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RICHARD W. BRILL

Southwest Fisheries Center Honolulu Laboratory
National Marine Fisheries Service, NOAA
P.O. Box 3830, Honolulu, HI 96812
Present address: *Department of Zoology*
The University of British Columbia
2075 Wesbrook Mall, Vancouver, B.C. V6T 1W5, Canada

EFFECTS OF A THERMAL DISCHARGE ON REPRODUCTIVE CYCLES IN *MYTILUS EDULIS* AND *MYTILUS CALIFORNIANUS* (MOLLUSCA, BIVALVIA)

One principal concern about thermal effluents is the effect of altered temperatures on the reproductive biology of organisms near the discharge (e.g., Hedgpeth and Gonor 1969). In marine mussels of the genus *Mytilus*, the role of temperature in regulating the reproductive cycle and the effects of temperature stress on the energy budget for growth and reproduction have been particularly well studied (Bayne 1975; Gabbott 1976; Seed 1976). *Mytilus edulis* has a seasonal cycle of gametogenic activity that is conditioned by temperature and is linked with the storage and utilization of reserve materials in the body (Bayne 1975). Metabolism and filtration rate show complete temperature acclimation from 5° to 20° C, and the scope for growth is relatively independent of temperature over this range (Widdows and Bayne 1971; Widdows 1973, 1978a). However, above 20° C the mechanisms of temperature adaptation break down, producing an increase in the metabolic rate, a decline in filtration rate, and thus a reduced scope for growth (Widdows 1976, 1978a). Above 25° C this scope is so reduced that there is no energy for growth, and energy reserves are depleted in order to survive (Widdows 1978b).

This study examined the effect of a thermal discharge from a coastal steam-electric power plant on reproduction in *M. edulis* and *M. californianus* in central California. The reproductive cycles and gonadal weights of these mussels in the warmwater outfall and in control regions of naturally occurring temperatures were compared using body component index methods. Water temperatures in the outfall exceeded 20° C much of the late summer and early fall, while plant intake temperatures were usually in the 12°-15° C range and rarely exceeded 17° C.

Methods

This study was conducted at the Pacific Gas and Electric Company fossil-fuel power plant at Morro Bay, Calif. (Figure 1). The 1,030-MW plant used ocean water for once-through cooling and discharged warmed water into a canal about 80 m long. The canal released water into the surf, forming a plume with an isotherm 5° C above naturally occurring temperatures of about 0.6-3.0 acres sur-

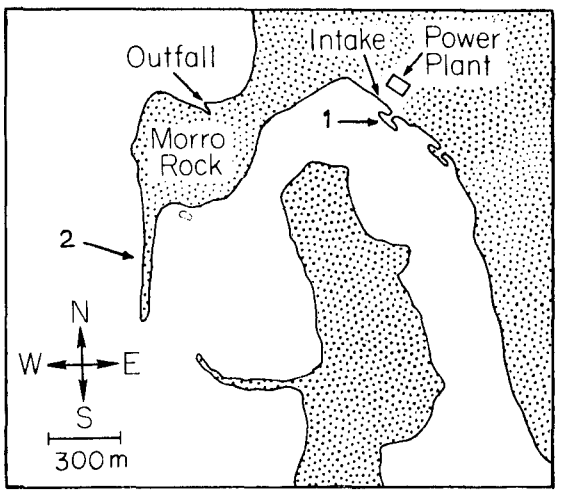

FIGURE 1.—Locations of the intake and outfall of the Morro Bay power plant in California and the collecting sites for mussels in this study. In addition to outfall samples of both species, control samples of *Mytilus edulis* were taken from site 1, and control samples of *M. californianus* were taken from site 2.

face area depending on plant load and weather conditions. The mean temperature differential between the intake and outfall was about 7° C and ranged from about 3° C in spring to about 15° C in late summer and fall (Figure 2). The intake showed a seasonal cycle of low temperatures around 11°-12° C in winter and high temperatures around 14°-15° C in summer and fall. Mean outfall temperatures exceeded 20° C from May through January and varied seasonally from around 18° C in spring to around 26° C from July through October. Daily temperature fluctuations in the outfall were much greater (up to 11° C) than those in the intake (up to 3° C). Also, heat treatment every few weeks to kill organisms fouling the cooling tubes raised outfall temperatures in places to as high as 36° C for about an hour.

