Impact of Wastewater on Reproduction of *Amphiodia urtica*

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The red brittlestar (*Amphiodia urtica* (Lyman 1860)) is one of the most common benthic invertebrates on the continental shelf in the Southern California Bight (Jones 1969, Thompson et al 1987). Densities over 4,000 individuals/m^2 have been measured, but average near 1,200 individuals/m^2 when favorable environmental conditions exist (Thompson et al 1993). Optimal depths for *Amphiodia* spp. are 48 to 102 m, particularly when sediments are composed of coarse silts or fine sands (median grain sizes between 0.035 to 0.093 mm) (SCCWRP 1994a, Bergen 1996). Although this species is often the numerically dominant invertebrate on the continental shelf, little is known about its life history or reproductive cycle.

Unlike other areas of the Southern California Bight, *A. urtica* is rare or absent near municipal wastewater outfalls, even when depth and sediment grain size are favorable. Its abundance was greatly reduced in moderately contaminated areas near municipal wastewater outfalls compared to reference areas in Santa Monica Bay and on the Point Loma shelf in 1990 (SCCWRP 1994b). Abundances at these moderately impacted areas were generally an order of magnitude less than at reference locations. Likewise, a gradient of decreasing *Amphiodia* spp. abundance was measured approaching the County Sanitation Districts of Orange County wastewater outfall (SCCWRP 1995b). The factors limiting its abundance near wastewater outfalls are, as yet, unknown.

Reproductive effects, such as a reduction in fertility or fecundity, are possible explanations for the decline in *A. urtica* abundance near wastewater outfalls. Thompson and Bergen (SCCWRP 1994b) examined gonads and measured the size of female oocytes in an assessment of its growth and reproduction off Point Loma and in Santa Monica Bay. At the reference sites, adults greater than 1.0 mm in oral width (OW) consistently contained developed gonad and were considered reproductively mature. Large oocytes were found in spring, summer, fall, and winter indicating that spawning may occur year-round at some low level. At Point Loma, there were proportionately more small oocytes in the spring and more large oocytes in the fall and winter. However, the number of adults were greatly reduced at the moderately impacted sites and an insufficient number of females were collected for an assessment of reproduction at those locations. Hence, more information is needed on the impact of wastewater discharge on *A. urtica* reproduction.

The objective of the current study was to describe the reproductive cycle of female *A. urtica* and to determine if it has been impacted near a municipal wastewater outfall.

MATERIALS AND METHODS

Sediment samples were collected near the City of San Diego Wastewater Treatment Plant outfall off Point Loma from a moderately impacted station (PLI) and a reference station (PLR) (Figure 1). These were the same stations sampled by Thompson and Bergen (SCCWRP 1994b) and are part of the City of San Diego's Ocean Monitoring Program (Stations A9 and B3, respectively). At each station, sediments were collected monthly between July 1993 and June 1994 using a 0.1 m^2 chain-rigged Van Veen grab and sieved through a 1.0 mm mesh screen. Relatively large *A. urtica* were selected from the material retained on the screen. Sampling continued until approximately 50 specimens were collected. These were then preserved in glass jars filled with 10% borax-buffered Formalin-seawater solution. After 48

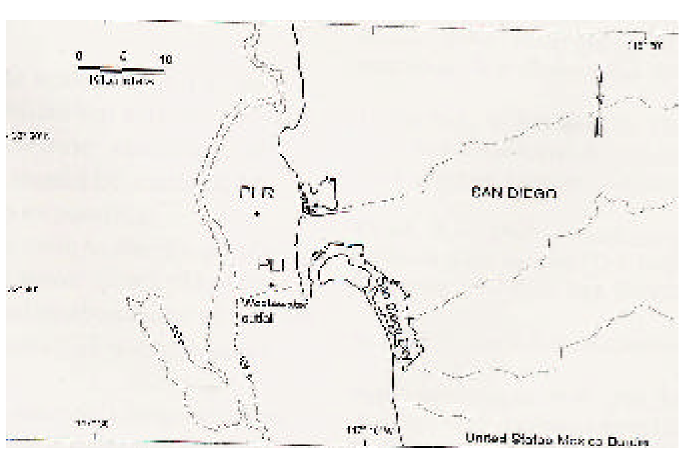

FIGURE 1. Location of sampling stations for *Amphiodia urtica* off Point Loma, California, in 1993 - 1994.

