatching of resting eggs of *Tortanus forcipatus*. Mar. Biol. (Berl.) 31:31-35.

KASAHARA, S., S. UYE, AND T. ONBÉ.
1975. Calanoid copepod eggs in sea-bottom muds. II. Seasonal cycles of abundance in the populations of several species of copepods and their eggs in the Inland Sea of Japan. Mar. Biol. (Berl.) 31:25-29.

LANDRY, M. R.
1975a. Dark inhibition of egg hatching of the marine copepod *Acartia clausi* Giesbr. J. Exp. Mar. Biol. Ecol. 20:43-47.
1975b. Seasonal temperature effects and predicting development rates of marine copepod eggs. Limnol. Oceanogr. 20:434-440.

LEES, A. D.
1955. The physiology of diapause in arthropods. Camb. Univ. Press, Engl., 151 p.

MILLER, C. B., J. K. JOHNSON, AND D. R. HEINLE.
1977. Growth rules in the marine copepod genus *Acartia*. Limnol. Oceanogr. 22:326-335.

PERTZOVA, N. M.
1974. Life cycle and ecology of a thermophilous copepod *Centropages hamatus* in the White Sea. [In Russ.] Zool. Zh. 53:1013-1022.

SAZHINA, L. I.
1968. Hibernating eggs of marine Calanoida. Zool. Zh. 47:1554-1556. (Transl., 1969. Fish. Res. Board Can. Transl. Ser. 1259.)

SLÁMA, K., M. ROMAŃUK, AND F. ŠORM.
1974. Insect hormones and bioanalogues. Springer-Verlag, N.Y., 477 p.

STROSS, R. G., AND J. C. HILL.
1965. Diapause induction in *Daphnia* requires two stimuli. Science (Wash., D.C.) 150:1462-1464.

TRINAST, E. M.
1976. A preliminary note on *Acartia californiensis*, a new calanoid copepod from Newport Bay, California. Crustaceana 31:54-58.

UYE, S., AND A. FLEMINGER.
1976. Effects of various environment factors on egg development of several species of *Acartia* in southern California. Mar. Biol. (Berl.) 38:253-262.

UYE, S., AND S. KASAHARA.
1978. Life history of marine planktonic copepods in neritic region with special reference to the role of resting eggs. Bull. Plankton Soc. Jpn. 25:109-122.

WATSON, N. H. F., AND B. N. SMALLMAN.
1971. The physiology of diapause in *Diacyclops navus* Herrick (Crustacea, Copepoda). Can. J. Zool. 49:1449-1454.

WIGGLESWORTH, V. B.
1972. The principles of insect physiology. 7th ed. Chapman and Hall, Lond., 827 p.

ZILLIOUX, E. J., AND J. G. GONZALEZ.
1972. Egg dormancy in a neritic calanoid copepod and its implications to overwintering in boreal waters. *In* B. Battaglia (editor), Fifth European Marine Biology Symposium, p. 217-230. Piccin Editore, Padova, Italy.

ZIMMERMAN, S. T.
1972. Seasonal succession of zooplankton populations in two dissimilar marine embayments on the Oregon coast. Ph.D. Thesis, Oregon State Univ., Corvallis, 212 p.