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SEASONAL CHANGES IN MUSSELS

There is widespread interest in the question of whether mussels are reliable indicators of contaminant levels and related biological effects. In this experiment, mussels were sampled at three dissimilar sites along the coast of southern California for over a year, and evaluated for changes in concentrations of metals and organics, degree of detoxification of these, and histological conditions. We found that, for the most part, seasonal changes in chemical and biological parameters were greater than the differences between stations although elevations of contaminant concentrations above control levels were discernible. There was no correlation between histopathology and contaminant levels. Most histological conditions correlated with the reproductive stage. Seasonal changes of reproductive stage and other histological conditions did not appear to coincide from year to year.

In ecological studies it is important to be able to distinguish between natural variations and changes caused by human activities. A large component of natural variation will be due to both daily and yearly biorhythms. Biorhythms have been shown to include cyclic changes in enzyme activities, energy reserves, hormone levels, resistance to disease, physical stamina and susceptibility to toxicants (Harris 1964; Hardeland 1973; Gordon and McLeay 1978).

Recent studies have indicated that many seasonal changes in the condition of organisms may be directly related to the reproductive cycles. This has been demonstrated histologically for both fish (Pierce *et al.* 1980) and mussels (Brown *et al.* 1982). In mussels which have recently spawned there is a considerable infiltration of hemocytes into reproductive tissue. These hemocytes are phagocytic and act to sequester cellular debris resulting from spent reproductive follicles. In addition to hemocytic infiltration of reproductive tissue there is also considerable infiltration of other tissues. For reasons that are not entirely clear, these other tissues are damaged during this stage (Brown *et al.* 1982). Damage occurs in the form of tissue vacuolation and destruction. In addition, the occurrence of kidney concretions, a storage excretory and detoxification mechanism for trace metals, has been shown to be linked to the reproductive cycle (George *et al.* 1980).

In addition to these biological variations, it has also been clearly demonstrated that there are seasonal changes in contaminant levels in mussels (Goldberg *et al.* 1980; Boesch *et al.* 1981).

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Because mussels are used as tools to determine relative levels of contaminants and associated biological effects, it is necessary to clearly define seasonal changes so that these can be standardized back to a baseline which incorporates seasonality.

For those reasons, and to better understand the dynamics of seasonality in general, we undertook a two year seasonal study of the histology and contaminant levels in mussels. In order to determine if anthropogenic influences could be distinguished from natural variations, we sampled these mussels from three areas including one considered to be relatively clean (Point Dume), another considered to be moderately contaminated (Redondo Beach), and one considered to be highly contaminated (White Point).

We have also determined the portion of contaminants that are detoxified on a seasonal basis, since this factor should provide clues as to seasonal differences in susceptibility to toxicants. In addition we have determined tissue differences in detoxification processes for trace metals.

METHODS

Mussels (*Mytilus californianus*) were collected intertidally from Point Dume, Redondo Beach breakwater and White Point in November 1980, March 1981, June 1981, September 1981 and December 1981. The mussels were returned to the laboratory, depurated for 24 hrs, shucked and dissected. A subsample of mussels (n = 20) was preserved in buffered formalin in preparation for histopathological analysis, while other subsamples of 10 mussels each were composited for analysis of total tissue chlorinated hydrocarbon concentrations, trace metal concentrations and detoxification/toxification studies (n = 10). All subsequent analyses were the same as those described previously (Brown *et al.* this report) except that results of histopathological ratings such as presented in Table 1, were summarized by combining data for moderate (++) and high (+++) ratings and then plotting according to season (Figure 5).

CONTAMINANT CONCENTRATIONS

Results from contaminant analysis of whole mussel tissue indicate that there were no clear seasonal patterns for trace metal levels (Figure 1). However, total DDT (DDT+DDE+DDD) concentrations were related to season with highest concentrations occurring in the November-December sampling and progressively lower concentrations occurring from March through September. This pattern was followed closely by concentrations of PCB's in mussels from White Point but to a lesser degree in those from Point Dume and Redondo Beach.

Copper concentrations were higher in tissues of mussels from Redondo Beach relative to those from Point Dume ($P < 0.05$; 2-tailed t-test for paired data). The source of this Cu may be the anti-fouling paints used to protect the undersides of boats in the harbor enclosed by the Redondo Beach breakwater, from where the mussels are collected (Young 1975). There was no evidence of increased concentrations of Cd, Cu or Zn in tissues of mussels collected from the White Point station, which was the sampling site located nearest to sediments that are known to be highly contaminated with trace metals (Word and Mearns 1979).

Concentrations of Total DDT were elevated 5- to 14-fold in mussels from White Point ($P < 0.05$; 2-tailed t-test for paired data) relative to those from Point Dume. The source of the DDT is known to be the JWPCP sewage discharged near White Point. Concentrations of PCB's were increased in mussels from both White Point ($p < 0.05$) and Redondo Beach ($p < 0.05$) but more so in those from Redondo. Sewage is a source of PCB's from near White Point but the source of PCB's from near Redondo Beach is unknown.

Table 1. Histological ratings of tissues of mussels collected in September 1981. This was a blind rating system since the examiner did not know the identity of the slide he was examining. Conditions are described in the text and shown in photos in Figure 6. A summary of frequency of moderate and high ratings for all seasons considered is given in Figure 5.

For reproductive stages: 0 = neuter, + = developing/mature prespawn, ++ = spawning, +++ = postspawn resorptive;
For digestive phases: 0 = holding, + = absorptive, ++ = disintegrating, +++ = reconstituting;
For other tissues: 0 = none, + = low, ++ = moderate, +++ = high levels.