Mytilus californianus and *M. edulis* were collected from the warmwater outfall and from nearby control areas of normal temperatures at about monthly intervals from November 1972 to November 1973 (Figure 1). *Mytilus edulis* were collected from shallow (1-2 m) subtidal rocks midway along the discharge canal and from the undersides of floats near the intake. *Mytilus californianus* were collected intertidally at 1-3 ft above mean lower low water at the mouth of the discharge canal and at equivalent tidal heights from the jetty at the Morro Bay harbor entrance. High surf made collecting impossible on the jetty and difficult at the mouth of the discharge canal at

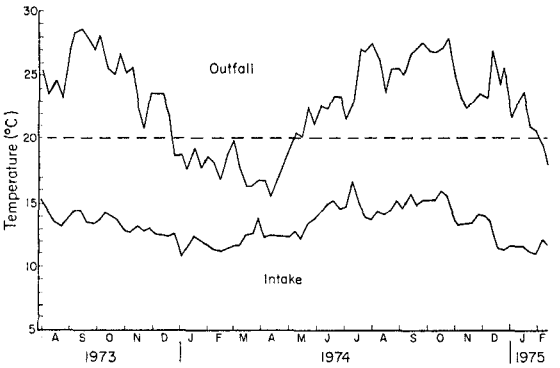

FIGURE 2.—Temperature records from the outfall and intake of the Morro Bay power plant. Weekly mean temperatures were calculated from continuous temperature recorders positioned at mean lower low water near the power plant's intake screens and discharge tubes. The dashed line marks the temperatures above 20° C, which are energetically stressful to *Mytilus edulis*. (Redrawn from Hines 1978.)

times during the winter. Neither outfall nor control *M. edulis* were exposed to significant surf at any time, but the outfall had much stronger currents (up to 0.7 m/s) than the control areas. Salinities in the control and discharge areas did not differ significantly from seawater.

The temperature records closely reflect the thermal environments of the samples of *M. edulis*, because they were collected at nearly the same locations as the recorders. However, the records do not represent as closely the thermal regimes of the samples of *M. californianus*, which were collected from intertidal positions above the recorders and were therefore exposed to air temperatures part of the time, or which were collected at locations distant from the recorders. Seawater temperatures for the control sampling site for *M. californianus* were sometimes 1°-2° C lower than intake temperatures, and temperatures at the mouth of the discharge canal where outfall samples were taken were often 2°-4° C lower than the records show due to dilution of the warmwater discharge by incoming surf.

Monthly samples of 12 mussels 70-110 mm long from outfall and from control populations of each species were processed. For each mussel, the shell length and the internal shell volume determined by the volume (milliliters) of water required to fill the empty valves were recorded. Total wet tissue weight (grams) and wet weight of the gonad tissue dissected from the mantle and body mass were recorded for each mussel. From these data the gonadal index was calculated as: (gonad wt ×

100)/total tissue wt. The body weight/shell volume index was calculated as: (total wt − gonad wt)/shell volume. The gonadal index reflects reproductive condition and the body weight/shell volume index reflects nutritional condition (Giese and Pearse 1974). Preliminary work showed that indexes calculated from wet and then dry weights did not have significantly different variances.

Results

Mytilus edulis and *M. californianus* longer than about 50 mm were sexually mature, and the gonadal indexes of both species had large variances. Because gonadal indexes of both species were calculated for a large size range (70-110 mm shell length) in each population, the covariance of gonadal index on shell volume was analyzed for each species. However, regressions of the arcsine transformation of the gonadal index on shell volume calculated for each monthly sample did not have significantly different slopes for either species (ANCOVA, $P>>0.05$): for *M. californianus* the common regression slope = 0.09, $F_{(18,190)} = 0.206$; for *M. edulis* the common regression slope = 0.08, $F_{(21,220)} = 0.217$. Therefore, mussel size was ruled out as a significant source of variability in gonadal index for this study. Rather, the variability was probably a result both of the difficulty in precisely dissecting the diffuse gonad from the body tissues and of a large degree of inherent reproductive asynchrony in the populations.

The gonadal indexes of *M. edulis* from the outfall and from the control populations showed the same distinct cycle of gonads increasing in size during summer and fall and dropping to a low in spring (Figure 3). However, gonadal weights of the outfall population were lower than the controls, as can be seen by the generally lower level of the outfall gonadal index, particularly in the April through November samples. Similarly, the body weight/shell volume index for *M. edulis* showed an annual cycle which peaked in summer and dropped in fall and winter to a low in spring (Figure 3). The phase of this body weight/shell volume index was slightly in advance of the gonad cycle. As with the gonad cycle, the outfall population had the same basic body weight/shell volume cycle as the control, but it showed a generally lower level than the controls and indicated that the outfall mussles were in poorer nutritional condition than the controls.

