EFFECTS OF THERMAL EFFLUENTS ON REPRODUCTION IN A SEA ANEMONE

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ABSTRACT

Reproduction of the sea anemone *Anthopleura elegantissima* was studied in the thermal-outfall canal of a large (1350-MW) Pacific Gas and Electric Company power plant and at an adjacent control site at Morro Bay, San Luis Obispo County, California. Female reproductive cycles, determined by oocyte diameters, were similar at both sites for 3 years; oocytes appeared in the fall, and spawning occurred in the late summer. Outfall females spawned as much as 1 month before control females, however. Spermatogenesis of outfall males was delayed and compressed compared with control populations, but outfall males developed quickly and spawned before control males. Anemones transplanted from the control to the outfall in November 1975 spawned by the end of March 1976; undisturbed animals from both sites did not spawn until the end of the summer. It is suggested that this species of sea anemone spawns during the summer period of highest annual temperature and that anemones in the outfall have become acclimated to the temperatures there, which are often 10°C above ambient.

The sea anemone *Anthopleura elegantissima* is a conspicuous and abundant intertidal animal along the central California coast (Hand, 1955). It occurs in large numbers in the thermal-outfall canal of the Pacific Gas and Electric Company's large (1350-MW) power plant at Morro Bay, San Luis Obispo County, California.

Ford (1964) showed that *A. elegantissima* exhibits an annual reproductive cycle in central California, with gametogenesis beginning in the winter, oocytes increasing in size through the spring and

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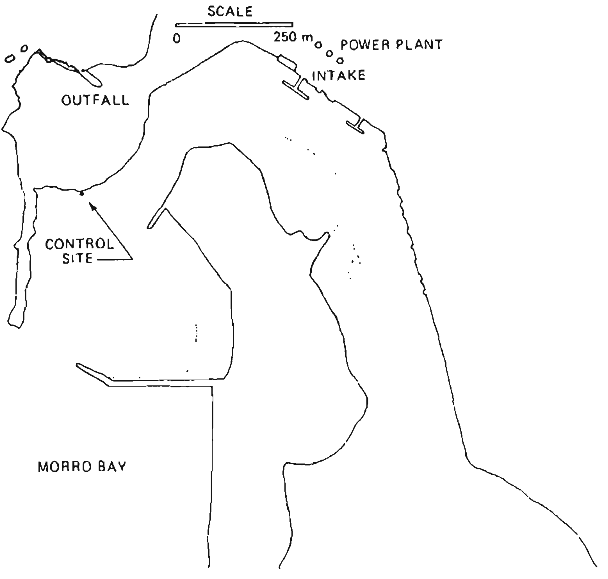

Fig. 1 Collection sites near a 1350-MW Pacific Gas and Electric Company power plant at Morro Bay, San Luis Obispo County, California.

summer, and spawning occurring in the early fall. He speculated that reproduction might somehow be cued by the increasing seawater temperature in summer.

My purpose in this study was to determine whether the timing of reproductive events in this species was altered by subjection to the elevated temperature of a power-plant thermal-outfall canal.

MATERIALS AND METHODS

Twelve to 24 anemones were collected at both control and outfall sites (Fig. 1) at 4- to 8-week intervals between Feb. 4, 1974, and Sept. 27, 1976. Animals were relaxed in 50 : 50 seawater and isotonic $MgCl_2$, cleaned, fixed in Bouin's fluid, dehydrated, embedded in paraffin, and sectioned at thicknesses of 8 to 10 μm on a rotary microtome. Sections were made transversely through the column at the level of the gonads, which run longitudinally from the basal disk to the bottom of the actinopharynx (Stephenson, 1928). The sections were mounted on glass slides, stained with picro-indigo carmine and basic fuchsin, and examined microscopically.

Oocytes from each identifiable female were measured with a calibrated ocular micrometer, after the method of Dunn (1975), to assess female maturation over time at both sites. At least 50 gametogenic cells were measured from one section of each female. As a correction for differences in the diameters of oocytes due to the plane of the section, only cells having a distinct nucleolus were included in the total. If fewer than 50 cells were present in the section, all gametogenic cells were measured regardless of position. Males were classified into arbitrary stages based on an assessment of their gonadal maturity (Table 1).