	POINT DUME[1]				REDONDO BEACH[2]				WHITE POINT[3]			
	0	+	++	+++	0	+	++	+++	0	+	++	+++
Reproductive Stage	0	4	15	1	1	0	8	10	0	7	9	4
Digestive Gland Phase	0	12	6	1	0	11	8	1	1	10	9	0
Nutritive Cells (Total)	25	11	3	0	30	8	1	0	22	4	4	0
Reproductive Tract	10	8	2	0	10	8	1	0	12	4	4	0
Digestive Gland	15	3	1	0	20	0	0	0	20	0	0	0
Vacuolation (Total)	8	19	14	20	14	16	18	12	16	11	18	16
Digestive Gland	0	0	4	15	0	0	9	11	0	2	8	10
Kidney	0	3	7	3	0	9	4	1	2	0	9	6
Pericardial Gland	3	5	2	2	5	0	4	0	6	0	0	0
Adductor Muscle	5	11	1	0	9	7	1	0	8	9	1	0
Hemocytic Infiltration (Total)	28	40	15	2	9	37	18	14	29	33	7	4
Reproductive Tract	10	6	4	0	1	6	3	9	10	5	2	3
Digestive Gland	6	11	2	0	2	7	6	5	9	10	1	0
Pericardial Gland	3	5	2	2	1	6	2	0	0	4	2	0
Gill Lamellae	6	8	3	0	3	11	2	0	8	7	0	0
Gill Interlamellar Tissue	3	10	4	0	2	7	5	0	2	7	2	1
Epithelial Sloughing: Kidney	1	3	6	3	0	10	3	1	1	5	8	3
Concretions: Kidney	3	9	1	0	4	7	2	1	12	3	1	1
Trematode Eggs (Total)	60	28	6	1	60	16	4	4	52	21	4	6
Digestive Gland	18	0	0	0	20	0	0	0	14	4	0	2
Kidney	13	0	0	0	13	1	0	0	16	1	0	0
Byssus	3	10	5	1	3	9	4	4	4	9	2	4
Gill Lamellae	16	1	0	0	16	0	0	0	14	1	0	0
Gill Interlamellar Tissue	8	7	1	0	8	6	0	0	4	6	2	0
Large Lysosomes: Digestive Gland	18	1	0	0	20	0	0	0	1	8	6	5

[1] 6 ♂♂ 14 ♀♀
[2] 4 ♂♂ 14 ♀♀
[3] 13 ♂♂ 7 ♀♀

* $p < 0.05$ (Chi Square)
** $p < 0.01$

METAL DETOXIFICATION

Seasonal variation in the distribution of trace metals between the enzyme-containing (ENZ) pool and the metallothionein-containing (MT) pool was also considered (Figure 2). As discussed previously (Brown *et al.* this report) the ENZ pool contains a normal complement of Cu and Zn because these are required components of metalloenzymes. Excess levels of Cu and Zn, over the requirements of the metalloenzymes, are partitioned onto MT; these would be toxic if they were present in the ENZ pool at levels above the normal requirements of metalloenzymes. Cadmium is a nonessential trace metal and is therefore usually partitioned into the MT pool where it is rendered nontoxic.