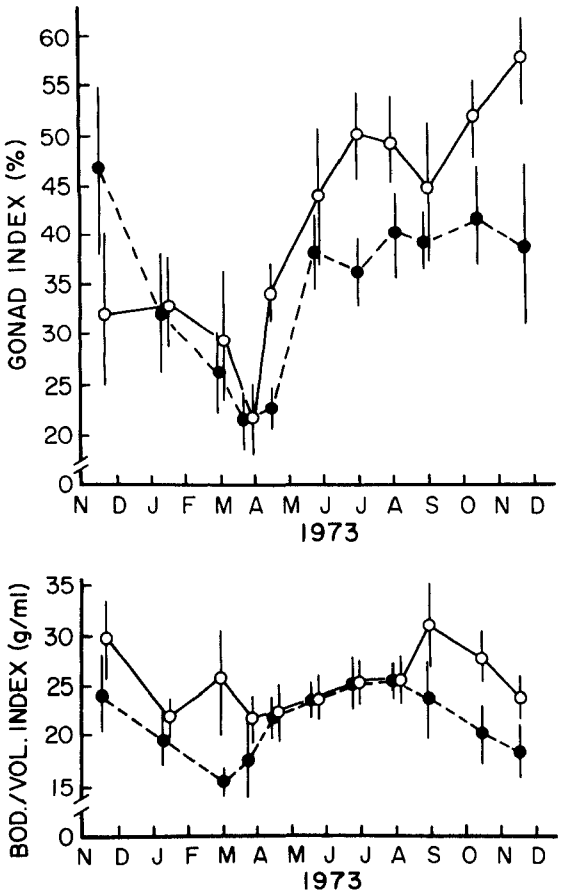

FIGURE 3.—Monthly mean values for the gonad index and body weight/shell volume index for *Mytilus edulis*. Circles = control population; dots = outfall population. Vertical lines are the 95% confidence limits of the means. Each sample was 12 mussels.

In contrast to *M. edulis*, the gonadal index of *M. californianus* did not show a distinct annual cycle (Figure 4). The April and May control samples probably represented a peak of reproductive activity, but the erratic fluctuations of the index made this uncertain without histological information or field observations of spawning. Except for this brief spring peak, the outfall population showed a consistently higher level of ripeness throughout the year than the control mussels. The body weight/shell volume index of *M. californianus* appeared to show a slight annual cycle with a low in March and April and higher levels in late summer (Figure 4). However, this trend was not pronounced and did not appear to correlate with the gonadal index. Contrary to the trend shown by gonadal index levels, outfall body weight/shell volume indexes were consistently lower than the

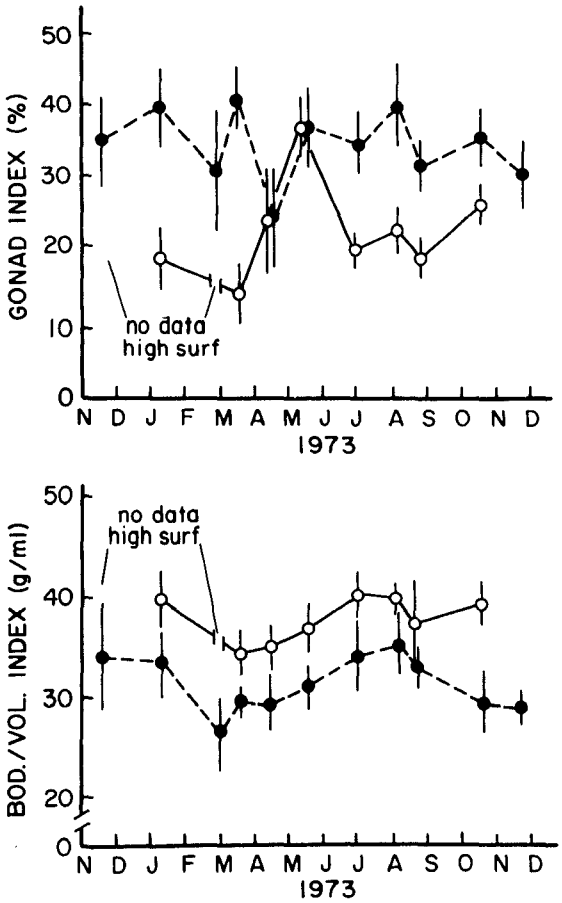

FIGURE 4.—Monthly mean values for the gonad index and body weight/shell volume index for *Mytilus californianus*. Circles = control population; dots = outfall population. Vertical lines are the 95% confidence limits of the means. Each sample was 12 mussels.

