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Race Rocks

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Race Rocks Ecological Reserve

Distribution of Anthopleura elegantissima

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ABSTRACT

The following research essay deals with the distribution pattern of the sea anemone Anthopleura elegantissima. The research essay was carried out at the intertidal levels of Race Rock islands in the Juan de Fuca straits. The aim of the research was foremost to find out the distribution pattern of the Anthopleura in a mathematically comprehensive way and using that information, to identify the Anthopleura elegantissima's sub-species level. The distribution pattern and zonation will give us insight into the enviornmental, biotic as well as abiotic, factors influencing the pattern.

In determining the pattern both Poisson and Plotless methods were involved. Poisson methods give results very much dependant on the size of the sampling units. On the other hand Plotless methods are independant of unit sizes and at least one source of error is eliminated. We employ both methods and the accuracy of one against the other is judged by formal statistical tests.

INTRODUCTION.

Lester B. Pearson College is situated on the north shores of Pedder Bay. Pedder Bay lies on the southern tip of Vancouwer island, approximately Thirty miles out of Victoria city. Just south of the mouth of Pedder Bay lie the islands of Race Rocks. Race Rocks is a valuable, provincial government, marine, ecological reserve. The following research essay was carried out at the west, north-west intertidal areas of Race Rocks. (see map # 2, on page # 24.).

The research essay deals with the distribution of a single particular species of anemones, Anthopleura elegantissima. The distribution pattern is analyzed mathematically from data obtained by a number of different techniques. The mathematical description of the pattern is obtained and tests done on the results to see Their extent of accuracy or validity. Anthopleura elegantissima is an Inter-tidal species and the two techniques applied to it were Poisson and Plotless methods at low tide.

The interactions of individuals of a species with their biotic and abiotic results in a certain pattern of distribution of individuals in the habitat occupied by a population of the species. This pattern may vary from one of random distribution to one tending either towards uniform spacing of individuals or towards aggregating (clumping) of individuals. The pattern exhibited by a population is often indicative of the operation of specific environmental factors affecting the behaviour, survival or growth of individuals.

The common intertidal sea anemone, _Anthopleura elegantissima_, occurs in two forms, the clonal aggregating and the solitary form. Lisbeth Francis 1979.

The aim of the research essay was to mathematically discover and describe this pattern in an accurate way which would lead to an insight into the biotic/abiotic factors influencing the distribution pattern of _Anthopleura elegantissima_.

Intertidal sea anemone, _A. elegantissima_, occurs in two forms, the clonal aggregating form and the solitary form. Clonal aggregation results from asexual reproduction and the solitary form results from sexual reproduction. The clonal form is well-suited to life higher in the mid-intertidal with resistance to physical stresses, for example drag by ocean waves and desiccation. The solitary form lives lower in the sub-tidal and is more resistant to predation and less able to withstand physical stress or intense intraspecific competition. All this information is available to us by previous research studies done on _Anthopleura elegantissima_; two prominent names in the study of this particular field being Lisbeth Francis (1979) and Kenneth P. Sebens (1982). Having this previous knowledge, the distribution pattern revealed by the mathematical analysis can be successfully used to identify the _Anthopleura elegantissima_ as either the clonal or the solitary form.

A line transect perpendicular to the

sea-shore combined with a land profile will give us the zonation of the anemone which we can then classify as intertidal or sub-tidal etc. The zonation will reveal the physical stresses encountered by invertebrates with habitat located in that zone which we can then compare with the dispersion pattern, identify the form of _A elegantissima_ and see how the latter has developed in evolutionary terms or has adapted to the maximum possible to it's advantage to suit it's habitat.

In assessing the distribution pattern, two different techniques are employed; Poisson methods and Plotless methods. The former is sample-size dependant but the latter is independant of sample size. On each technique confidence (statistical tests are carried out that reveal the percentage error of each and confidence levels of each. The two serve as counter-checks and reveal the usefulness of one against the other in pattern analysis. Therefore, last but not the least, this study can lead to an estimation value of each technique and can then be used as a guide for the future choice of techniques for the exploration of intrapopulation dispersion of _A. elegantissima_ and/or other intertidal invertebrates with overlapping habitats, similar life-cycles or similar distribution pattern as _A elegantissima_.

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LIMITATIONS:

The entire experiment and data collection was done on the 25th of May 1986, due to the limiting factor of low-tides occuring in day-light hours. Hence the experiment could not be repeated and different data collected on another day. So the research reveals the distribution pattern at a fixed point in time and not the variations, growths and changes in pattern over a period of time.

Anthopleura elegantissima, solitary and clonal form occur commonly on the west coast of North America. Not only the two forms occur at different tidal heights but the solitary form has not been known to exist north of Bodega Head. (See map # 4) — Lisbeth Francis 1979. However another sea-anemone, *Anthopleura xanthogrammica* overlaps *A. elegantissima* in biogeographical distribution as well as in microhabitat. Thus we must learn the distinguishing features of the two *Anthopleura* before we set out to do field work. For the distinguishing features description see the section: THEORY OF WORK; *ANTHOPLEURA*. Care needs to be taken to identify *A. xanthogrammica* from *A. elegantissima* and to exclude the former when collecting data.

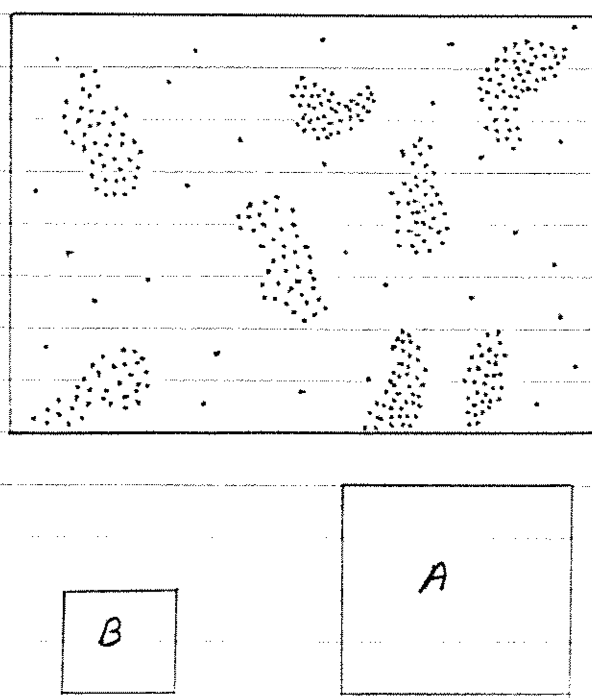
When using Poisson methods for intrapopulation dispersion some limiting factors have to be taken into consideration. First of all Poisson distribution is expected to apply for populations in which the density of individuals is low compared to what it could conceivably be on the basis of the available surface area, or volume. Thus at the outset we start with the basic assumption that the density of Anthopleura elegantissima is low compared to what it could be in an intraspecific competition-free habitat. Thus Poisson techniques are invalid for studying patterns in situations such as dense grassland. Poisson techniques are also invalid for dense, sessile invertebrates. Whether A elegantissima's population can be classified as dense or too dense for Poisson techniques, the accuracy of the results would show.

A elegantissima is not a sessile invertebrate since contact with non-clonemates or other species of Anthopleura does elicit a response, "tentacular nematocyte discharge" as well as a slow moving away from the stimulus. (Stephen C. Ertman and Demorest Davenport, Dec 1981). Thus the results of Poisson techniques will act as a standard classifying test showing whether A elegantissima is dense and sessile enough to make the technique an invalid one.

Poisson methods involve analysis by unit area or volume samples related to the size of the sampling unit employed. Analysis of the same population with samples of different size may give different results. (see section DEFINITION OF TERMS, page # 7). Hence the sampling unit's size has to be choosen very carefully for the sake of accurate results. A convenient criterion is that the sample size be small enough for many of the samples to contain no individuals of the species. Lastly the samples must be taken randomly throughout.

EFFECT OF QUADRAT SIZE ON DETECTION OF PATTERN ANALYSIS:-

The effect of quadrat size is most significant in pattern analysis when the pattern is one of highly aggregation or clumping. The detection of randomness or aggregation depends on the size of the sample unit being used. If the dispession of the population in the figure below were measured by repeated sampling with quadrat B, the marked aggregation of the population would show. If quadrat A were used the comparison of the number of individuals per quadrat with the Poisson distribution would indicate that the population has a regular or uniformly distributed pattern. This was illustrated graphically by Greig-Smith (1952) by using a series of progessively larger quadrats to measure distribution in an artificial situation in which ~~dis~~ individuals were represented by coloured disks.