h, the specimens were transferred into 70% ethanol. In the laboratory, between 13 and 15 of the largest female specimens were chosen for histological examination. The oral width (OW) and disk diameter (DD) of each was measured prior to gonad dissection. The largest specimens were chosen since they were more likely to be reproductively mature and hence, could provide sufficient tissue for histological examination. In October, 1993, and again in February and May, 1995, two additional Van Veen grab samples were collected at each station and all *Amphiodia* spp. which were retained on a 0.3 mm mesh screen were enumerated and measured as part of a tangential study off Point Loma.

To prepare slides for histological examination, female gonads were removed and decalcified with EDTA and mild HCl. Gonads were dehydrated with a series of graded alcohols, cleared with xylene, and mounted in paraffin. Cross sections (5 µm thick) of gonads were prepared using a microtome, mounted on a microscope slide, and stained with Harris' Hematoxylin and Eosin B.

Fifty oocytes per female were measured using a compound microscope and ocular micrometer. Only oocytes sectioned through the nucleolus were measured and for those with distorted shapes, the largest diameter was measured. To avoid measurement duplication, transects were established across each gonad cross-section. Transects were selected at random to minimize any location bias. Several cross-sections per female were required to obtain a sample size of 50 oocyte measurements.

In total, 131 sediment samples were collected during this one year study. Altogether, 352 *A. urtica* specimens were chosen for histological examination from which 17,600 oocytes were measured. More samples were required at PLI compared to PLR to capture enough females for dissection. The number of grab samples per month required to collect gravid females at PLI ranged from 6 to 11 and averaged 8.5. In contrast, the number of grab samples per month at PLR ranged from 2 to 3 and averaged 2.4. The difference in sampling effort was primarily due to the reduced overall abundance at PLI for this species.

Data analysis included an assessment of differences in parental size as measured by OW. All OW and oocyte diameter measurements were recorded to the nearest 0.001 mm. Differences in parental size between station locations were tested using a Mann-Whitney Rank Sum procedure since the selection of parents was not random and the variances was not homogeneous. Differences in parental size among months within each station was tested using analysis of variance (ANOVA) after testing for normality (by Kolmogorov-Smirnoff) and homogeneity of variances (by Levene Median test). A Student-Neuman-Keuls *post hoc* comparison (SNK Test) was used to determine significant differences among the months. Size-frequency histograms with oocyte size classes of 0.005 mm were used to assess trends in the seasonal distribution of oocyte diameter. A G-Test was used to determine if the proportion of selected oocyte size ranges from PLI differed from PLR.

RESULTS

Small but significant differences were observed in the OW of adult female *Amphiodia urtica* used for oocyte examination between moderately-impacted and reference stations (PLI and PLR, respectively) when all samples for each location were combined (Mann-Whitney $T = 26219.5$, $df = 177,179$, $P < 0.01$) (Table 1). Mean monthly brittlestar OW ranged from 1.44 to 1.58 mm at PLR and from 1.56 to 1.68 mm at PLI. Coefficients of variation for OW during each month were typically less than 10% at both stations. No apparent seasonal trend was observed in the OW of the mature female adults used for histological examination. All months from PLI were considered to be similar (ANOVA $F = 0.751$, $P = 0.688$). However, at PLR the monthly mean OW of adult female *A. urtica* were statistically different (ANOVA $F = 2.46$, $P = 0.007$). A SNK Test indicated that the OW of adults sampled during January 1994 were significantly smaller than adults sampled during September, 1993 or June, 1994.