Reciprocal transplants of anemones were made between the outfall and control sites on Nov. 17, 1975. Eight cages with 1.25-cm-mesh stainless-steel screen fronts, sides, and tops; masonite floors; and neoprene mesh backs held together by nylon fishing line were built. Sixty anemones collected from one clone in the control area were distributed evenly among four cages. Two cages were hung at zero tidal height at the power-plant intake structure (caged experimental control group), and two were hung in the outfall canal (transplants). Similar procedures were followed for four cages of outfall animals. The cages were collected on Mar. 25, 1976, and the anemones were analyzed histologically.

RESULTS

Anthopleura elegantissima at the Morro Bay control site reproduced sexually on an annual cycle (Fig. 2). Although the January 1974 control collections were anomalous and there were no females in the samples again until July, it is clear that oocyte diameter reached a peak in summer, followed by spawning between August and September. No oocytes were visible again in prepared sections until the end of December 1974, but they increased in size through the spring and summer, and spawning occurred again in August 1975. The third year followed this pattern, with oocytes first visible in October and a gradual increase in diameter leading to spawning between August and September 1976.

In the power-plant thermal outfall at Morro Bay, distinct annual cycles of gametogenesis and spawning were obtained (Fig. 3). No gonads were found in the first samples collected (Feb. 4, 1974), but

TABLE 1

MATURATION STAGES OF MALES

Stage	Description
Stage 1	First appearance of spermatogonia
Stage 2	Spermatogonial proliferation; swelling of spermatogonial vesicles to occupy one-fourth of mesenterial width
Stage 3	First appearance of spermatocytes; further vesicular swelling (up to one-half of mesenterial width)
Stage 4	First appearance of tailed sperm; vesicles occupy up to three-fourths of mesenterial width
Stage 5	Gonads ripe, occupy entire width of mesentery
Spawning	Sperm vesicles breaking down; many tailed sperm present
Residual	Only a few unspawned sperm remain

this may be an artifact of early collecting technique (i.e., all small anemones from a single clone). Beginning in March, oocytes appeared and grew through the summer. An examination of live material indicated that some females were spawning on July 21, 1974. Subsequent analysis of the histologically prepared material supported this finding. Spawning continued for more than a month; samples taken Aug. 18 showed two females ripe and one having spawned. By Sept. 17, only residual oocytes remained. Small oocytes appeared again at the end of October and developed through the winter and spring, reaching peak size by July 11, 1975. Spawning was not observed until Aug. 8, by which time it was complete, except for a few residual gametes that persisted until early October. The third year showed the same course of development.

Spermatogonia were present in samples from the Morro Bay control site in February, and the first few tailed sperm had appeared by March 1974 (Fig. 4). Ripe gonads occurred by April, and spawning had begun by Aug. 18. Although spawning was apparently complete by Sept. 17, residual gametes remained through the end of October. The 1975 pattern was quite similar to that found in 1974, with ripe sperm appearing as early as April and spawning beginning in August. A few late-spawning individuals were collected on Nov. 17 although the third year's cycle had already begun. The cycle was repeated in 1976.

In the outfall, only 14 of 27 samples taken over the course of this project had male gonads present; all these occurred between late spring and early fall. Males ranging from early spermatogonial proliferation to first presence of tailed sperm were found in April 1974, and the population was ripe by May. Although the June