From the diagram the remedy of using a quadrat small enough to include no samples at all is justified. Thereby we will gain a truer and more accurate portrayal of the distribution pattern.

8 DEFINITION OF TERMS:

Ecology is the study of the relationship of an organism to it's enviornment. Groups of organisms together with their physical enviornment comprise *ecosystems*. Plants and animals do not exist by themselves but interact with both the physical and biological aspects of their enviornment. The physical or *abiotic* enviornmental factors are moisture, temperature, light quality and quantity, wave action on sea shores, mineral nutrients etc. The biological or *biotic* factors include life expectancy and survivorship rates and interactions of an individual with those of it's own kind and individuals of other species.

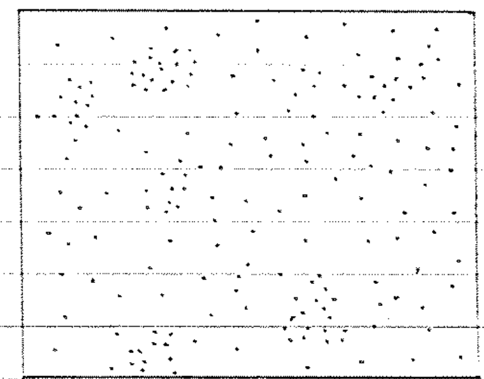
A population is a group of individuals of the same species that interbreed and occupy a given area at a given time. Because a population consists of many individuals giving birth and dying over a period of time, a population has a birth rate and a death rate. Because a population usually consists of individuals of different ages, it has an age structure. And because individuals occupy different points in time and space, a population has dispersion in time and space. Therefore in the research essay since pattern at a fixed point in time is analyzed, the pattern disclosed is the distribution pattern and not the dispersion pattern, for which we would have to repeat the same experimental technique a number of times, at time intervals to disclose the variations in pattern with time.

9 POPULATION PATTERNS: Individuals of a single species are scattered across the space they occupy. The pattern will depend on the enviornmental forces acting in the certain habitat, the repsoduction mechanism, sexual or asexual, and the repsoductive capacity. The most common type of spacing observed is clumping or aggregating. Clumping may result because individuals of many species tend to form social groups. Examples include caribou and elk which live in herds, gulls and swallows have colonial nesting. Clumping also results because enviornments are not commonly uniform. Human communities are a good example of clumping since they are formed in groups along rivers, valleys, junction of major highways etc. forming a socially interacting community. In some clumped distributions however organisms tend to maintain a minimal spacing between individuals within which they do not tolerate other individuals. This minimal distance is especially important for the proper growth and development of sessile animals and plants.

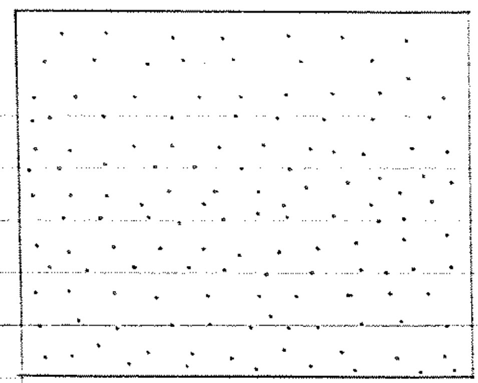
Less common than clumping is uniform distribution. In uniform distribution individuals of a population are more or less evenly spaced due to the fact that these animals tend to divide and defend the habitat between them. In desert communities or in dense pure strands of grass or trees, plants tend towards uniform spacing because of competitive elimination or because older plants produce toxins that inhibit the establishment of their own seedlings within the spread of their crown or the reach of their roots.

Rarely found in nature is random distribution in which the location of one individual has no influence on the location of another. In this situation individuals exhibit no tendency to attract or repel, and the habitat is so uniform that it plays no part in determining the location of individuals. Some invertebrates of the forest floor and some forest trees are randomly distributed.

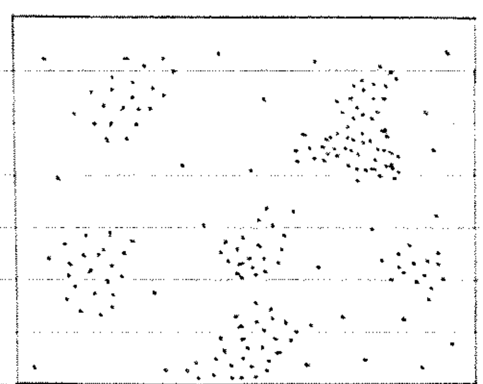
POPULATION DISTRIBUTION.



RANDOM



UNIFORM OR REGULAR.



AGGREGATED OR CLUMPED.

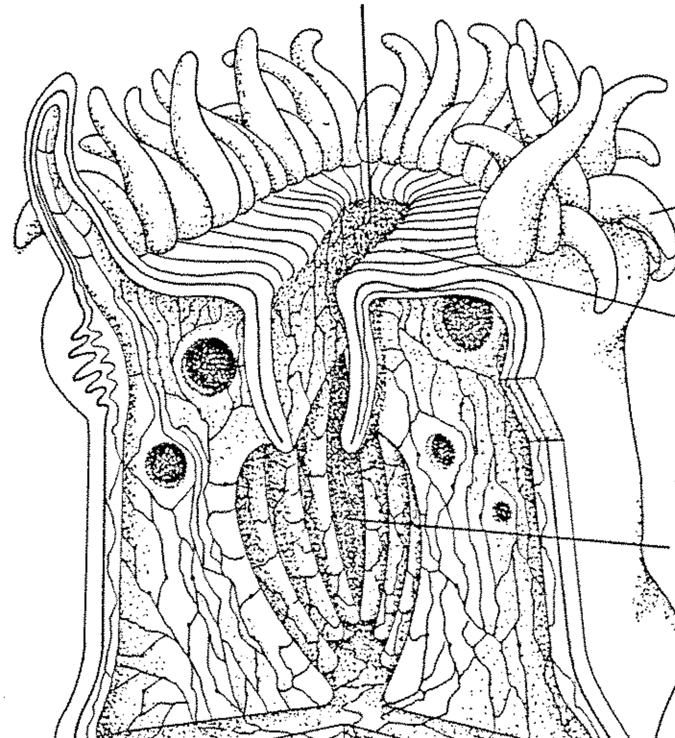
THEORY OF WORK : ANTHOPLEURA.

Anthopleura is a sea anemone which comes under the classification of *Anthozoa*. The *Anthozoa* include sea anemones, sea pens and corals and have completely forsaken the medusa stage of the hydroids and jelly fishes, retaining only the attached polyp form. They are by far the most conspicuous and successful of the cnidarians with over 6000 species, 2000 of which are anemones.

The sea anemones have improved upon the basic polyp form by having the gut cavity divided into vertical sections by the insertion of membranes known as septa which extend from the body wall inward towards the centre of the gut. There is a distinct "pedal disk" in most species which is used to attach the animal firmly to it's chosen substrate. The column or stalk of the thick body is crowned with tentacles sorrounding an oral disk. The disk has an elastic mouth opening in it's centre, which acts as a kind of permanent lid, closing the end of the tubular body.

Anemones are not considered mobile invertebrates though they are able to move very, very slowly by sliding on their pedal disks if environmental conditions indicate a change. This can be in response to a more abundant food supply, a need for greater shelter or less direct sunlight to avoid drying out if exposed at low tide.

PHYLUM CNIDARIA.
CLASS ANTHOZOA
SUB-CLASS ZOANTHARIA.
ORDER ACTINARIA.
FAMILY ACTINIIDAE.



Anthopleura elegantissima is abundant on the west coast of North America. It occurs in two forms, the clonal aggregating form and the solitary form. The obvious difference results from the presence of asexual reproduction in the clonal form and sexual reproduction in the solitary form. The clonal form is well-suited to life higher in the mid-intertidal. Asexual reproduction results in moderately small individual size and close association with clonemates, improves it's resistance to physical stresses (drag and dessication) and makes it a superior competitor in exposed habitats higher up in the intertidal where species diversity and predator pressure are low. The larger solitary animals live in more protected microhabitats lower in the mid-intertidal and sub-tidal, and are more resistant to predation and less able to withstand physical stress or intense intraspecific competition.

The above information is cited from Lisbeth Francis (1979). 19: 669–681.

The two forms also have different biogeographic ranges. (See map #4).

These differences in phenotype frequencies, biogeographic range and microhabitat, reproduction, all suggest that the clonal anemones and the solitary anemones, known as *Anthopleura elegantissima* are actually sibling species pairs or they are sub-species.