The size-frequency distribution of OW from adult *A. urtica* subsampled for histological examination were compared to the entire adult populations sampled to determine if the differences in OW between PLR and PLI were a result of sampling bias (Figure 2). The comparisons represent a combination of three months: October, February, and May. At PLR, the size-frequency distribution of OW between the histology subsample and the observed adult population were relatively similar (Figure 2a). Adults in both the population subsampled for histology and the observed population recorded peak frequencies of 1.45 to 1.60 mm diameter. The size-frequency distribution of OW for adults at PLI demonstrated that the subsample for histology and the observed population were dissimilar (Figure 2b). The OW of histology subsampled specimens were skewed towards larger individuals compared to the observed population at PLI. The OWs of the majority of adult *A. urtica* subsampled for histology were greater than 1.5 mm diameter while the OWs of the majority of adults from the observed population were less than 1.5 mm diameter.

Oocyte diameter size classes at both stations ranged from 0.005 to 0.100 mm over the entire year (Figure 3a and b). Every month contained small (< 0.03 mm) and large (>

0.07 mm) oocytes. The mean oocyte diameter for the entire year at PLI was slightly larger (0.045 vs. 0.031 mm) and less variable (61.2 vs. 73.7 % CV) compared to PLR. The difference between their means was statistically significant (Mann-Whitney T = 55542769.5, $P < 0.001$). Mean OD and maximum OD were independent of OW when compared on a monthly basis.

TABLE 1. Mean oral widths of female *Amphiodia urtica* examined histologically, by month, from moderately impacted (PLI) and reference (PLR) stations off Point Loma, California.

	Station PLI			Station PLR		
		Oral Width (mm)			Oral Width (mm)	
Date	n	Mean	SD	n	Mean	SD
Jul 93	15	1.59	0.15	15	1.55	0.12
Aug 93	15	1.55	0.16	15	1.47	0.08
Sep 93	15	1.56	0.12	15	1.58	0.10
Oct 93	15	1.62	0.16	14	1.54	0.08
Nov 93	15	1.68	0.15	15	1.50	0.12
Dec 93	15	1.61	0.11	15	1.53	0.09
Jan 94	15	1.61	0.17	15	1.44	0.13
Feb 94	15	1.65	0.12	13	1.52	0.08
Mar 94	15	1.61	0.14	13	1.54	0.12
Apr 94	15	1.62	0.16	15	1.51	0.11
May 94	14	1.60	0.14	14	1.55	0.10
Jun 94	14	1.60	0.14	15	1.57	0.10

SD=standard deviation.

The relationship between large and small oocytes changed over time at both stations. At PLR, nearly 67% of the oocytes measured were less than 0.03 mm diameter in July (Figure 4a). This proportion dropped to fewer than 31% in November as oocytes moved into larger size classes. The proportion of small oocytes of this size steadily increased from December through February as new gamete formation began (Figure 3a). In February, 94% of all oocytes at PLR were less than 0.03 mm diameter. A very similar pattern of changes in the proportion of small oocytes over time was observed at PLI (Figures 3b and 4a). Both sites exhibited coincident peaks in maximum numbers of small gametes and a distinct progression of oocyte-diameter size classes. The most noticeable difference between the two sites was that PLR had proportionately more small oocytes nearly every month sampled. The frequency of small oocytes observed at PLI was significantly different than those at PLR ($G = 100.56$, $P < 0.001$).

The relative changes in the large (> 0.07 mm diameter) oocytes at both stations were, for the most part, inversely related to the trends observed from changes in the small oocytes described above (Figures 3a and b, and 4b). When the proportion of small oocytes decreased, the proportion of large oocytes increased. PLR exhibited a dramatic shift in oocyte diameter from large to small gametes between December and January indicating that the larger oocytes had either been spawned or resorbed. PLI lagged by one month compared to PLR; the shift from large to small oocytes occurred between January and February. In addition, there were proportionately more large oocytes every month at PLI. At least 10% of all oocytes were greater than 0.07 mm