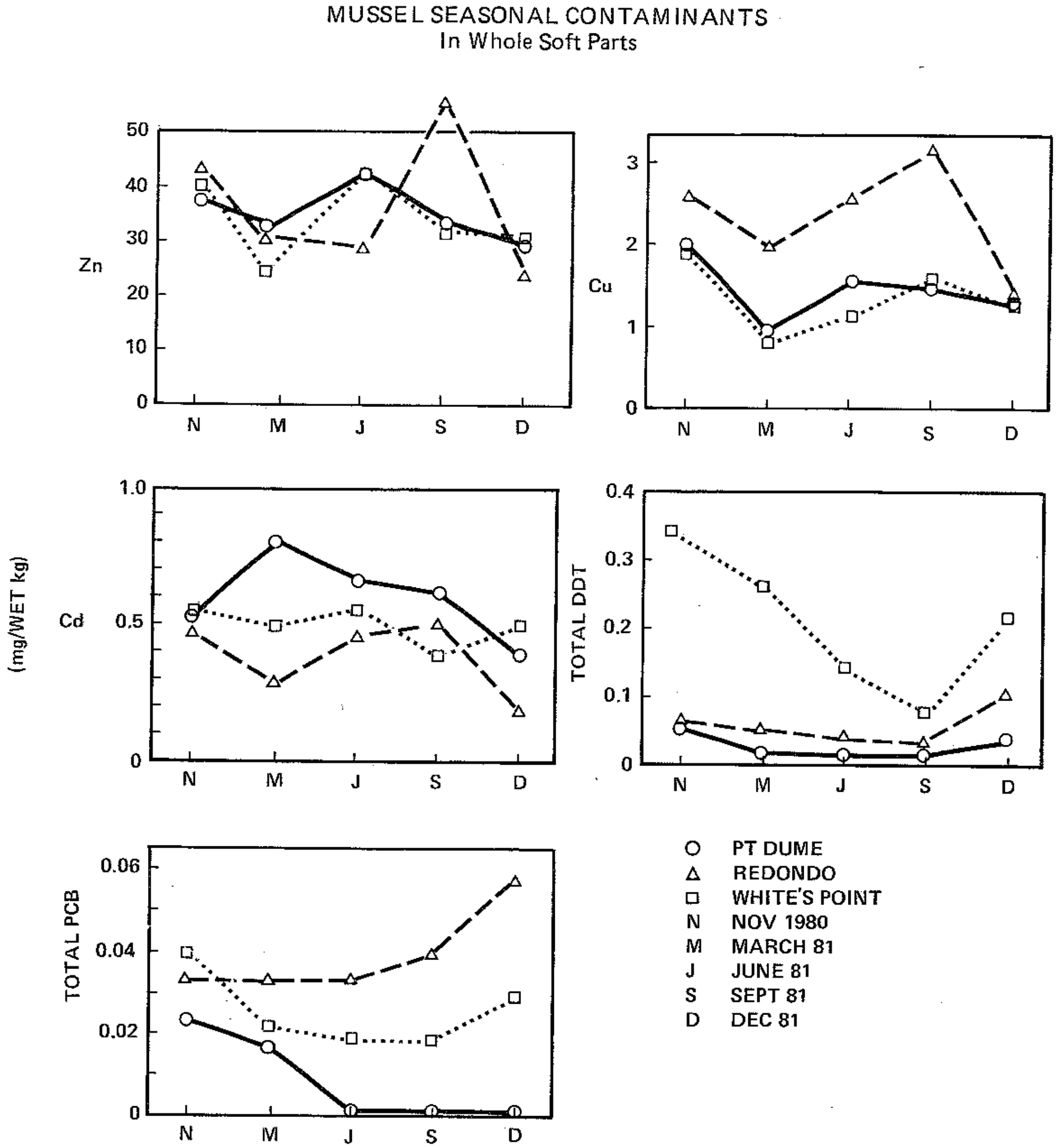


Figure 1. This figure shows the seasonal changes in contaminant concentrations in whole soft parts of mussels collected from three sites in coastal southern California. There were seasonal changes in chlorinated hydrocarbon, but not trace metal concentrations in tissues of mussels. Concentrations of Cu and total PCB's (1242 + 1254) were most highly elevated in mussels from Redondo Beach while total DDT (DDT+DDE+DDD) was most highly elevated in mussels from White Point.

The results indicate that there is little excess Zn in mussels from any of the three stations considered, because only a small portion occurred in the MT pool (Figure 2). Copper almost always occurred at higher concentrations in the MT pool relative to the ENZ pool, indicating that it was present at concentrations which greatly exceeded the requirements of metalloenzymes in the ENZ pool. Studies on urchins (Jenkins *et al.* this report) and fish (Brown *et al* this report) indicate that large reserves of Zn and Cu in the MT pool are normal. Most Cd usually occurred in the MT pool indicating that this non-essential metal was effectively detoxified.

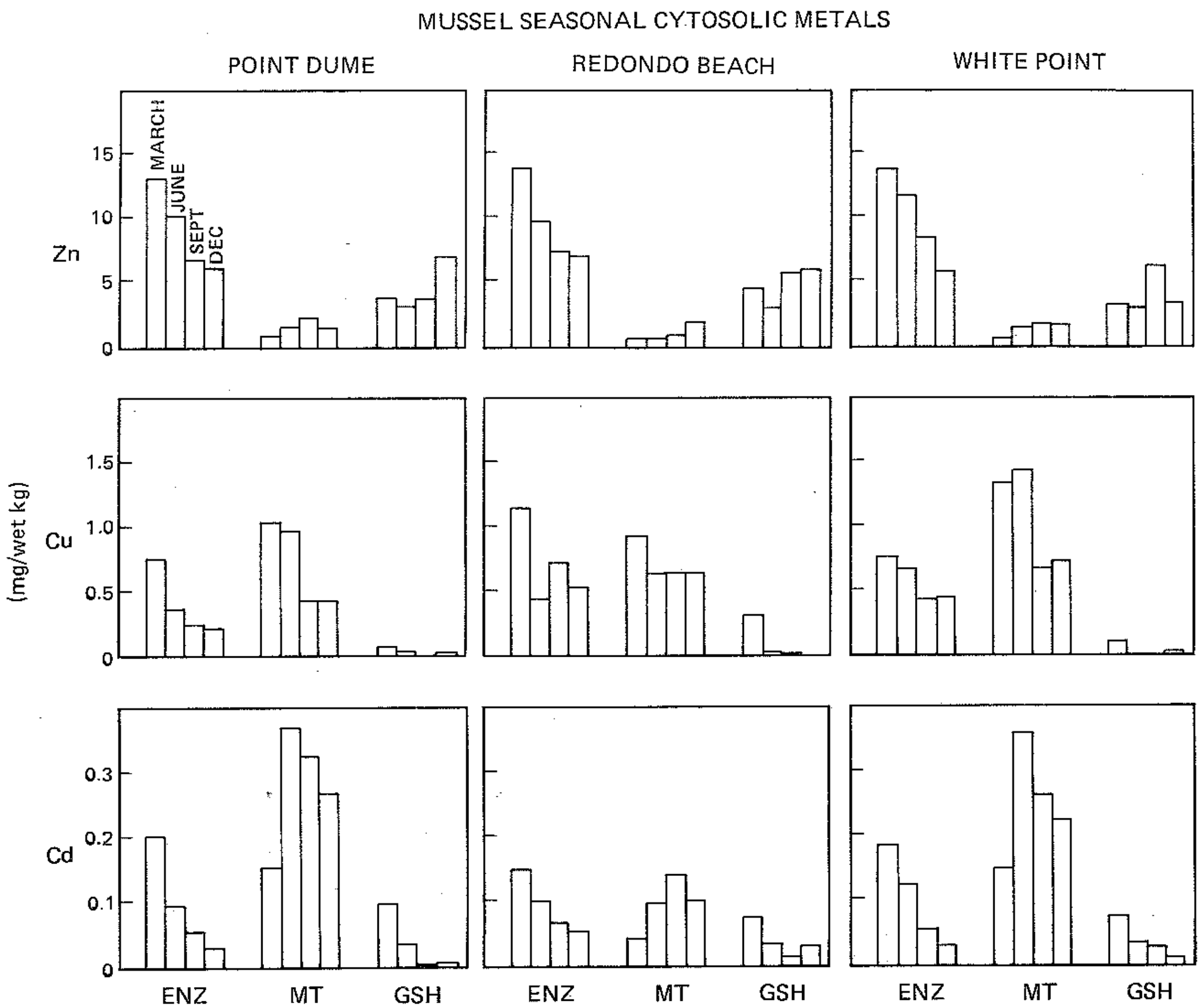


Figure 2. The seasonal variations in the distribution of metals between the ENZ, MT, and GSH pools are shown in this figure. There were seasonal variations in the concentration of metals associated with the ENZ, MT, and GSH pools. These changes were most consistent in the ENZ pool where there was a progressive decrease of metals from March to December. These results show that it would be difficult to determine degree of spillover of metals from MT to ENZ, without first establishing the normal seasonal concentrations in the ENZ pool.

There were definite seasonal differences in the levels of metals in the three cytosolic pools. With few exceptions, there was a progressive decrease from March to December of Zn, Cu, and Cd in the ENZ pools of mussels from all three stations. The pattern of changes of metal concentrations in the MT pool were more variable seasonally, but consistent between the three stations at most sampling times.