The clonal *A. elegantissima* reproduces by binary longitudinal fission, the fission leaving a noticeable scar which develops body wall adhesive verrucae and returns to normal appearance over a month or more. (Kenneth P. Sebens, 1982). This form of reproduction offers a rapid means of colonizing available habitat space, eliminating the need for innoculae of both sexes, and conferring greater absolute fitness on a particular asexual founder compared to a member, of a sexual pair. (Bonner 1958, Williams 1966, 1975, Maynard Smith 1971, 1978, Williams and Mitton 1973,

<u>Anthopleura elegantissima</u> contains photosynthetically active <u>zooxanthellae</u> and respond both biochemically and behaviourally to the combined enviornment stresses of exposure to sunlight and photosynthetically generated hyberbarametric oxygen, O_2. It also contains enzymes superoxide dismutase (SOD) and catalase which act in concert as defenses against oxygen toxicity, parallel the distribution of chlorophyll. <u>A. elegantissima</u> shows a finely controlled contraction behaviour which shades the zooxanthelae and reduces O_2 production but which leaves the column tissue directly exposed to sunlight. However the body column contains disproportionately high SOD and catalase activities as defenses against photodynamic damage. (James A. Dykens and J Malcolm Shick, 1984). The <u>Anthopleura</u> also responds by attaching broken, small pieces of sea-shells to it's body column and using them as sun-shades.

In the clonal anemone, <u>Anthopleura elegantissima</u>, direct tentacular contact between clonemates fails to elicit discharge of the nematocytes, the stinging cells. However contact between non-clonemates induces discharge. An interesting point that arose out of the research carried out on nematocyte discharge by Stephen C. Ertman and Demorest Davenport in 1981, was that <u>A xanthogrammica's</u> mucus on a glass rod failed to elicit a discharge by *A. elegantissima*. However the latter's mucus affected a significant discharge in *A. xanthogrammica*.

A. xanthogrammica and *A. elegantissima* though differing in their zonation levels and microhabitats, overlap in biogeographical range. Hence it is important to distinguish between the two species.

A. elegantissima has a beaded column with a diametre of 3 to 8cm. The pale tentacles have pink to purple tips caused by the symbiotic algae existing in it's tentacles. It is intertidal.

A. xanthogrammica is much larger with a column diametre of 25cm. It has a dark olive-brown column with green tentacles. It is subtidal.

THEORY OF INTRAPOPULATION DISPERSION TECHNIQUES.

A. POISSON METHOD:

Experiments yielding numerical values of a random variable X, the number of successes during a given time interval are called Poisson experiments. In our case the random variable X is the number of Anthopleura elegantissima occuring per quadrat and the interval is in length of quadrat, which is 0.250m.

The probability distribution of the Poisson random variable X, representing the number of successes occuring in a given time interval or specified region is given by :-

$$\text{Poisson probability of } X = \frac{e^{-\mu}\mu^{x}}{x!} \qquad x = 0, 1, 2, 3 \ldots$$

where μ is the average of a number of variables X's in the entire experimental range and e = 2.71828. In our case μ is the average number of _Anthopleura elegantissima_ per quadrat.

In the research essay Poisson method data is used in two different ways: the Variance/Mean ratio technique and the Observed/Expected sample frequencies techniques.

a. VARIANCE / MEAN RATIO:

This method is based on the fact that in Poisson distribution, the variance σ, is equal to the mean or the average μ. The variance is the average of the squares of the deviations of the values of X from the mean μ. With a uniform distribution fewer regions of unusually low or high density occur than expected by chance. Hence all the values of the variable x will be close to the mean μ and the variance will be less than the mean giving a value of less than 1.0 for populations tending towards uniform distribution.

The relationship of Variance to Mean is the result of the particular spread of values about the mean that occur for data from a randomly distributed population. The ratio has a value of 1.0 for randomly distributed population. For a clumped population, the spread will be greater, with many samples having values less or greater than the mean, and the variance will hence be greater than the mean. Therefore the ratio will be greater than 1.0 for population tending towards clumping.

The variance formula is given by :-

$$\sigma^2 = \frac{\Sigma f(x - \mu)}{n - 1}$$

n = no of sample quadrats.
f = frequency of each variable x value.

However to find the accuracy or validity of the Variance / Mean ratio result, we find the significance level by a t-test. If $\bar{x}$ and s^2 are the mean and variance symbols respectively of a random sample of size n or number of samples n taken from a normal population having the mean μ and unknown variance σ^2 then :-

$$t = \frac{\bar{x} - \mu}{s/\sqrt{n}}$$

t is a value of a random variable T having the t-distribution with $\nu = n-1$ degrees of freedom.

For our purposes $\bar{x} = \mu$ as calculated though μ is the mean (normal) for a normal population. $s^2 = \sigma^2$ The normal and the normal mean $\mu = 1.0$. The t-value will have $n-1$ degrees of freedom and the significance level corresponding to this value may be read from pre-calculated tables.

ii. OBSERVED AND EXPECTED SAMPLE FREQUENCIES.

This method involves the calculation of a set of sample frequencies expected on the basis of random pattern for the observed mean and sample number and a comparison of the expected frequencies with those actually observed.

The expected relative frequency, which is going to be a decimal fraction, is given by the Poisson expression of:

$$f(X) = \frac{e^{-\mu}\mu^{x}}{x!}$$ where μ is the mean and x is the random variable representing the number of Anthopleura per quadrat.

A final relative frequency is determined for all samples with numbers greater than that of the last sample size for which the above calculation was made by taking the difference between the sum of the previously calculated relative frequencies and 1.0. The expected relative frequencies must be multiplied by the observed total number of sample n to obtain expected numbers based on the total.

The direction of any deviation of observed values from those expected on the basis of the Poisson distribution can be determined by inspection. If the deviation is towards a uniform distribution, the observed data will contain fewer high and low values than expected. If the deviation is towards a clumped distribution, there will be more high and low values than expected.

The degree of correspondence between observed and expected sets of data may be determined by a chi-square test.

$$\text{Chi-square} = \frac{\Sigma\,(\text{Observed} - \text{Expected})^2}{\text{Expected}}$$

The calculated value may be compared with tabled values for the desired confidence level and the number of degrees of freedom, which will be equal to n-4.

B. PLOTLESS METHODS:

Plotless methods, as opposed to Poisson methods, do not rely on the size of the sampling unit. For the most part, these techniques utilize point-to-plant or plant-to-plant distance measurements.

1. POINT-TO-PLANT DISTANCE RATIO.

This test devised by Holgate 1965, involves the measurement of distances from a random point to the first nearest plant or investebiate and to the second nearest plant or investebiate, (P_1) and

(P_2) respectively. The two measurements for each plant are squared and combined into a ratio of (P_1) squared divided by (P_2) squared. The co-efficient of aggregation (A) for this method is the mean of these ratios from all sampling points.

$$A = \sum \frac{(P_1)^2}{(P_2)^2} \Big/ n \qquad n = \text{number of sampling points.}$$

The co-efficient should equal 0·500 for a randomly distributed population, less than 0·500 for populations tending towards uniform distribution and greater than 0·500 for populations showing aggregations.

A statistical test of the deviation may be done by a z-test from the observed co-efficient of 0·500.

If independant samples of sizes n_1 and n_2 are drawn from two large or infinite populations, discrete or continuous, with means μ_1 and μ_2 and variances σ_1^2 and σ_2^2 respectively, than the sampling distribution of the differences of means $\bar{X}_1 - \bar{X}_2$, is approximately normally distributed with mean and standard deviation given by:

$$\mu_{\bar{x}_1 - \bar{x}_2} = \mu_1 - \mu_2$$

$$\sigma_{\bar{x}_1 - \bar{x}_2} = \sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}}$$

Hence $$z = \frac{(\bar{x}_1 - \bar{x}_2) - (\mu_1 - \mu_2)}{\sqrt{\sigma_1^2/n_1 + \sigma_2^2/n_2}}$$

z is the value of a standard normal ~~value~~

variable Z.

For our purposes switching in the values gives the following simplified equation.

$$Z = \frac{0.500 - A}{0.2887/\sqrt{n}}$$ where n = number of sampling points.

and 0.2887 is the standard deviation of A values for a random population.

The level of confidence and the deviation from the value obtained can be read from pre-calculated values, and tables. They will have $\nu = n-1$ degrees of freedom.