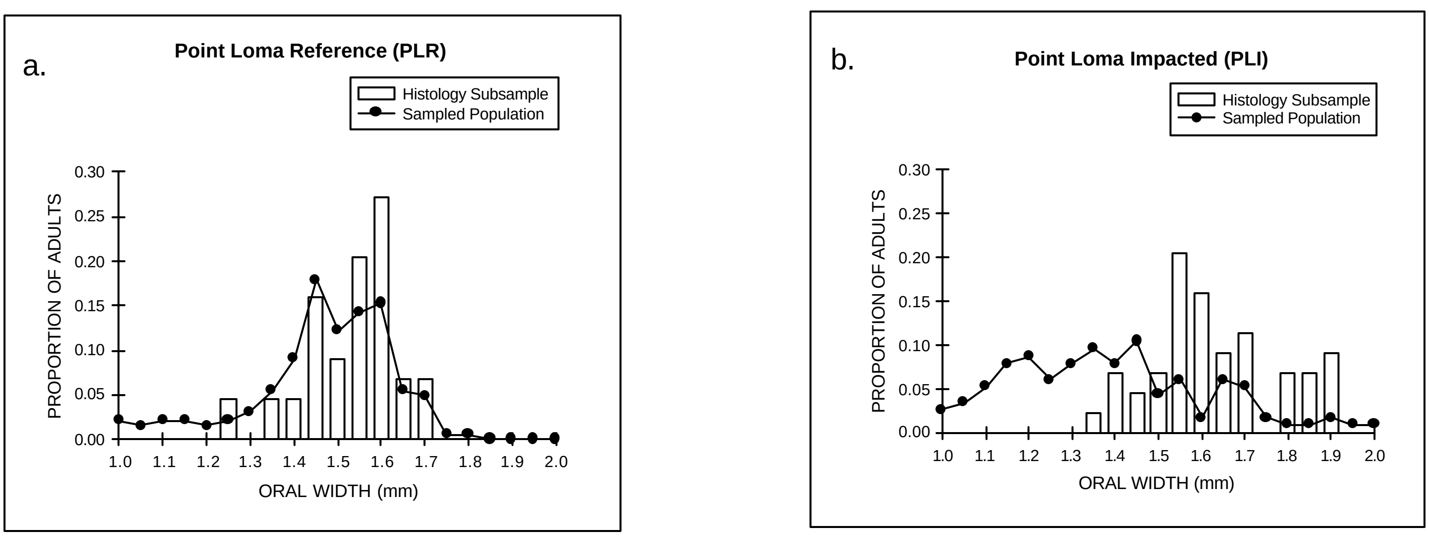

FIGURE 2. Distribution of *Amphiodia urtica* oral width (OW) measurements for the entire sampled population and the population subsampled for histological analysis at a) the reference site (PLR) and b) the moderately impacted site (PLI) off Point Loma, California.

diameter for 10 of 12 months at PLI. At PLR, only five of 12 months had 10% or more eggs greater 0.07 mm diameter. The difference in the frequency of larger oocytes at PLI compared to PLR was significant (G = 195.89, P < 0.001).

Following the distinct decrease in large oocytes in winter, an increase in *Amphiodia* spp. recruitment was observed in spring at the same stations from which specimens for the oocyte studies were collected (Figure 5). In February, 15% of all specimens collected PLI were considered new recruits (< 0.05 mm OW); by May, 69% were new recruits. The number of young recruits increased nearly 50 individuals/0.1 m^2. The population at PLR was more balanced between seasons and between age classes. Still, the number of new recruits increased from 12% to 26% of all specimens between February and May. The net recruitment at PLR was 21 individuals/0.01 m^2.

DISCUSSION

Based upon the results of this one-year study, *A. urtica* is predominantly a seasonal spawning ophiuroid. At the reference location, a distinct progression of oocyte diameter size classes can be observed in fall and winter. Between September and December, the proportion of oocytes greater than 0.07 mm diameter steadily increased from 10 to 36% of all female gametes. In January, a dramatic shift from larger to smaller sized oocytes occurred, presumably as a result of spawning. Large oocytes were present, but comprised less than 10% of all female gametes the remainder of the year.