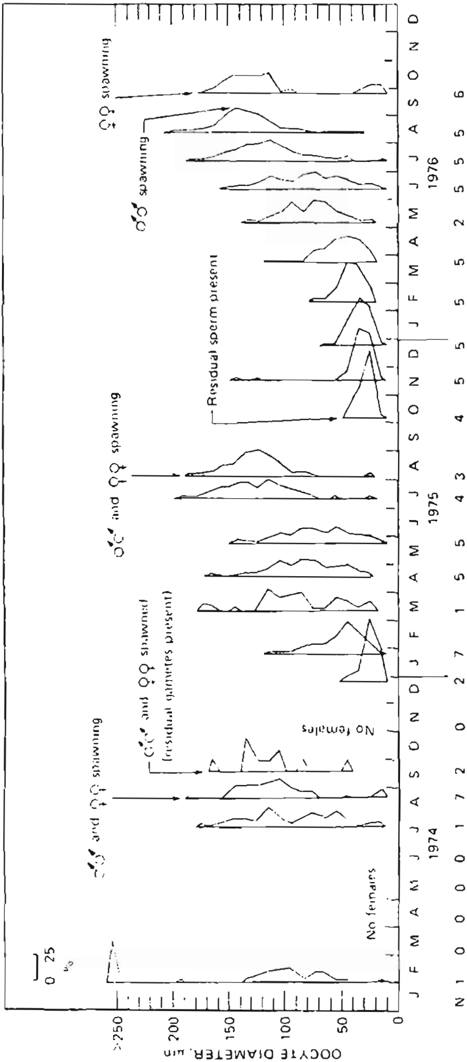

Fig. 2 Oocyte growth cycles of *A. elegantissima* at the Morro Bay control site.

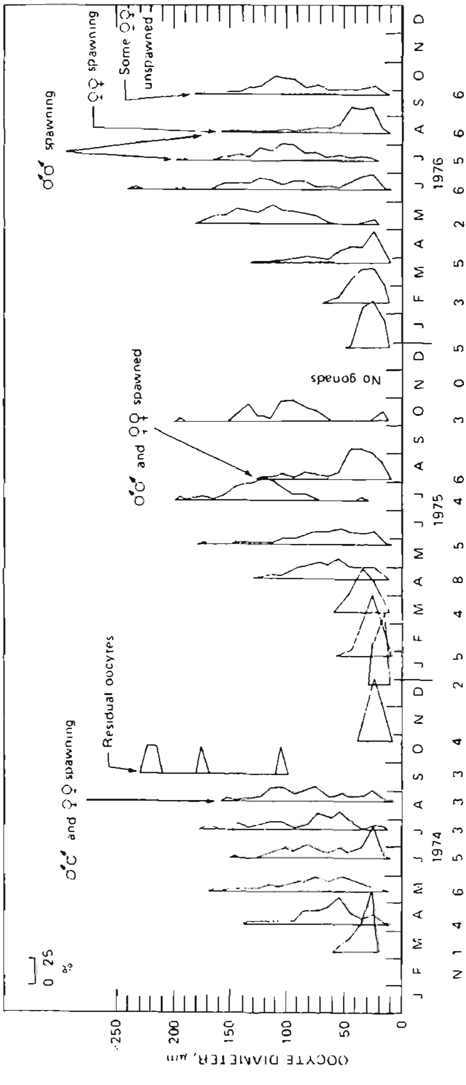

Fig. 3 Oocyte growth in the power-plant outfall.