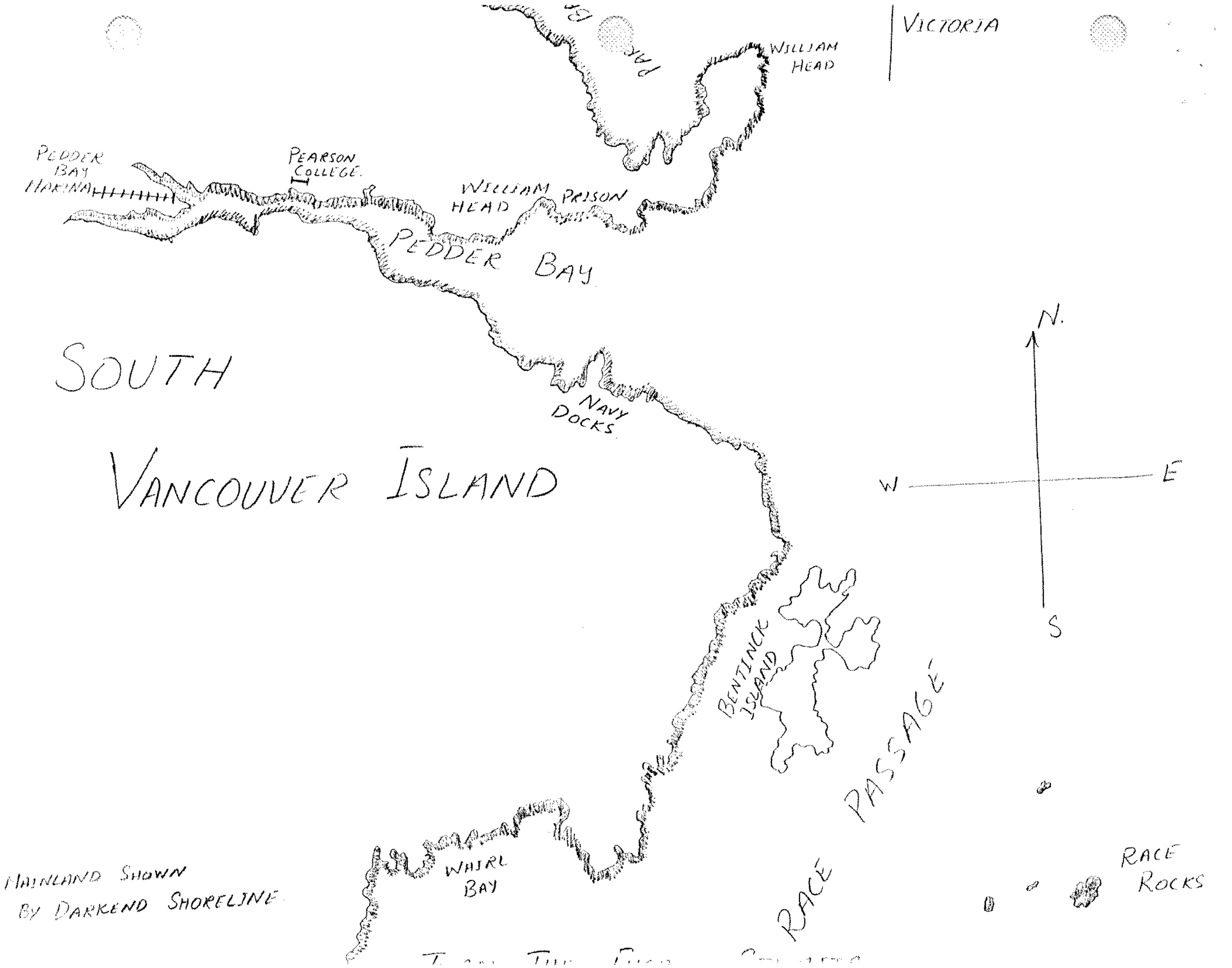
LIST OF MAPS

1. SOUTH VICTORIA, PEDDER BAY AND RACE ROCK ISLANDS.

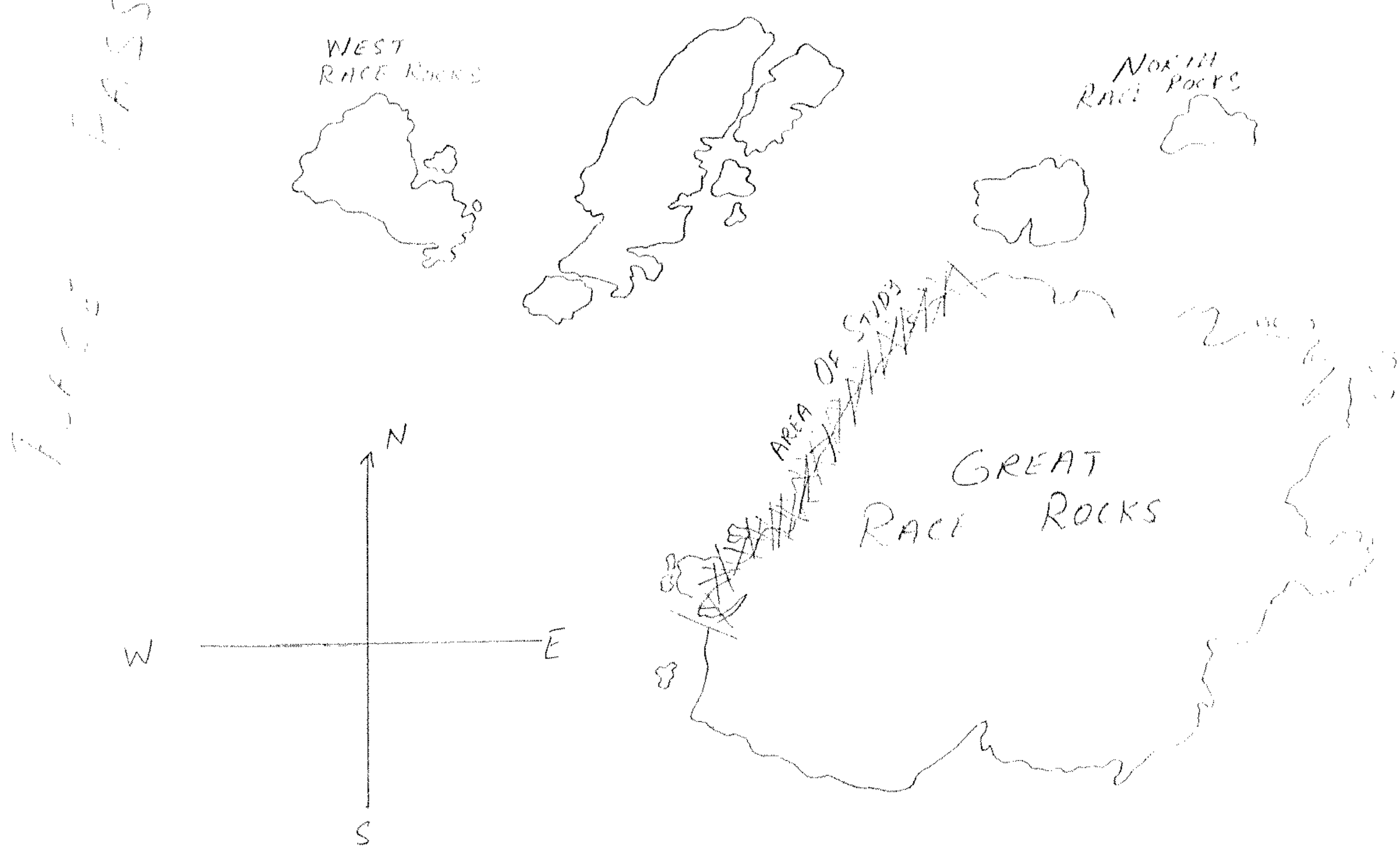
2. RACE ROCKS AND AREA OF STUDY.

3. TIDE TABLES FOR 25th MAY 1986.

4. BIOGEOGRAPHICAL DISTRIBUTION OF _ANTHOPLEURA ELEGANTISSIMA_ (SOLITARY AND CLONAL) AND _XANTHOGRAMMICA_ ALONG THE WEST COAST OF NORTH AMERICA.