Pulsed recruitment further indicates seasonal periodicity in the *A. urtica* reproductive cycle. Newly recruited *Amphiodia* spp. doubled at the reference station and quadrupled at the moderately impacted station within several months of spawned oocytes. This is consistent with observed springtime increases of the smallest *Amphiodia* spp. in Santa Monica Bay and off Point Loma in 1990 as well as off Orange County in 1991 (SCCWRP 1994b, 1995b). Similar to the results herein, relatively fewer large oocytes and young recruits occurred other times of the year during those studies.

Annual periodicities in reproductive cycles have been observed elsewhere for other species in the family Amphiuridae (Hendler 1991) including *Amphipholis squamata* (Hendler 1975). Size frequency results by Bowmer (1982) showed a similar progression and relative abundance of large sized oocytes in *Amphiura filiformis* during its three month spawning period (June through August). Gonad indices (gonad weight/disk weight) reported for *Amphiodia occidentalis* increased sharply from March through May and abruptly decreased after spawning in June (Rumrill and Pearse 1984). Fenaux (1970) described *Amphiura chiajei* as a seasonal spawner (autumn/winter), but reduced levels of mature gonads and developing planktonic larvae were observed year round. The exogenous cues which may stimulate oocyte growth and spawning in *A. urtica* are unknown. Several environmental factors have been shown in other species of ophiuroids including temperature, food availability, day length/lunar cycle, and currents (Hendler 1991).

There was no indication that reproductive cycles were inhibited at PLI compared to PLR. At both stations, similar patterns over time in the size-frequency distributions of oocytes were observed. The proportion of large oocytes increased during the fall, peaked in winter and decreased sharply in January and February at PLR and PLI, respectively. Overall, there was a greater proportion of larger sized oocytes at PLI compared to PLR; up to 20% more potentially ripe oocytes were measured every month at PLI. The significance of the increased proportion of larger oocytes and the one month lag in shedding those oocytes at PLI relative to PLR is unclear. The difference could be the result of interpopulation differences, subtle changes in exogenous cues, sampling bias, or the treated wastewater discharges. Previous laboratory studies (SCCWRP 1994c, 1995a) have not shown a significant increase or decrease in oocyte diameter from females exposed to sediments collected near outfalls compared to those exposed to sediments from reference areas. Thus, reductions in populations of *A. urtica* near wastewater outfalls do not appear to result from reductions in the growth of female gametes.

CONCLUSIONS

Amphiodia urtica can produce large oocytes year-round, but spawning peaks in winter. Based upon size-frequency distributions of 17,600 oocytes, gametes grow September through December and are shed in January and February. A substantial increase in newly recruited *Amphiodia* spp. was observed the following spring. Reference (PLR) and outfall (PLI) areas had similar patterns in their size-frequency distributions of oocytes. Thus, reductions in *Amphiodia* spp. populations near outfalls are not a result of impacts to the female reproductive cycle such as a reduction in oocyte growth.

a.