These results tend to indicate that it would be difficult to evaluate degree of spillover (discussed in Brown *et al.* this report) without clearly defining the normal partitioning of metals between pools for the season in which mussels were sampled. Reproductive stage may be an important factor influencing the retention and subcellular distribution of trace metals since most metallothionein in mussels occurs in the gonads (Figure 3).

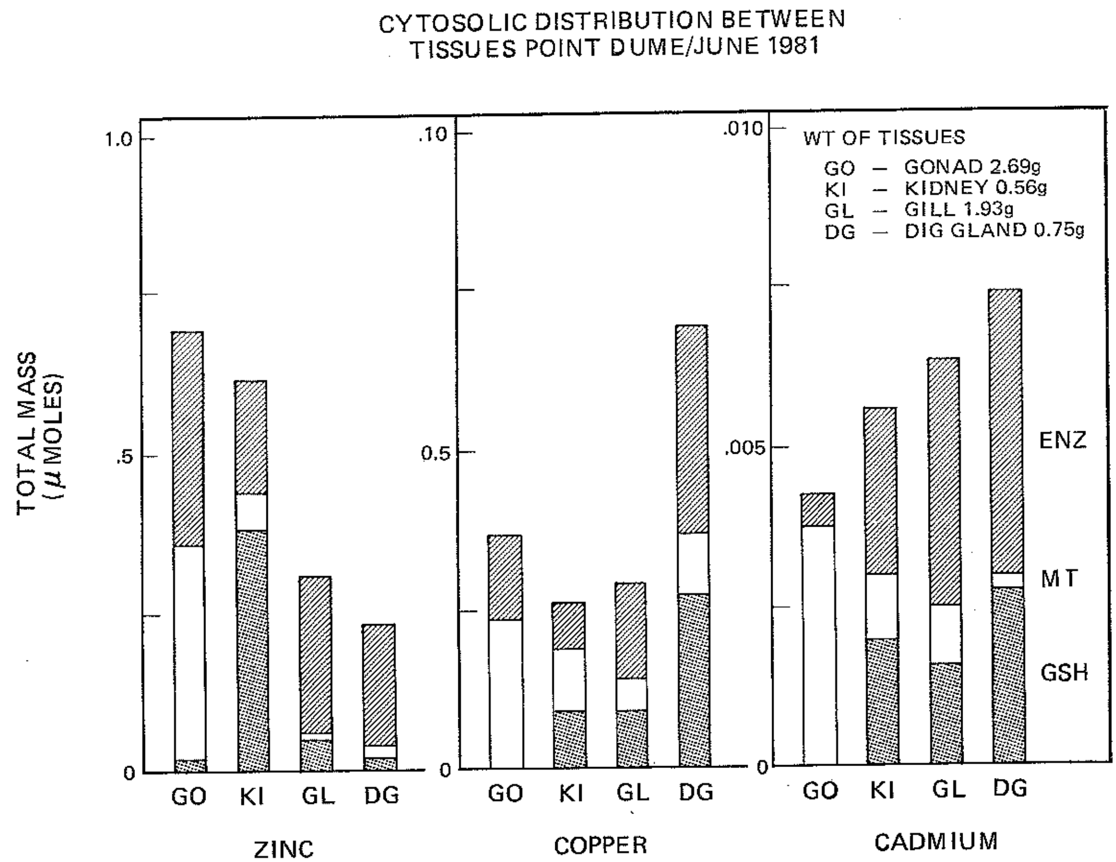


Figure 3. This figure shows the total amount of metals associated with each of the ENZ, MT, and GSH pools in mussels collected from Point Dume in June 1981. Most metallothionein occurred in their gonads indicating that the reproductive cycle could have an important influence on the degree of detoxification of trace metals.

CHLORINATED HYDROCARBON DETOXIFICATION

We also considered the partitioning of organic metabolites between cytosolic pools. Organics are metabolized by the mixed function oxygenase enzymes to highly reactive epoxides which conjugate with glutathione (discussed in Brown *et al.* this report). If glutathione, the site of final detoxification, becomes saturated, then epoxides can react with sites of toxic action includ ing sulfhydryls in the ENZ pool and the MT pool.

The cytosolic distribution of synthetic organic metabolites is shown in Figure 4. Most metabolites at all three stations occurred in the glutathione-containing (GSH) pool, and are considered to be nontoxic since they are partitioned away from sensitive cellular sites (e.g. ENZ and MT). Only a small portion of metabolites occurred in the ENZ and MT pools.

The concentrations of metabolites in mussels far exceeded the concentrations of parent compounds, similar to results for fish (Brown *et al.* this report). Mussels have been reported by some to have only a low ability to metabolize synthetic organic compounds, while others have reported significant increases of mixed function oxygenase enzymes in mussels exposed to