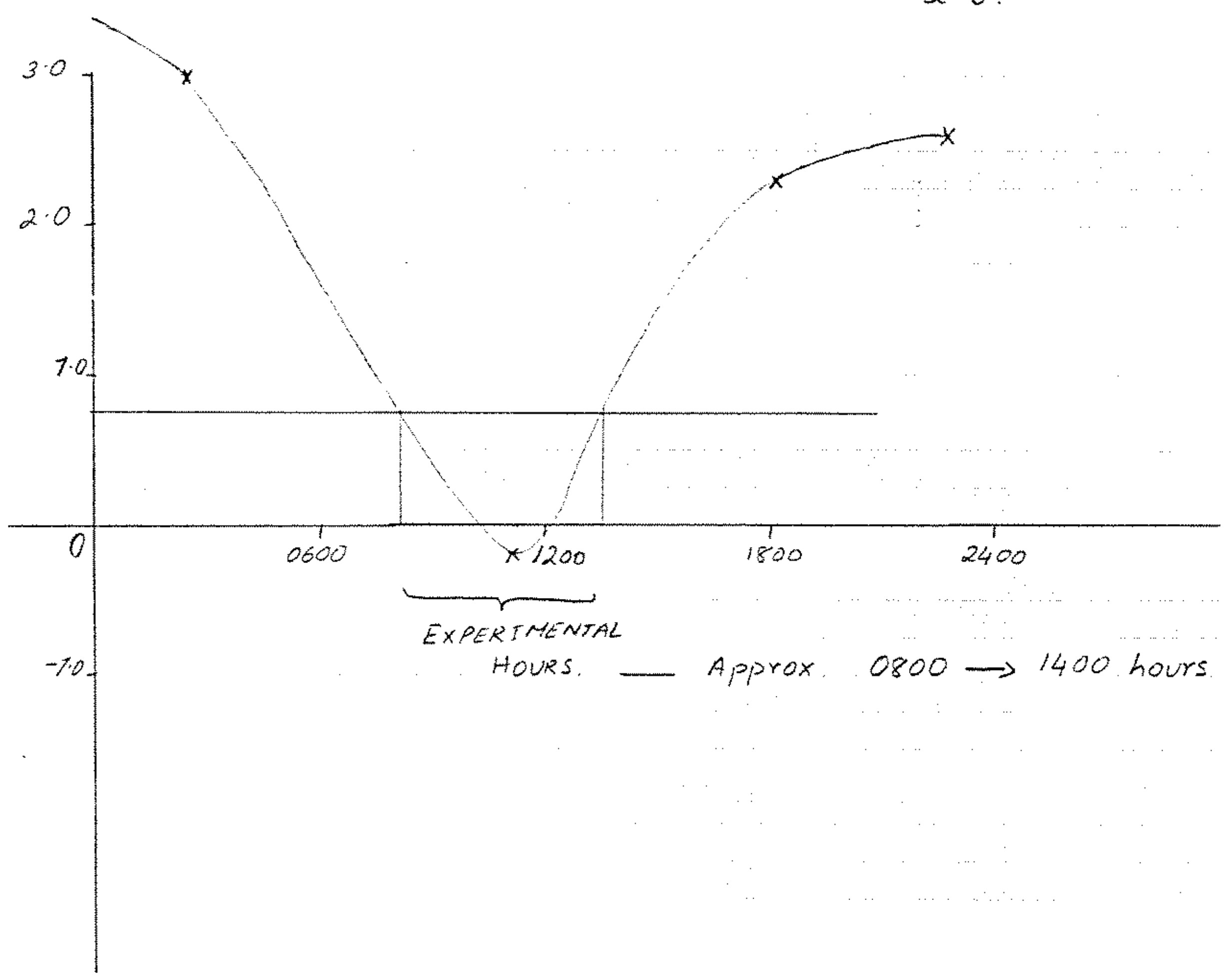

VICTORIA
WILLIAM HEAD
PEDDER BAY MARINA
PEARSON COLLEGE
WILLIAM HEAD PRISON
PEDDER BAY
SOUTH VANCOUVER ISLAND
NAVY DOCKS
N.
W
E
S
BENTINCK ISLAND
RACE PASSAGE
WHIRL BAY
RACE ROCKS
MAINLAND SHOWN BY DARKEND SHORELINE

RACE ROCK ISLANDS.



TIDE – TABLES. OF VICTORIA, B.C.
25th MAY 1986.

HOURS	HEIGHT.
0230	3.0
1115	−0.2
1815	2.3
2245	2.6.



— Approx. 0800 → 1400 hours.

BIOGEOGRAPHICAL DISTRIBUTIONS OF ANTHOPLEURA.

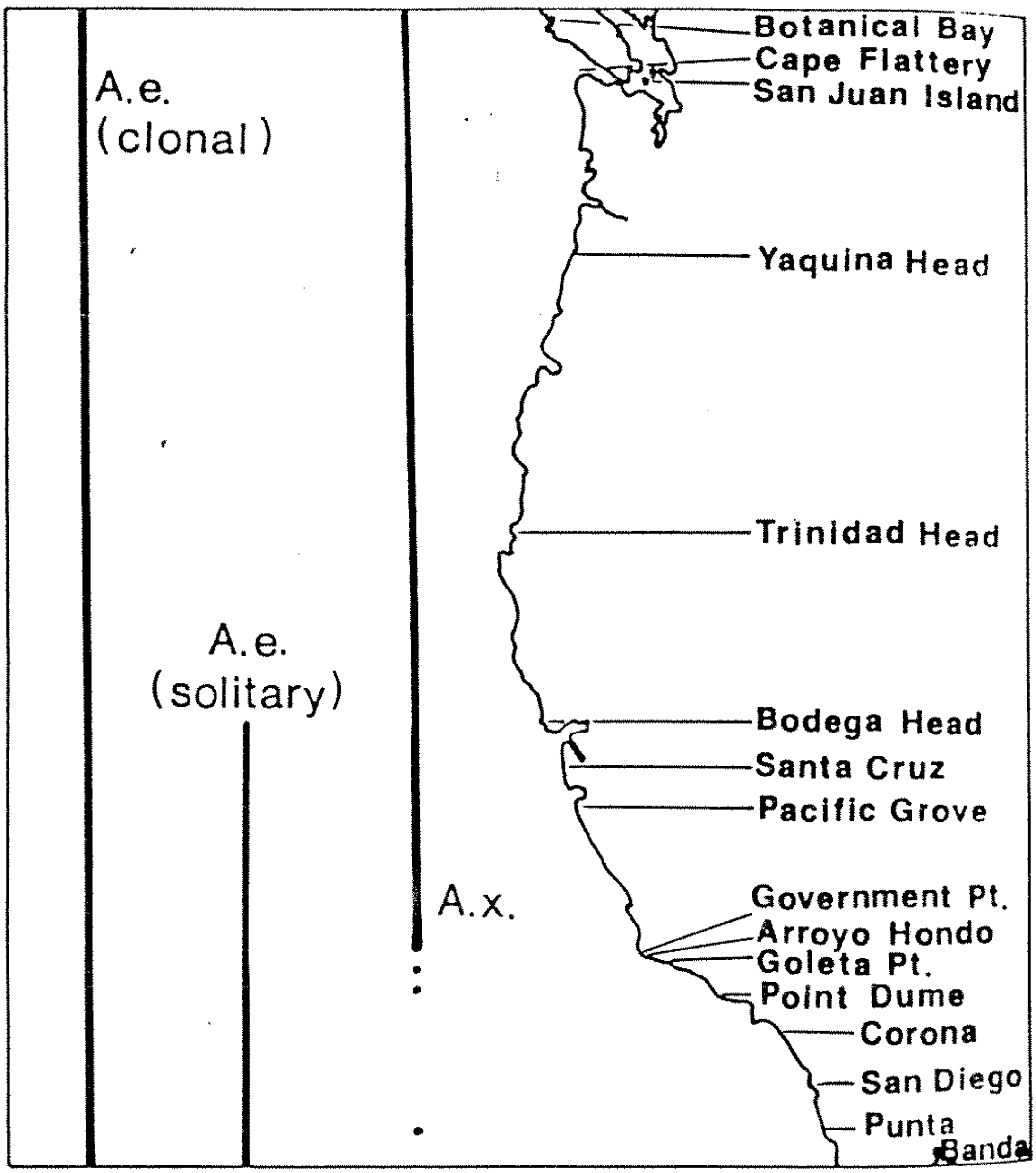


FIG. 2. The Pacific coast of North America showing survey sample sites and proposed ranges for the giant green anemone, *Anthopleura xanthogrammica*, the clonal aggregating anemone known as *A. elegantissima*, and the solitary anemone, also called *A. elegantissima*.

27

METHODS

PROCEDURE: Before collecting any data for Poisson or Plotless techniques, a simple line transect was carried out in the area of study and a zonation done on it. This was to give an idea about the distribution pattern which would help in setting up a hypothesis. It would describe the zonation of *Anthopleura elegantissima* and forms the preliminary study and research.