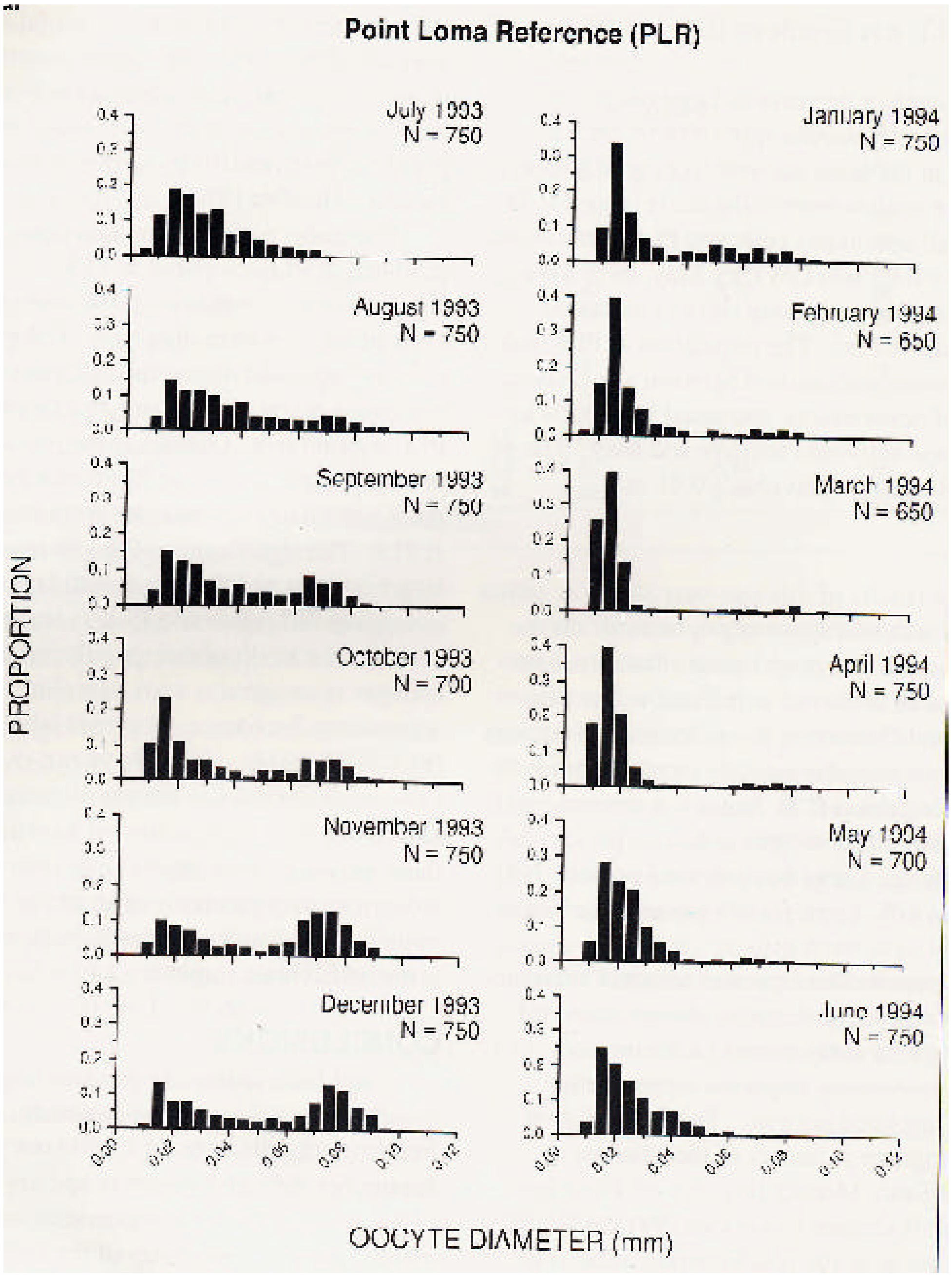

FIGURE 3. Distribution of *Amphiodia urtica* oocyte diameter measurements for

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