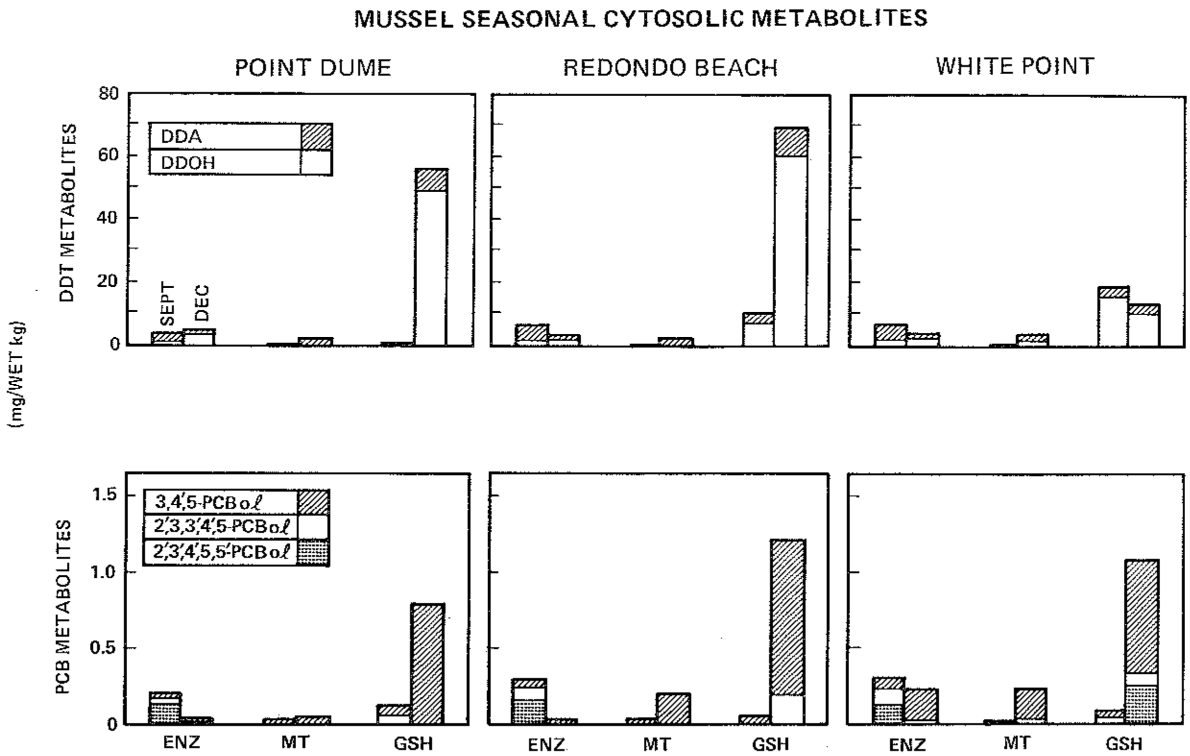


Figure 4. This figure shows the distribution of DDT and PCB metabolites between the ENZ, MT, and GSH pools in mussels collected from three sites in coastal southern California in September and December 1981. Most of the metabolites of DDT and PCB occurred in the GSH pool of mussels and therefore are considered to be detoxified. Comparison with Figure 1 shows that concentrations of metabolites in mussels greatly exceed those of parent compounds. Metabolite levels were much higher in December than in September.

aromatic hydrocarbons (Bayne *et al.* 1980). Disparity of results may be a result of (i) difficulties associated with extracting these enzymes (ii) the tissue examined for enzyme activity, and (iii) difficulties of extracting metabolites without the use of a heat-catalyzed base hydrolysis. Moore (1979) has found that the highest levels of activity of mixed function oxygenase enzymes may occur in the hemocytes of mussels. In this context, note that mussels from White Point, which had the lowest levels of metabolites in December, also had the lowest levels of infiltration of tissues with hemocytes (Figure 5, discussed later).

There may be seasonal differences in concentrations of metabolites. In the present study, concentrations of metabolites were higher in December than in September. This parallels the trend of higher concentrations of parent organic compounds in December compared with September and may be due to a greater availability of organics for metabolism. The reproductive stage may influence the degree of metabolism, either from loss of metabolites during spawning, or because some enzymes, which metabolize reproductive hormones, also metabolize chlorinated hydrocarbons (Torok 1976). As shown later, mussels from White Point had statistically significant lower reproductive activity than those from Point Dume.

HISTOLOGY

A synopsis of the histological ratings of condition of tissues is presented in Figure 5. A between-stations comparison was done for a variety of conditions using a 2-tailed t-test for