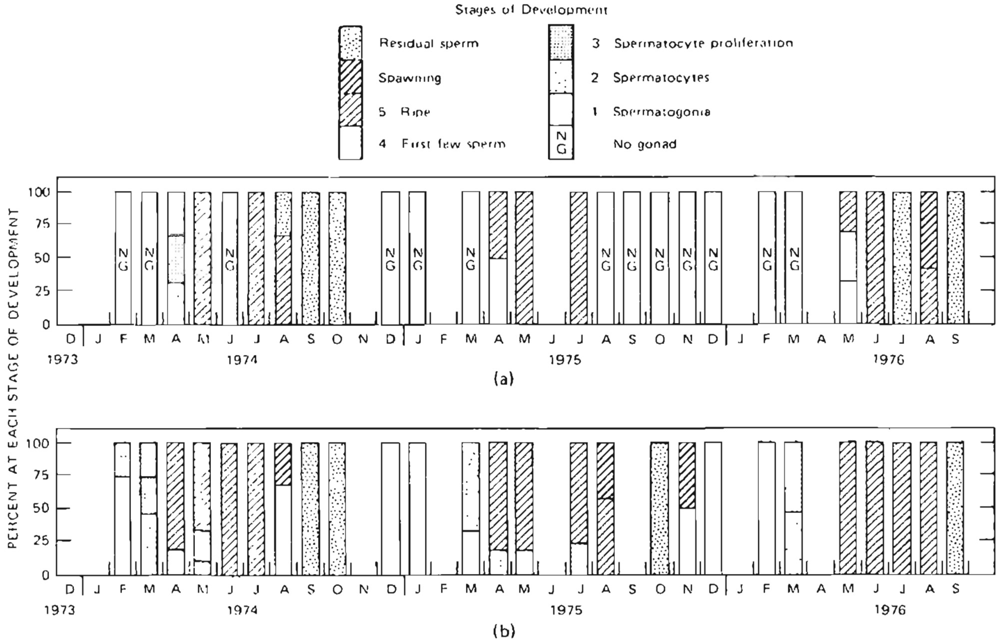

Fig. 4 Percentages of male *A. elegantissima* at each maturation stage. (a) At the power-plant outfall. (b) Control.

samples contained no males (possibly a sampling artifact), males with mature sperm were present in July, and by August some of the population had spawned. Residual gametes persisted until October. In 1975, no gonads could be found until April, when anemones with only early spermatogonia occurred along with those with ripe gonads. Mature sperm remained through July, but by Aug. 8 there were no identifiable males. This situation remained until May 1976, when again a variety of stages of maturity appeared at once. Although only residual gametes were found on July 13 (this indicated that some of the population had spawned), the August sample again contained ripe anemones, as well as some which were spawning. By September, only residual sperm were found.

Outfall temperatures were significantly higher than those of the control area throughout the project, except during April and May 1975 (Fig. 5). Control temperatures showed a rise through the spring to a summer plateau in all 3 years and a subsequent drop-off each winter. Outfall temperatures showed a similar pattern, but at a considerably higher level. Fluctuations in outfall temperatures were caused by variations in power-plant activity and occasionally by high spring tides, which backflushed the outfall canal to some extent.

Of the anemones collected on Mar. 25, 1976, from the transplant cages, the size-frequency histograms (Fig. 6) of outfall (where five of the six anemones examined had gonads) and outfall caged but not transplanted (three of seven had gonads) animals were very similar to those of unmanipulated anemones from the Morro Bay control site (where five of six had gonads). Of the control-site anemones transplanted to the outfall, however, two showed a much larger average oocyte diameter, and three more had spawned. Unfortunately, the transplant cages hung in the intake area became silted up, and the anemones were lost.

DISCUSSION

Numerous studies have correlated seasonally changing sea temperatures with the timing of reproductive events in marine invertebrates (see Kinne, 1970; Giese and Pearse, 1974, for reviews). Hedgpeth and Gonor (1969) suggested that temperature is the major environmental factor controlling reproductive cycles of benthic marine invertebrates, but they added that photoperiod and other factors are also important. Thorson (1946), Giese (1959), and others cautioned that a distinction must be made between the effect of gradual temperature changes on gametogenesis and that of a sharp temperature change on spawning.