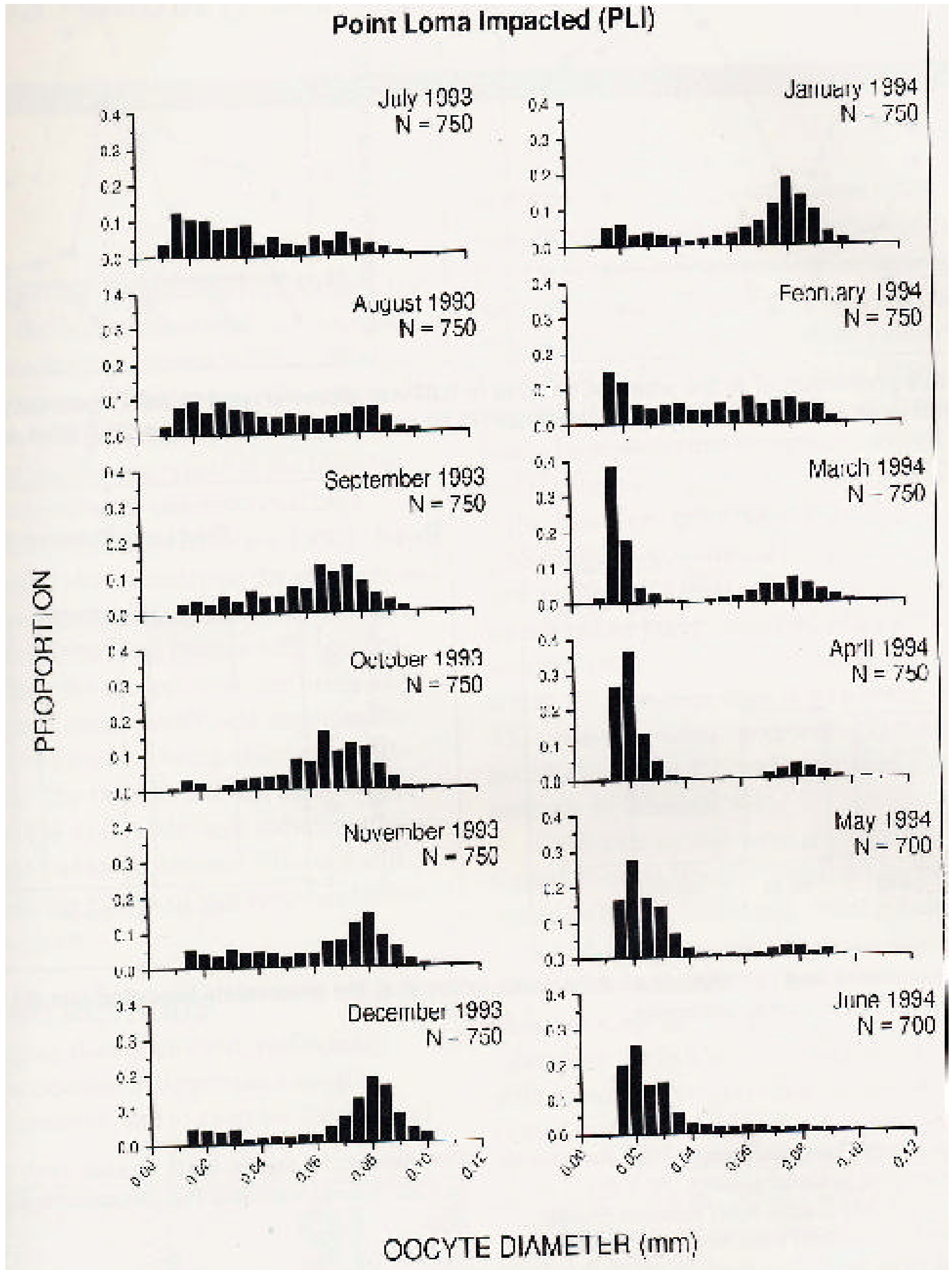
b.



a) the reference site (PLR) and b) the moderately impacted site (PLI) off Point Loma, California.