MUSSEL SEASONAL HISTOLOGY
Percentage With Moderate and High Rating

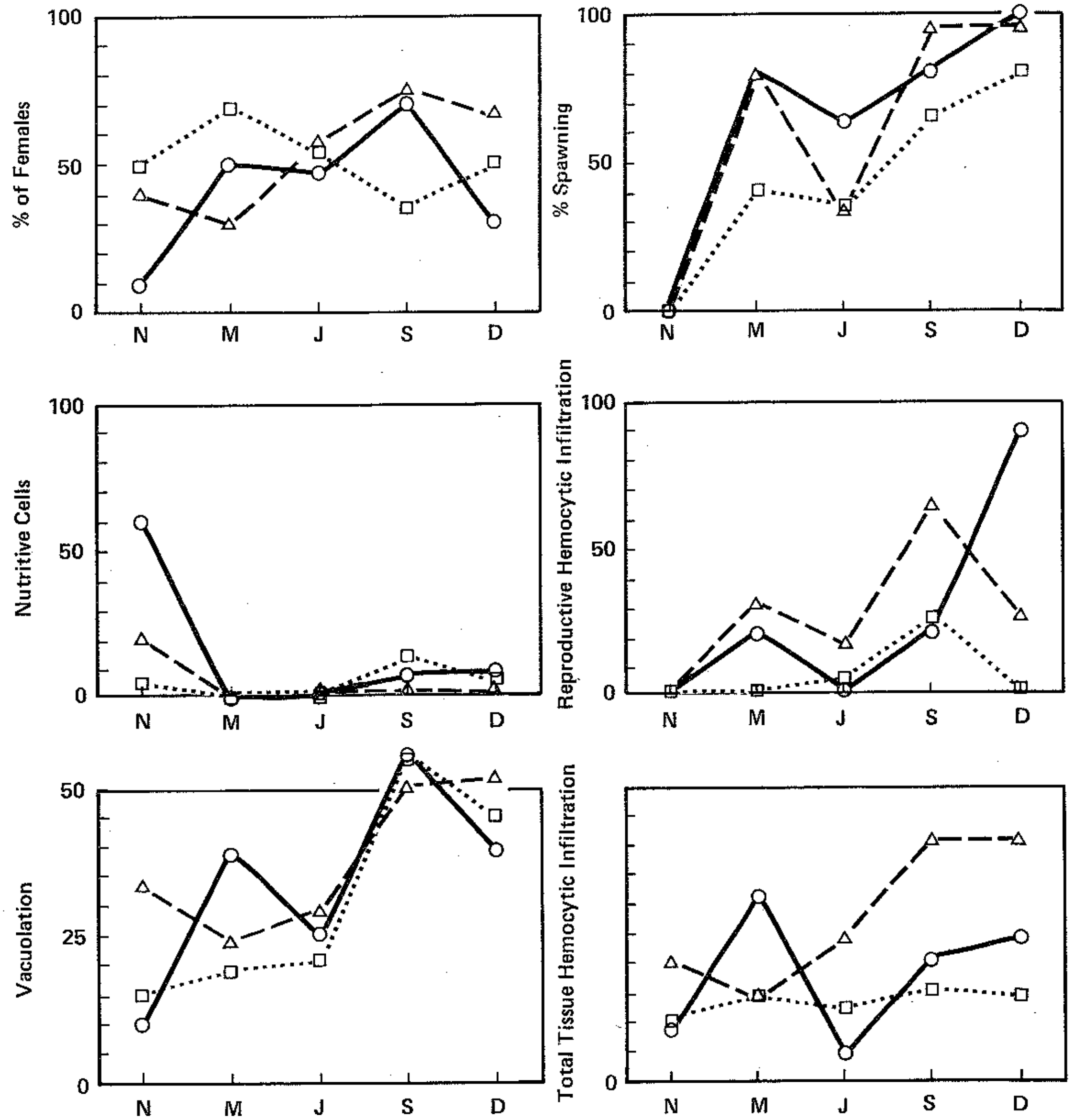


Figure 5. The histological condition of a number of tissues of mussels collected from coastal southern California are shown in this figure. For each histological parameter, the percentage of individuals at each station with moderate and high ratings (see Table 1) were plotted against season. Spawning activity increased from zero spawners in November 1980 to highest frequency of spawners in December 1981. Relative levels of nutritive (glycogen-containing) cells were highest in November 1980 when spawning was lowest. Vacuolation of tissues paralleled degree of spawning activity. Hemocytic infiltration of reproductive tissue appeared to be lowest in November 1980 when spawning activity was lowest and at White Point where spawning was lowest over the duration of the experiment. Sloughing of the kidney epithelium also paralleled spawning activity. Kidney concretions were highest in Redondo Beach mussels. Large lysosomes occurred in digestive tubules of both Point Dume and White Point mussels. Trematode eggs were common in mussels from all stations.

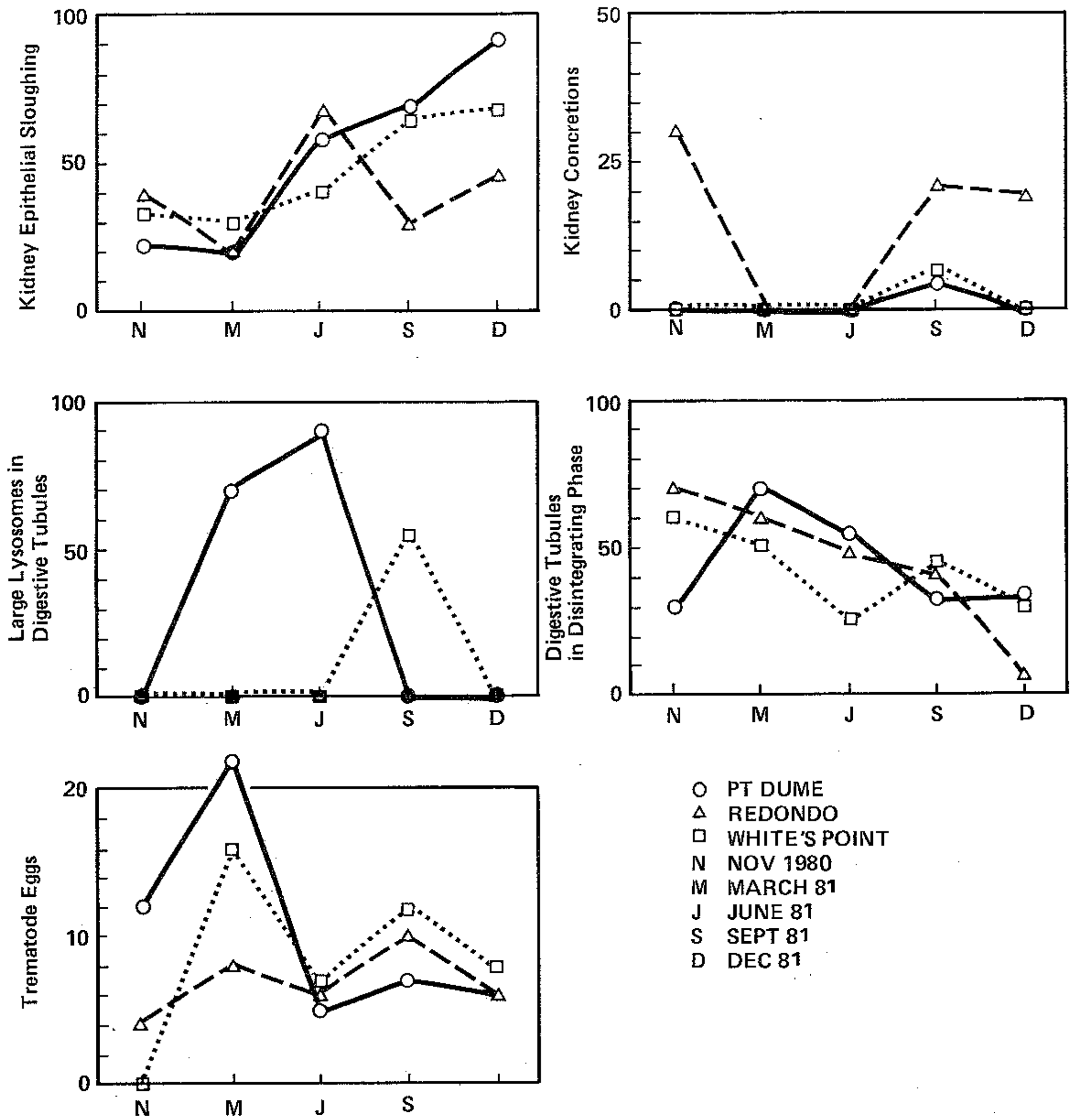


Figure 5. continued

paired data. Over the course of the study, November 1980 to December 1981, there was no statistical difference between the number of males or females present at the three stations.