A transect is a line, normally straight along which observations are made in a systematic fashion. It is a form of regular sampling. A straight transect was taken from peg number 6, (see location on map # 2) and extended down to the zero-level tide mark at low tide. The quadrat choosen was a 0·25m × 0·25m square. The quadrat was moved edge to edge, that is no gap or interval between one quadrat and the next and the number of *Anthopleura elegantissima* (clonal) counted in each, care being taken to avoid counting *A. xanthogrammica*.

Three line transects were done on the same peg, one perpendicular to the rocks, the other two at 45° angle on each sides of the first one. The second and third line transects serve as comparisons while the first one is used for zonation.

For the Poisson method, random sampling is done along the rocks including a number of quadrats containing no *Anthopleura* at all. Again a 0·25m square quadrat is choosen and tossed at random along the entire north, north-west side of island of Race Rocks. Wherever it lands, the number of *Anthopleura elegantissima* inside are counted. If an anemone is more than or 50% inside, it is counted, otherwise ignored. The data collected is then treated by two methods, the Variance / Mean ratio method and the Poisson distribution method.

For the Plotless technique, data is obtained randomly as well. The test ~~includes~~ involves the measurement of distances from a random point to the first nearest anemone (P_1) and the second nearest anemone (P_2). Random points can be selected by tossing a coin and taking the centre of the coin as the random point and measuring the distance from it. This method as we see, is not dependant on the size of any sampling unit, length, volume etc. and hence a source of error is eliminated. Sometimes the *Anthopleura* are quite squeezed together. In this case, error in measurements can be reduced by measuring the distance from the random point to the opening in the oral disk. Readings will then be accurate to one decimal place or 0·1cm.

RAW DATA

Line Transect – peg # 6.
x = no of continuous quadrats.
f_1 = no of Anthopleura in each quadrat in 1st line transect (used for zonations).
f_2 = Second transect
f_3 = Third transect.

x	f_1		f_2	f_3
0	0		0	0
1	0		0	0
2	0		0	0
3	0		0	0
4	0		0	0
5	0		0	0
6	0		0	0
7	0		0	0
8	0		0	0
9	0		1	0
10	22	TIDE POOL	0	0
11	65		0	0
12	0		0	0
13	2		0	0
14	4		0	7
15	12		0	6
16	40		0	11
17	35		0	28
18	9		0	60
19	13		1	16
20	39		75	9
21	16		78	22
22	15		45	13
23.	0		15	10.

Line transect data contd.

x	f_1	f_2	f_3
24	0	12	0
25	0	4	4
26		1	6
27		0	0
28		0	0.

POISSON METHOD:-

x = no of *Anthopleura elegantissima* in a quadrat.

f = frequency of quadrat having that number of *Anthopleura* in it.

--- CONTD ---

x	f	x	f
0	8	15	5
1	7	16	4
2	2	17	4
3	1	18	3
4	3	19	3
5	2	20	4
6	5	21	3
7	5	22	4
8	3	23	1
9	6	24	2
10	5	25	3
11	3	31 → 40	16
12	4	41 → 50	8
13	5	51 → 60	10.
14	6		

Plotless Method : Point-to-Plant ratio.

n = number of random sampling points.
P_1 = distance to the nearest *A. elegantissima*.
P_2 = distance to the second nearest *A. elegantissima*

n	P_1 (in cm)	P_2 (in cm)
1	16 cm	18·0
2	8·5	12·0
3	2·3	7·0
4	10·5	11·0
5	4·3	6·0
6	3·0	4·0
7	2·7	4·5
8	2·0	2·3
9	2·2	6·0
10	2·0	3·5
11	2·4	3·9
12	3·2	4·4
13	1·3	3·8
14	1·9	2·8
15	12·0	15·5
16	3·2	3·5
17	2·1	2·8
18	2·3	2·9
19	1·4	2·6
20	2·0	3·6
21	3·8	6·0
22	3·0	4·0
23	2·5	3·0
24	2·6	3·1
25	4·2	4·7
26.	3·0	4·3.

STATISTICAL TECHNIQUES FOR ANALYSIS

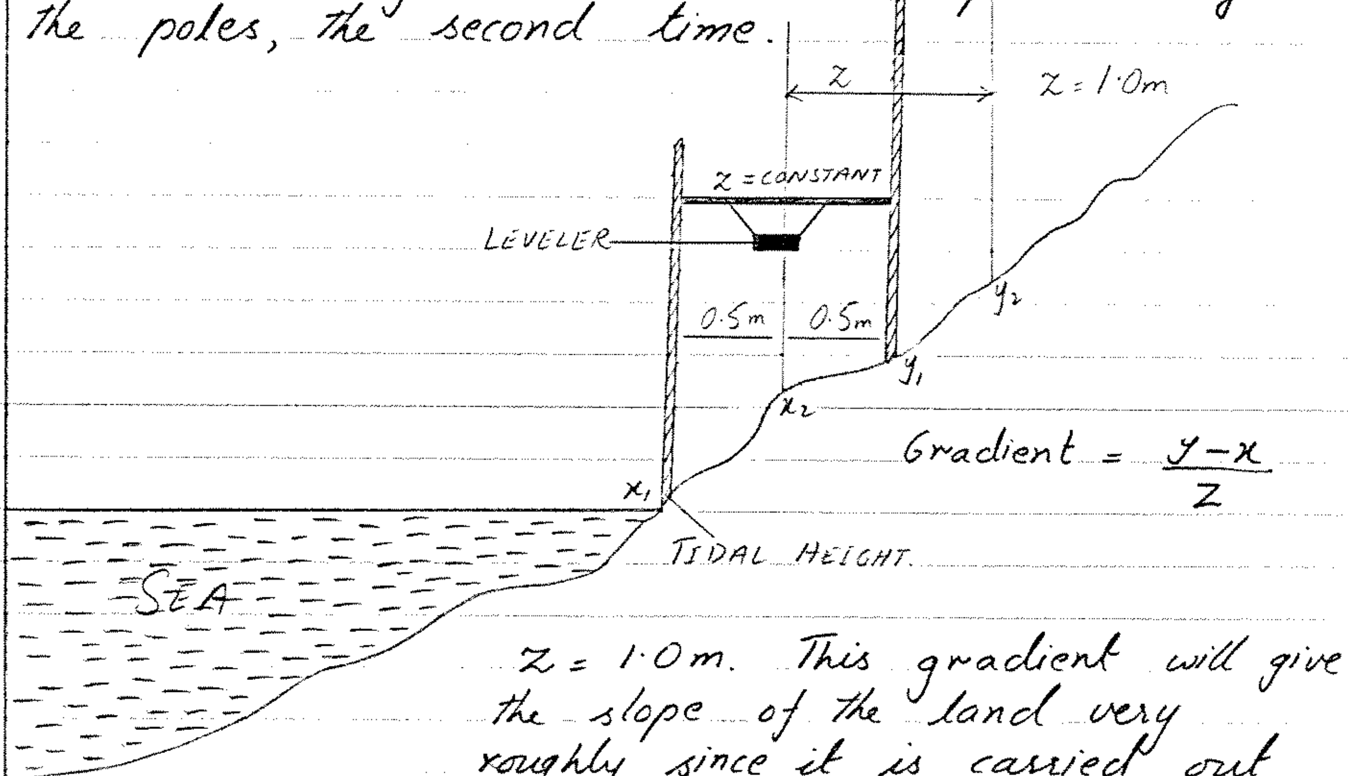
For the Variance/Mean ratio the calculations were done by hand with a calculator as well as computer programmes were written for it as well as the t-test that follows it. The two results were the same. For the Observed/Expected frequencies, part of the calculations (the factorial (!) and power calculations) were done with computer programmes already available. The chi-square test was done with a calculator, and the values and confidence levels read off from tables. For the computer programmes, special thanks to Sylvia Lange.

The Point-to-Plant ratio was calculated manually with a calculator as well as the z-test that follows it.

PRELIMINARY STUDIES

Before collecting the actual data, a few preliminary studies were carried out to give an idea of the land profile as well as to find the zonation of Anthopleura elegantissima so as to help in the setting up of a hypothesis. The following diagram may help to explain the procedure which led to the development of a fairly crude land profile of the study area.

The lighter lines show the position of the poles, the second time.



Z = 1·0m. This gradient will give the slope of the land very roughly since it is carried out at 0·5m intervals.

SEA-LEVEL OR TIDAL HEIGHT = 1·8m.