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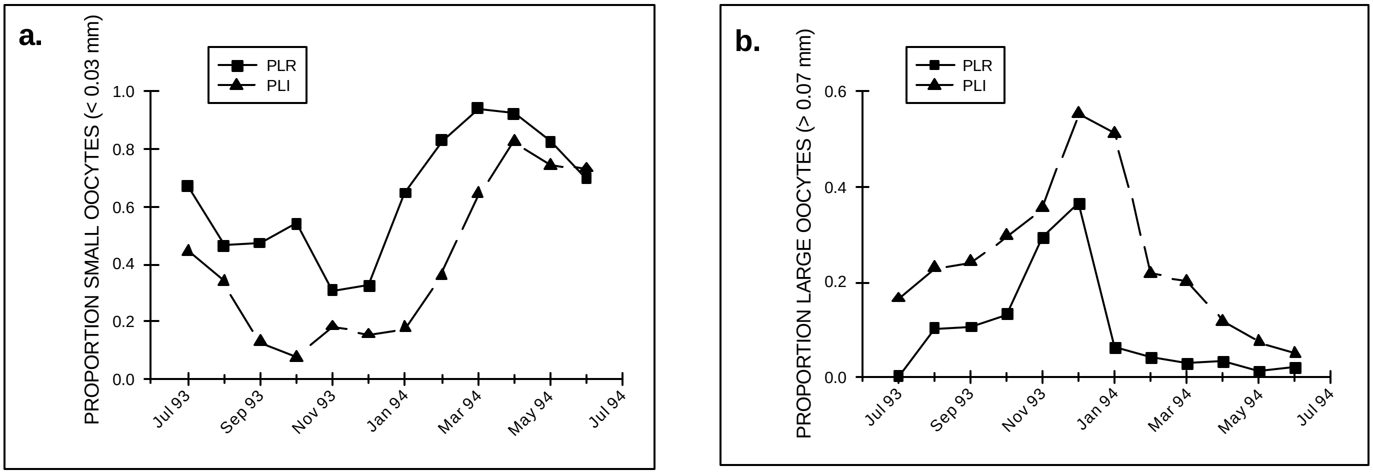

FIGURE 4. Relative proportion of a) the smallest oocytes (< 0.03 mm diameter) and b) the largest oocytes (> 0.07 mm diameter) for *Amphiodia urtica* by month at the reference (PLR) and moderately impacted (PLI) sites off Point Loma, California.

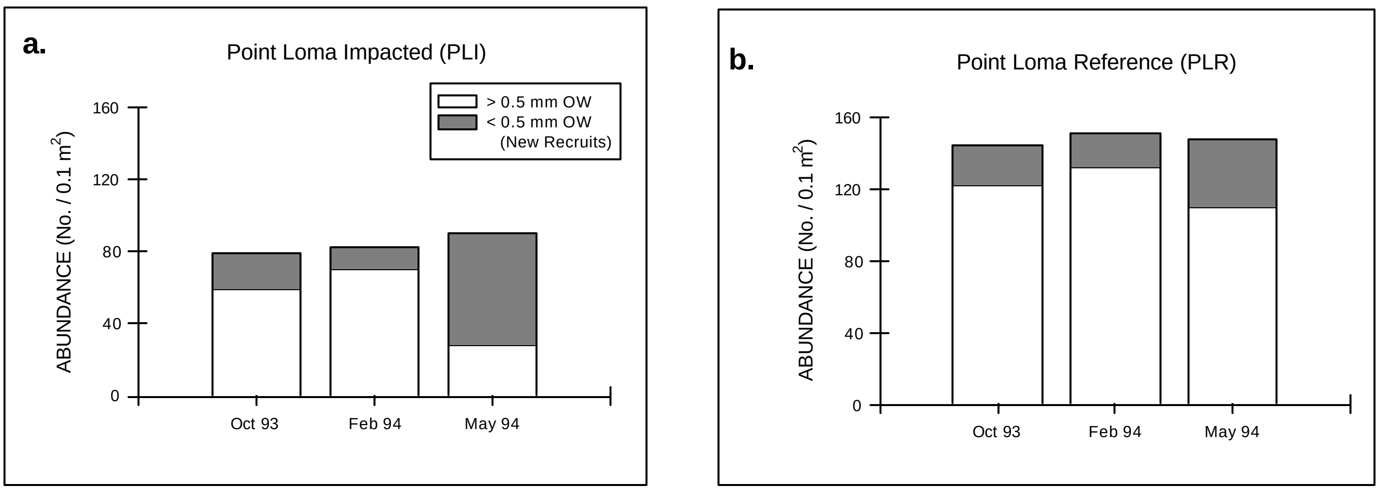

FIGURE 5. Total abundance and recruitment of *Amphiodia urtica* at a) the moderately impacted site (PLI) and b) the reference site (PLR) off Point Loma, California.

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