The only condition which was statistically significant ($p < .05$) using this comparison for paired data was a reduction in the number of spawning and postspawn resorptive reproductive tracts in mussels from White Point relative to those from Point Dume (Figures 5 and 6). Reproductive tracts were considered to be spawning if they had fully developed follicles and gametes, a paucity of germinal cells, and gametes present in the reproductive ducts. They were considered to be postspawn resorptive if follicles were large, but depleted of gametes, and replete with hemocytes. The hemocytes function in the sequestration of spent reproductive follicles, and their subsequent transport to the digestive tract where they are redigested, or, to the kidney where they are excreted. Postspawn resorptive reproductive tracts were found only in the September 1981 sample.

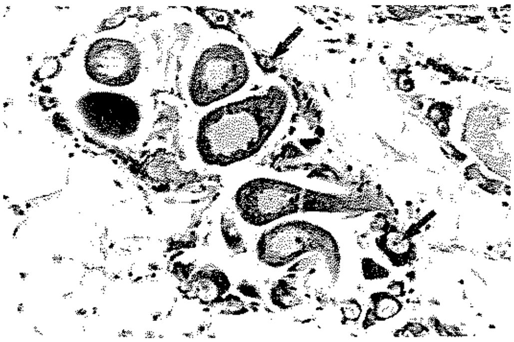
The cause of the reduced reproductive activity in mussels from White Point is not clear. It is unlikely that it was a result of metals since these were not elevated in White Point mussels. There was no evidence of reduced reproductive activity due to chlorinated hydrocarbons, since there was no correlation between tissue concentrations of DDT and degree of spawning activity when a linear regression test was utilized incorporating DDT and spawning data from all three stations and all sampling times. In addition, although DDT compounds were elevated in whole mussel tissue at White Point, DDT metabolites, which comprise most of the measured form of DDT, were lower in White Point mussels than in those from the other stations while spillover from GSH to ENZ or MT was low in mussels from all stations, including the ones from White Point (Figure 4). Reduced reproductive activity could be due to indirect effects of contaminants on food supply, but this has not been demonstrated. Other unknown influences may also be present.

It is of considerable importance that reproductive tracts were in a vastly different stage on November 21st, 1980 than on December 9th, 1981. This tends to indicate that reproductive cycles are irregular and may not coincide at a particular sampling time from year to year. Thus, any attempt to correct for effects of reproductive cycle, by sampling at the same time of year each year, may be fruitless.

Probably most frequent samplings are necessary to clearly define the reproductive cycles of mussels. It is tempting to speculate, based on the data given here, that September represents the end of a yearly reproductive cycle; i.e. the reproductive tracts are being resorbed in September 1981; that November represents the beginning of the next reproductive cycle; i.e. the reproductive tracts in November 1981 are immature and developing; and that early December represents the beginning of the next spawning season which, based on the infrequent number of samples collected here, appears to continue until the end of summer.

Presence of nutritive cells, which stain according to glycogen content, is related to the reproductive stage (Bayne 1976). In this present study, these were most prominent in sexually immature mussels collected from Point Dume in November 1980 (Figures 5 and 6). They were less prominent at other seasons because mussels were more mature sexually, and glycogen is utilized as an energy source for development of reproductive tracts. Since glycogen is clearly discernable only in November, it would be necessary to determine its level at this time in order to compare the relative energy depletion between stations relative to environmental stresses. The problem with this approach, however, is that it assumes reproductive stages are completely synchronized between stations. Even subtle differences (a few weeks or less) in reproductive