It should be noted however that this profile of the area of study in a two dimensional way was done on a different day than the actual data collection.

x (in m)	Δy (in m)
0·0	1·8
0·5	0·4
1·0	0·15
1·5	0·05
2·0	0·15
2·5	0·04
3·0	−0·04
3·5	0·0
4·0	0·02
4·5	0·08
5·0	0·30
5·5	0·78
6·0	0·40

The instruement for the purpose was made by taking three wooden poles and hammering in nails at every 10cm mark. The third pole had two nails hammered in at a distance of 1·0m from each other, and had a leveler attatched to it. This was a home-made device. Starting from the edge of the water the poles were placed together as shown in the diagram and the two readings in height and their differences taken. The poles were moved along 0·5m at a time, so now we have the gradient of the rock face at 0·5m interval. The profile of the land is roughly sketched or plotted on a graph paper.

Zonation is done by having a simple line transect perpendicular to the rock face on peg # 6 and then placed or super-imposed over the profile sketch. This will give the zonation, an idea of the level and the height where the

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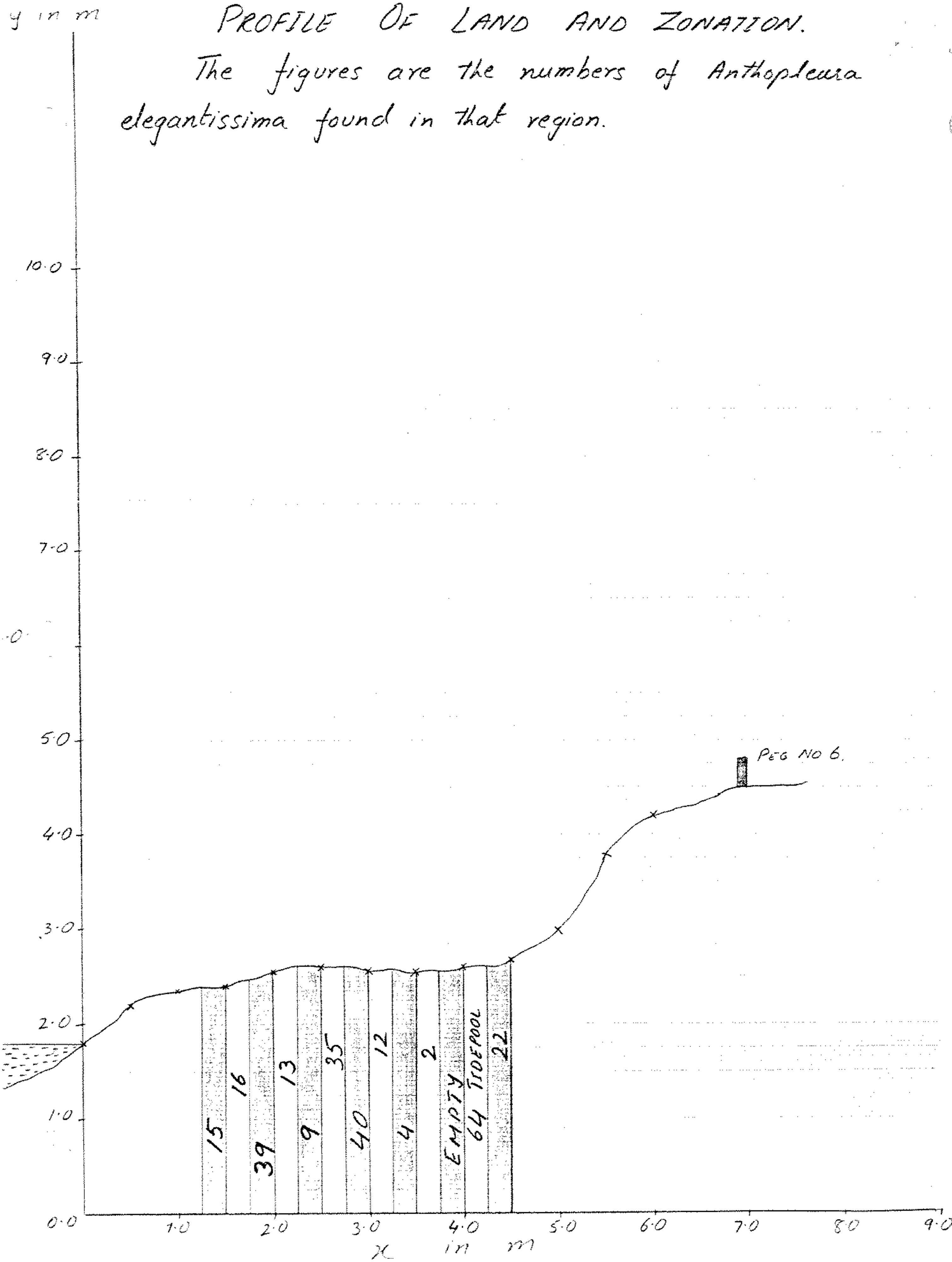
Anthopleusa elegantissima are going to be concentrated. This will classify the anemone as either an intertidal or a sub-tidal one.

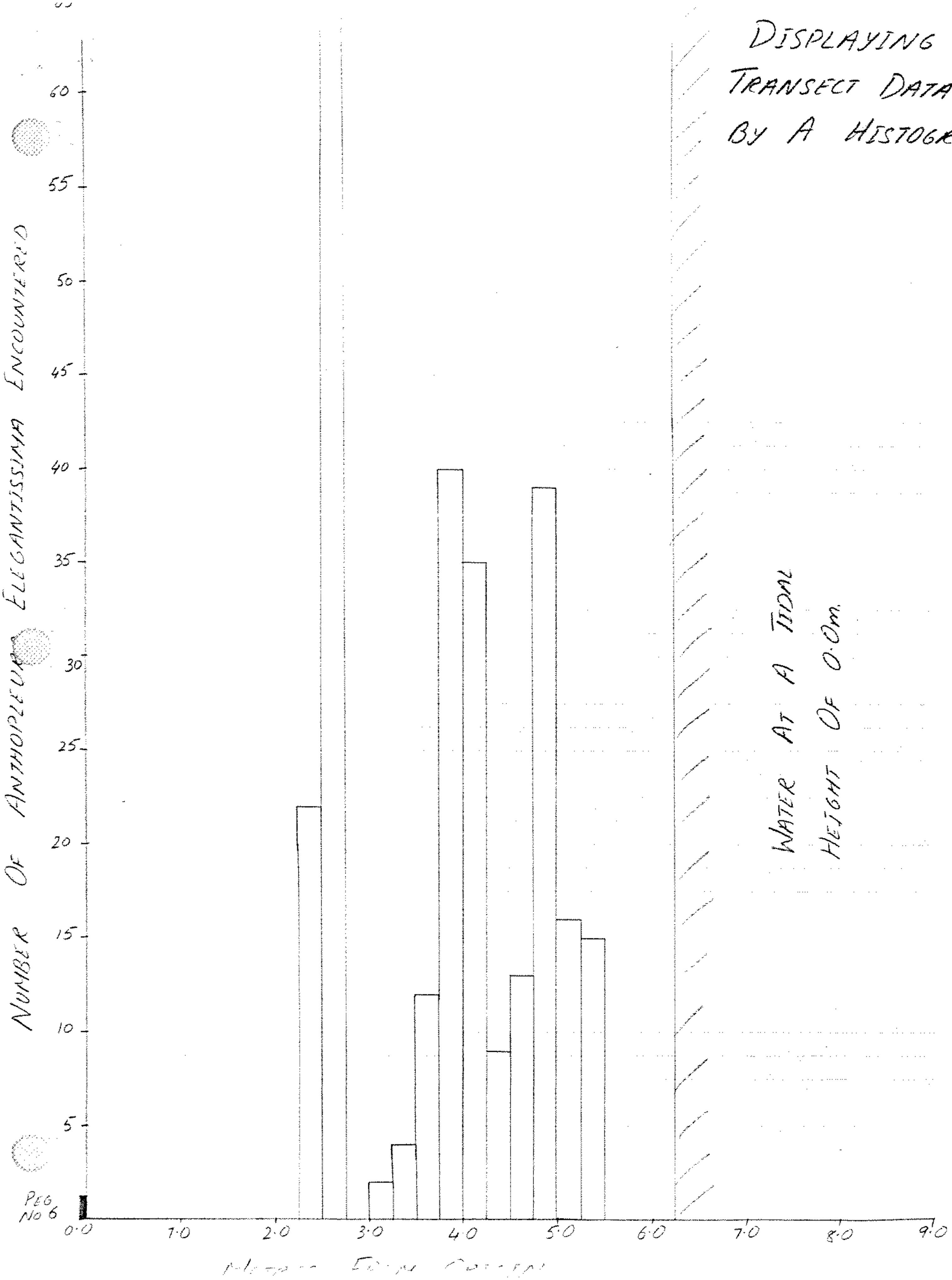
From the results and the graph sketch, the zonation of the anemone is obvious and suggests that it is intertidal, majorly concentrated at heights between 2·3m to 2·6m. The majority of the *Anthopleusa* occur here, at an intertidal level. One obvious reason that can be suggested from the graph is that this area is the flatest or in a plain and thus the cracks are better able to hold the water of out-going tide thus preventing desiccation of the anemone as opposed to sloping nearby rock.