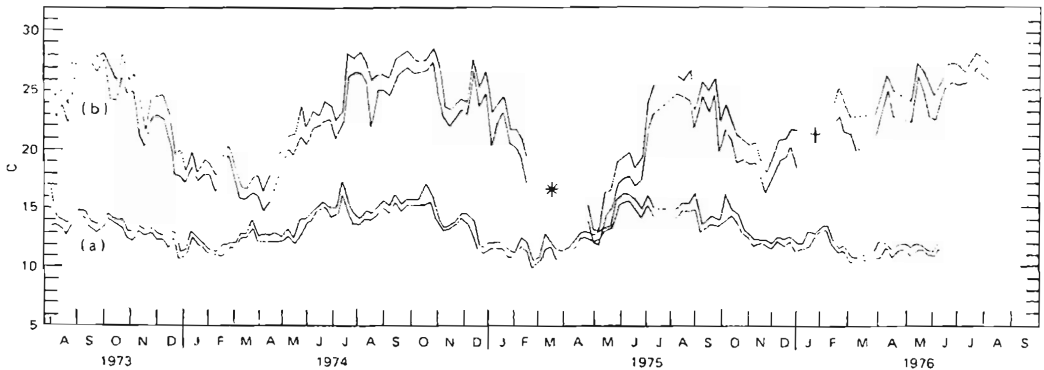

Fig. 5 Comparison of seawater temperatures of Morro Bay control (a) and outfall (b) during this study. Temperatures were measured with Ryan 45-day temperature recorders and are ±2 standard errors of weekly means (28 6-hr means estimated from continuous traces). Breaks in the outfall curves (b) in 1975 (*) occurred because a recorder was lost and in 1976 (†), because of recorder malfunction.

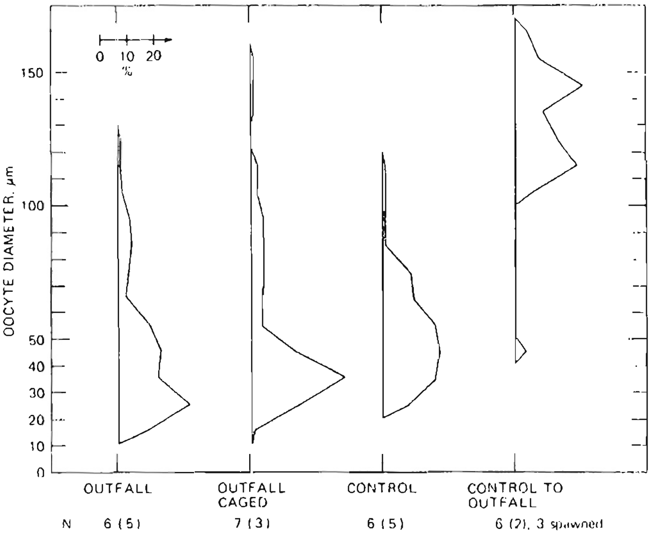

Fig. 6 Oocyte size-frequency histograms from the Mar. 25, 1976, transplant collections. N is the number of animals examined, and the numbers in parentheses are those having gonads.

Although temperatures are significantly higher in the Morro Bay outfall than at the control site (Fig. 5), the reproductive cycles of females appear to parallel each other well, except for the timing of spawning. The observed early spawning of outfall animals was consistent from one year to the next and suggests a possible effect of the warm-water regime on this critical aspect of reproduction. Comparing the timing of spawning of outfall anemones (Fig. 3) with the temperature data shows that, in 1974 and 1975, anemones spawned within 2 weeks of the time that mean temperatures first exceeded 25°C. In both years this temperature was reached abruptly. In 1976, spawning did not occur until the mean temperature had stabilized at 26°C; earlier brief fluctuations above this point did not bring about spawning. It is possible that anemones are unable to retain and nourish oocytes at sustained temperatures above this threshold level because of the metabolic demands placed upon them by these high temperatures.

In the Morro Bay control populations, female spawning also occurs during periods of maximum temperature, but not with the same timing exhibited by anemones in the outfall. Spawning took place in the control samples during periods of mean temperatures between 14 and 16°C, but it did not occur until a month after these temperatures had been reached. In 1974 and 1975, these temperatures were reached in June, but spawning did not occur until July. In 1976, these temperatures were reached in July, and the population did not spawn until September. A similar spawning pattern was seen by Ford (1964) in populations from Marin County and by Jennison (1977) in Sonoma County, California.