Figure 6. These photos show some of the histological conditions which were found in mussels collected from coastal southern California.
a. Developing female reproductive tract with immature ova (arrows) budding from the germinal epithelium of the reproductive follicles; Redondo Beach, November 1980. 200X
b. Spawning female reproductive tract with mature ova (arrows) in the reproductive follicles; White Point, December 1980. 200X
c. Postspawn resorptive male reproductive tract with heavy infiltration of hemocytes into reproductive follicles (arrows); Redondo Beach, September 1981. 200X
d. Digestive tract with tubules in the disintegrating phase with no vacuolation and high levels of nutritive cells (arrows); Point Dume, November 1980. 400X
e. Digestive tract with tubules in the disintegrating phase with high numbers of large lysosomes (arrows) and a moderate degree of vacuolation. Point Dume, June 1981. 400X
f. Digestive tract with tubules in the disintegrating phase and a high degree of vacuolation. White Point, December 1981. 400X
g. Kidney with low degree of epithelid vacuolation and a moderate degree of sloughing of the epithelium into the lumen. Point Dume, March 1981. 250X
h. Kidney with concretions (arrows) and a high degree of epithelial vacuolation. Redondo Beach, September 1981. 250X
i. Byssus with high numbers of trematode eggs (arrows); White Point March 1982. 200X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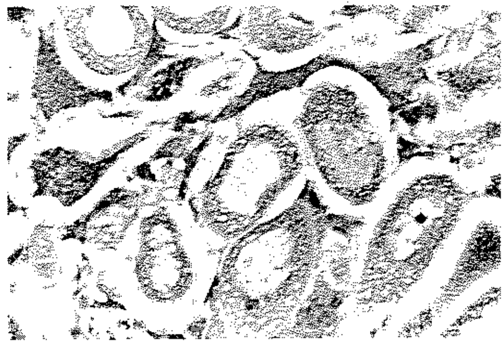
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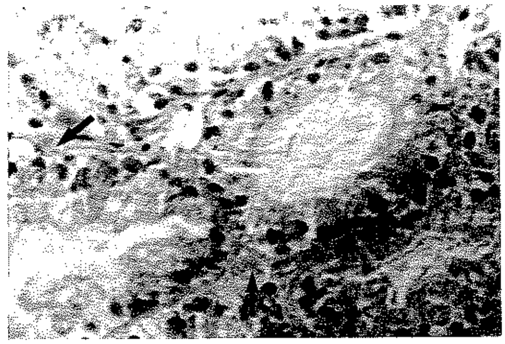
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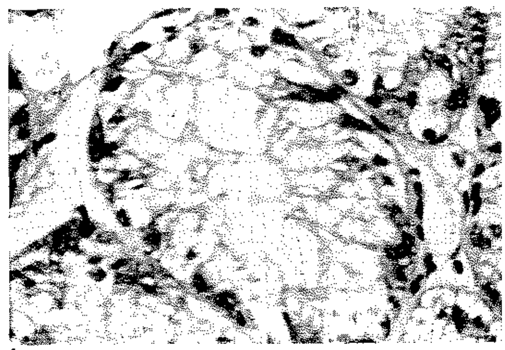
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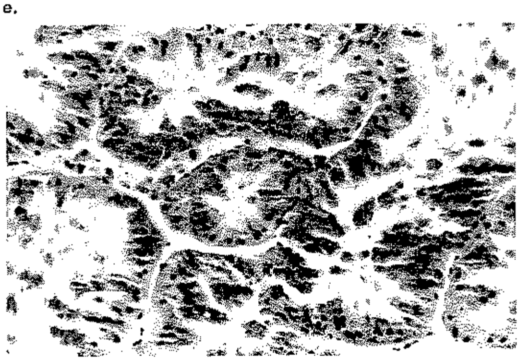
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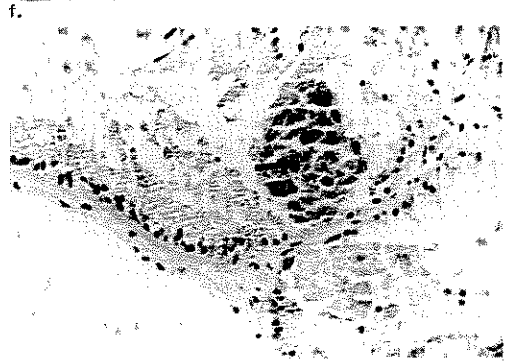
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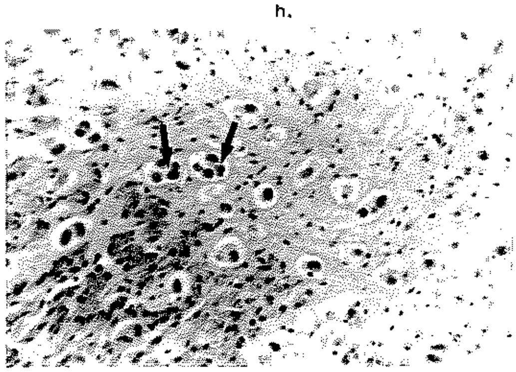
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stage could dramatically alter glycogen levels. Therefore, for clear determination of differences in nutritive cell staining, weekly sampling might be required at each station so that peak levels could be determined.

Degree of vacuolation of tissues (Figures 5 and 6) was directly correlated with reproductive stage ($p < 0.05$; $r = 0.700$). Vacuolation most likely reflects degree of starvation or utilization of energy reserves, and therefore is more apparent with more advanced reproductive stage (Widdows, personal communication). There was no difference in degree of vacuolation between stations over the course of the entire study.

Hemocytes function in the resorption of spent reproductive follicles and, therefore, any assessment of the condition of mussels in terms of hemocytic infiltration must take into account the reproductive stage of the organism. Hemocytic infiltration of reproductive tracts and total tissues of mussels was not different between stations (Figures 5 and 6). However, hemocytic infiltration of reproductive tracts and other tissues correlated ($p < 0.05$; $r = 0.678$ and $r = 0.538$, respectively) with degree of spawning activity. White Point mussels, which had the lowest degree of reproductive activity, also had the lowest degree of hemocytic infiltration of the reproductive tract and other issues.

The degree of sloughing of kidney epithelium (Figures 5 and 6) did not correlate with the degree of spawning activity. Kidney epithelium is sloughed as part of the excretory process. Levels of kidney concretions (Figures 5 and 6) were highest in mussels from Redondo Beach, but were not statistically significant over the course of the experiment. Kidney concretions are a means of storage and detoxification of trace metals. Therefore, the tendency for higher levels of these in mussels from Redondo Beach may be a result of the higher Cu levels.

Large lysosomes were present in digestive tubules of mussels from Point Dume in March and June 1981, and in those from White Point in September 1981 (Figures 5 and 6). These are reported to occur in response to starvation or spawning (Widdows, personal communication) and may result from aggregations of smaller lysosomes because of high degrees of loading of these during periods of starvation. However, in this study, levels of large lysosomes did not correlate with either degree of vacuolation or reproductive stage, nor did they correlate with the portion of digestive tubules in the digestive (disintegrating) phase. Thus, both their cause and significance remains unknown.

Degree of parasitism was not different between the three stations over the duration of the experiment (Figures 5 and 6). In two of the five samplings, parasitism was highest at the Point Dume control station, suggesting it is not related to degree of environmental contamination.

Although a number of histological parameters were correlated with the reproductive cycle, none correlated with the presence of contaminants. Inspection of the data presented in Figure 5 indicates that the station differences in spawning activity were small relative to the seasonal differences. Therefore, since a number of histological parameters are linked to the degree of spawning activity, any contaminant-related effects may be masked by the observed seasonal variability. Given the variability present because of the reproductive cycle, it is unlikely that histological examination of mussels could be a useful means of assessing the biological impact of contaminants.

CONCLUSIONS

Seasonality must be taken into account when using mussels to evaluate relative concentrations of chlorinated hydrocarbons, but not when evaluating relative concentrations of trace metals.

Concentrations of Cu and total PCB's were highest in mussels from Redondo Beach, while total DDT was highest in mussels from White Point. Seasonal differences in a number of histological parameters appear to be related to the reproductive cycle. The reproductive cycle appears to be irregular from year to year making it difficult to devise procedures to account for seasonal influences. Furthermore, the seasonal changes are greater than the differences between stations examined in this study, indicating that the variability inherent in mussels would tend to reduce the visibility of contaminant impacts.

ACKNOWLEDGEMENTS

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