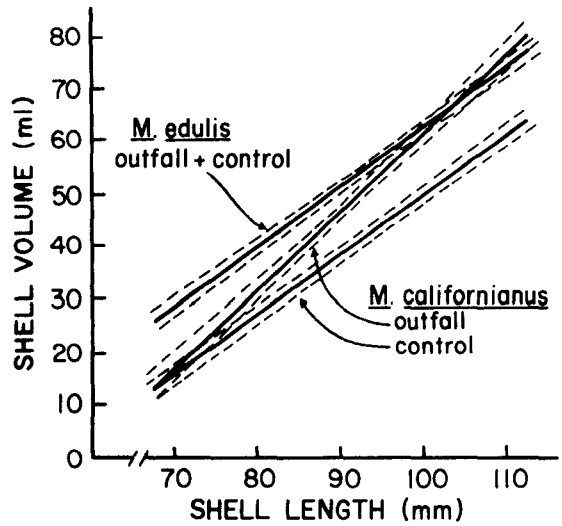

FIGURE 5.—Regressions of shell volume on shell length for all *Mytilus edulis* sampled and for outfall and control samples of *M. californianus*. Dashed lines indicate the 95% confidence limits of the mean predicted shell volumes. For each population of mussels the sample size, correlation coefficient, and regression equation with the 95% confidence limits of the slopes and intercepts in parenthesis was as follows. *Mytilus californianus* outfall: n = 132, r = 0.94, Vol = 1.48(±0.09)Length − 85.8(±8.5); control: n = 96, r = 0.91, Vol = 1.13(±0.11)Length − 63.0(±9.0). *Mytilus edulis* outfall: n = 132, r = 0.94, Vol = 1.07(±0.07)Length − 47.2(±5.2); control: n = 132, r = 0.85, Vol = 1.12(±0.12)Length − 48.4(±10.8); combined: n = 264, r = 0.88, Vol = 1.10(±0.07)Length − 47.9(±6.2).

controls, indicating that the control mussels were in better nutritional condition.

For any given body weight, a larger shell volume will result in a lower body weight/shell volume index. Therefore, the relationship of shell volume to shell length was examined for each of the mussel populations. Over the size range of mussels sampled in the study, this relationship was closely approximated by linear regressions, even though it would probably have been curvilinear if much smaller mussels were included in the samples. Shell volumes of outfall *M. californianus* were proportionally larger than the controls over most of the sizes sampled (Figure 5), such that the slope and the intercept of the regression of shell volume on shell length for outfall mussels were significantly different from those of the regression for the control mussels (t-tests, $P<0.05$). However, for *M. edulis* the slopes and the intercepts were not significantly different between regressions of shell volume on shell length for outfall and control populations (t-tests, $P>0.05$). Therefore, a single, combined regression for both populations of *M. edulis* was calculated (Figure 5). The difference in the shells of *M. californianus* may partly account for the apparent differences in the nutritional condition of the outfall and control populations.

Discussion

The reproductive cycle of *M. edulis* varies with geographical location, but reproductive activity is generally correlated with rising water temperatures (Kinne 1970; Seed 1976). Bayne (1975) showed that gametogenesis is regulated by changing temperatures in terms of increasing "day-degrees." In the present study *M. edulis* from both the outfall and control populations also

showed the same cycle of increasing gonad activity in the late spring and early summer when temperatures were increasing, in spite of the temperature differential between the two areas. However, the outfall population did not attain as high gonadal index levels as the control, probably because stressful temperatures above 20° C were reached in June, leaving less energy available for gamete production (Widdows 1976; 1978a, b). Food availability interacts with temperature to influence the energy budget of mussels (Bayne 1973; Widdows 1978a, b), but food availability estimated by dry weight of suspended matter is not significantly different in the outfall and intake water at Morro Bay (Hargreaves 1977). In July through October outfall temperatures exceeded the energetically extremely stressfull level of 25° C (Widdows 1978a, b), and the body weight/shell volume index declined to levels well below the controls. Although the reduced gonadal index of the outfall population at Morro Bay strongly indicated a reduced reproductive output, *M. edulis* under stress apparently conserve the gonad up to a point at the expense of other tissues, so that stressed mussels continue to produce some gametes (Gabbott and Bayne 1973; Bayne 1975). However, gametes from stressed mussels result in embryos and larvae that are less viable than those produced by adults not under stress (Bayne 1972).