Despite the significant temperature differences between outfall and control areas, we see the same general pattern of a winter temperature decline and a summer increase at both sites. If anemones time their reproductive events by temperature cues, it is likely that a predictable pattern of temperature change would be more important than the absolute temperature level (Giese and Pearse, 1974). Galtsoff (1961) observed that different physiological races of the same species may spawn at different temperatures. Outfall anemones, although not necessarily of a different race than those in the control, may, nevertheless, be acclimated to the warm-water regime in which they are found. Possibly they are responding at 25°C to the same cues that affect the control animals at 15°C. The difference in response time (2 weeks in the outfall to 1 month in the control) may be simply a function of the more rapid metabolic rate of outfall anemones caused by the higher absolute temperatures in which they occur. The delay in spawning of outfall females in 1976 might have resulted from the gradual rise in temperature, which permitted a period of metabolic accommodation. Thus the precipitous spawning that was observed in 1974 and 1975, when the temperature threshold was reached abruptly, did not occur.

Males of *A. elegantissima* at the Morro Bay control site develop over an annual time course similar to that of the females, with early gametogenic stages proliferating in February and March, ripe sperm apparent by April, and spawning occurring between August and September in each of the 3 years studied (Fig. 4). Males in the outfall, however, showed an abbreviated reproductive season, with no early stages visible before April of each year. This appears to be caused by the high temperatures in the outfall. Male development is suppressed until late spring, possibly for metabolic reasons, but sperm mature in outfall populations by May each year. It is not unreasonable to assert that males of *A. elegantissima* can develop quickly. Using autoradiography, Beeman (1970) determined that it would take a minimum of 10 days to develop free spermatozoa from primary spermatocytes in the opisthobranch *Phyllaplysia taylori*. Only 9 days were required in the sea urchin *Strongylocentrotus purpuratus* (Holland and Giese, 1965).

Despite the delayed and compressed cycle exhibited by outfall males, these anemones spawn at about the same time as do their control counterparts. Anemones in the outfall began spawning sometime after July 21 and had finished by Sept. 17, 1974, and control animals spawned during the same period. In 1975, no spawning was observed in the outfall, but, since ripe gonads were found on July 11 and no gonads could be seen by Aug. 8, we can infer that spawning occurred between these two dates. In the control, anemones were spawning on Aug. 8. Not until 1976 was there an appreciable difference in the timing of spawning of the two populations. All outfall anemones collected on July 13, 1976, had spawned; others in the same clone were still ripe or spawning on Aug. 11; but all had spawned by the end of September. In the control, complete spawning took place between Aug. 11 and Sept. 27.

In the transplant experiment, outfall, control, and outfall caged but not transplanted anemones all showed very similar oocyte size-frequency histograms (Fig. 6). Control anemones that were transplanted to the outfall underwent a rapid maturation, however. Of six anemones sectioned, two were ripe, and three more had recently spawned. This occurred during a period of increasing temperature in the outfall (the temperature at the time of the March collection was around 22°C), and it appears that increasing temperature accelerated vitellogenesis and induced spawning in anemones from the cold-water control area. Anemones acclimated to the outfall do not normally spawn until summer, when the outfall reaches its average maximum temperature of 25°C, but by March 1976 the control anemones that were transplanted into the outfall were already 7°C past the 15°C maximum that the control area reaches in summer.

CONCLUSIONS

The sea anemone *Anthopleura elegantissima* reproduces on an annual cycle at Morro Bay, with gametogenic cells being visible first in late fall and winter and spawning occurring in late summer in natural populations.

Males of *A. elegantissima* from a thermal-outfall canal exhibit a delayed onset of gametogenesis but spawn as much as 1 month before those in natural populations. Females in the outfall begin gametogenesis at the same time as control animals but spawn earlier.

Control females transplanted to the outfall in November 1975 underwent accelerated vitellogenesis and spawned by the end of March 1976, 4 to 5 months ahead of either unmanipulated control or acclimated outfall anemones.

Control anemones spawn during the period of peak summer temperatures, which may reach 15°C. Outfall anemones spawn after temperatures in the thermal canal reach 25°C. It is suggested that outfall anemones have become acclimated to the warmer water (which is regularly 10°C higher in temperature than that in the control area) and are responding at 25°C to the same temperature cues that influence spawning in control populations at 15°C.

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