In *M. californianus* the relationship of temperature to the energy budget for growth and reproduction is not well studied as it is in *M. edulis*, nor have the critically stressful temperatures been determined for *M. californianus*. *Mytilus californianus* is reported to reproduce year-round with peak periods of more intense spawning at various times, particularly in spring and fall (Seed 1976). In the present study the control population showed a peak of gonadal activity in spring, corresponding with the period of rising ambient temperatures. The outfall population showed higher gonadal index levels than the controls year-round, indicating that in *M. californianus* higher absolute temperatures, rather than a temperature change as in *M. edulis*, stimulate gametogenesis and increased reproductive output. However, the body weight/shell volume index of *M. californianus* in the outfall was consistently lower than the control population. If *M. californianus* conserves its gonad at the expense of other tissues under the increased energetic stress of elevated temperatures in the same manner as *M. edulis*, this would explain the lower body weights of the outfall mussels.

It is not clear why all but the smallest outfall *M. californianus* in my samples had relatively larger shell volumes at the same shell length than the controls. The difference may reflect greater shell erosion of the control mussels, resulting from high surf levels on the jetty, rather than reflecting a temperature effect on the form of shell growth. Seed (1968) showed that shell growth in *M. edulis* is extremely variable, depending upon population density and physical conditions. *Mytilus californianus* also shows great variation in shell form from one locality to another (Coe and Fox 1944), and intertidal height and latitude also affect shell growth (Dehnel 1956).

It is often difficult to apply results from controlled laboratory conditions directly to field situations, where there are multiple and fluctuating variables. I must acknowledge that the ability to interpret the results of the present paper speaks well for the realistic analysis of energetics and stress in marine mussels in recent laboratory work by others. However, very few marine invertebrates have received this level of study critical for the assessment of complex sublethal effects of environmental disturbances such as thermal effluents.

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ANSON H. HINES

Department of Zoology, University of California
Berkeley, Calif.
Present address: *Center for Coastal Marine Studies*
University of California
Santa Cruz, CA 95064

INCIDENCE AND DISTRIBUTION OF PISCINE ERYTHROCYTIC NECROSIS AND THE MICROSPORIDIAN, *GLUGEA HERTWIGI*, IN RAINBOW SMELT, *OSMERUS MORDAX*, FROM MASSACHUSETTS TO THE CANADIAN MARITIMES

Since the first discovery by Laird and Bullock (1969) of piscine erythrocytic necrosis (PEN) in the red blood cells of the Atlantic cod, *Gadus morhua*; seasnail, *Liparis atlanticus*; and longhorn sculpin, *Myoxocephalus octodecemspinosus*, 15 genera of fishes, including 17 marine species along the North Atlantic coast of North America have been found to be affected by PEN. Sherburne (1977) reported PEN in the alewife, *Alosa pseudoharengus*, and smelt, *Osmerus mordax*. Walker and Sherburne (1977) reported PEN in the Atlantic herring, *Clupea harengus harengus*; Atlantic tomcod, *Microgadus tomcod*; spot, *Leiostomus xanthurus*; tautog, *Tautoga onitis*; rock gunnel, *Pholis gunnellus*; sea raven, *Hemitripterus americanus*; fourspot flounder, *Paralichthys oblongus*; and winter flounder, *Pseudopleuronectes americanus*. Sherburne and Bean (unpubl. data) have found PEN in pollock, *Pollachius virens*; Atlantic menhaden, *Brevoortia tyrannus*; American shad, *Alosa sapidissima*; and blueback herring, *A. aestivalis*.

PEN has been confirmed by electron microscopy as an erythrocytic icosahedral cytoplasmic deoxyribovirus (EICDV) infection in two of the above species—the Atlantic cod (Walker 1971; Appy et al. 1976; Walker and Sherburne 1977) and the Atlantic herring (Philippon et al. 1977; Reno et al. 1978).

During our investigations of PEN in the Atlantic cod, other marine species were examined for evidence of PEN, especially those forming the diet of the cod. One of these was the rainbow smelt, *Osmerus mordax*. Smelt were examined for both PEN and the pathogenic microsporidian parasite, *Glugea hertwigi*.

This report shows the incidence and geographical distribution of PEN and *Glugea hertwigi* in smelt populations from Massachusetts to the