PROFILE OF LAND AND ZONATION.

The figures are the numbers of *Anthopleura elegantissima* found in that region.



DISPLAYING TRANSECT DATA BY A HISTOGRAM.



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RESULTS AND ANALYSIS.

1. POISSON TECHNIQUE:

Two-tailed test hypothesis.
Null Hypothesis H_0 states that *Anthopleura elegantissima* of the area under study are randomly distributed.
Alternate hypothesis H_a states that *Anthopleura elegantissima* have a clumped distribution.

a. VARIANCE / MEAN RATIO.

x = Number of *Anthopleura* per quadrat.
f = frequency of quadrats having x number of *Anthopleura*.
m = mean number of *Anthopleura* / quadrat.

$$m = \frac{\Sigma(fx)}{n}$$

n = Total number of sample quadrats = $\Sigma(f)$
x−m = Deviation of each x value from the mean.

$$s^2 = \text{variance} = \frac{\Sigma(x-m)^2}{n-1}.$$

DATA.

NUMBER / QUADRAT x.	OBSERVED FREQUENCY f.	fx.	DEVIATION (x − m)	$(x-m)^2$	$f(x-m)^2$
0	8	0	−19·62	384·94	3079·52
1	7	7	−18·62	346·70	2426·90
2	2	4	−17·62	310·46	620·92
3	1	3	−16·62	276·22	276·22
4	3	12	−15·62	243·98	731·95
5	2	10	−14·62	213·74	427·48
6	5	30	−13·62	185·50	927·50
7	5	35	−12·62	159·26.	796·30.

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DATA (CONTD)

NUMBER/ QUADRAT x.	OBSERVED FREQUENCY f	fx.	DEVIATION $(x-m)$	$(x-m)^2$	$f(x-m)^2$
8	3	24	−11.62	135.02	405.06
9	6	54	−10.62	112.78	676.68
10	5	50	−9.62	92.54	462.70
11	3	33	−8.62	74.30	222.90
12	4	48	−7.62	58.06	232.24
13	5	65	−6.62	43.82	219.10
14	6	84	−5.62	31.58	189.48
15	5	75	−4.62	21.34	106.70
16	4	64	−3.62	13.10	52.40
17	4	68	−2.62	6.86	27.44
18	3	54	−1.62	2.62	7.86
19	3	57	−0.62	0.38	1.14
20	4	80	+0.38	0.14	0.56
21	3	63	+1.38	1.90	5.70
22	4	88	+2.38	5.66	22.64
23	1	23	+3.38	11.42	11.42
24	2	48	+4.38	19.18	38.36
25	3	82.5	+7.88	62.01	186.27
31→40	16	568	+15.88	252.17	4034.72
41→50	8	364	+25.88	669.77	6358.16
51→60	10.	555	+35.88	1287.37	12873.70
TOTAL	135	2648.5	−104.98	5022.90	35422.62

CALCULATIONS :-

$$\text{Mean } m = \frac{\Sigma(fx)}{\Sigma(f)} = \frac{2648.50}{135} = 19.62.$$

$n = \Sigma(f) = 135$ Mean $m = 19.62$.

$$\text{Variance } s^2 = \frac{\Sigma f(x-m)^2}{n-1} = \frac{35422.62}{135-1} = 264.35.$$

$$\text{VARIANCE / MEAN RATIO} = \frac{264.35}{19.62} = 13.47 = 13.5.$$

Variance / Mean ratio is known as the index of aggregation.

Index of aggregation = 1.0 — Random distribution.
Index of aggregation < 1.0 — Uniform distribution.
Index of aggregation > 1.0 — Clumped distribution.

The Variance / Mean ratio or the index of aggregation turns out to be 13.5. The results mathematically tell us that the Anthopleura elegantissima has a clumped distribution.

Now to test the accuracy of the results obtained we must put them to the t-test.

First we must find the standard error of deviation.

$$\text{st. error of deviation} = \sqrt{\frac{2}{n-1}} = \sqrt{\frac{2}{135-1}}$$
$$= 0.122.$$

$$\text{t-test} = \frac{\text{Observed } s^2/m - 1.0}{0.122} = \frac{13.5 - 1.0}{0.122}$$
$$= 102.459 = 102.5.$$

The t-value has $n-1 = 135-1 = 134$ degrees of freedom and it's value is 102.5. If we look up the standard t-tables, 134 degrees of freedom corresponds to more than 99.5%, a level of confidence. From this result we can successfully discard the null hypothesis which states that Anthopleura elegantissima is randomly distributed and accept the alternate hypothesis.

1. POISSON TECHNIQUES:-

b. OBSERVED AND EXPECTED FREQUENCIES:

The data obtained will give us known or observed frequencies. We will calculate the expected frequencies from the Poisson expression:-

$$e^{-m}.\frac{m^x}{x!}$$ where m = the mean = 19.62. (previously calculated).

e^{-m} will be a constant = $3.01403294 \times 10^{-9}$
x is the number of Anthopleura per quadrat sample.

Expected frequency = $\left(e^{-m}.\frac{m^x}{x!}\right)n$.

where $n = \Sigma(f)$ = Total number of sample quadrats.
And observed frequency = f/n $n = \Sigma(f)$.
Hence mathematically speaking, the sum of all the observed final frequencies should be equal to 1.000.

DATA:-

x = number of *Anthopleura elegantissima* per quadrat.
f = frequency of that quadrat.
$n = \Sigma(f)$ = Sum of all f-values = 135.
Constant $k = e^{-m} = 3.014 \times 10^{-9}$

Observed frequency $(O) = \frac{(f)}{\Sigma(f)} = \frac{(f)}{(n)}$

DATA — (CONTD).

x.	f.	POISSON EXPRESSION	POISSON FREQUENCY.	EXPECTED # OF QUADRATS (E)	OBSERVED # OF QUADRATS. (O).	$(O-E)^2$	$\frac{(O-E)^2}{E}$
0	8	$k.m^0/0!$	5.913×10^{-8}	5.972×10^{-6}	7.921×10^{-2}	0.0063	1067.8
1	7	$k.m^1/1!$	5.913×10^{-8}	5.972×10^{-6}	6.931×10^{-2}	0.0048	813.6
2	2	$k.m^2/2!$	5.801×10^{-7}	5.859×10^{-5}	1.980×10^{-2}	0.0004	6.79
3	1	$k.m^3/3!$	3.794×10^{-6}	3.832×10^{-4}	9.901×10^{-3}	0.00009	0.235
4	3	$k.m^4/4!$	1.861×10^{-5}	1.880×10^{-3}	2.970×10^{-2}	0.0008	0.425
5	2	$k.m^5/5!$	7.302×10^{-5}	7.375×10^{-3}	1.980×10^{-2}	0.00015	0.020
6	5	$k.m^6/6!$	2.388×10^{-4}	2.412×10^{-2}	4.951×10^{-2}	0.00065	0.028
7	5	$k.m^7/7!$	6.694×10^{-4}	6.761×10^{-2}	4.951×10^{-2}	0.00033	0.0059
8	3	$k.m^8/8!$	1.641×10^{-3}	1.657×10^{-1}	2.970×10^{-2}	0.0185	0.112
9	6	$k.m^9/9!$	3.578×10^{-3}	3.614×10^{-1}	5.941×10^{-2}	0.0912	0.252
10	5	$k.m^{10}/10!$	7.02×10^{-3}	7.09×10^{-1}	4.951×10^{-2}	0.4350	0.614
11	3	$k.m^{11}/11!$	1.252×10^{-2}	1.265	2.970×10^{-2}	1.5260	1.206
12	4	$k.m^{12}/12!$	2.047×10^{-2}	2.067	3.960×10^{-2}	4.1104	1.989
13	5	$k.m^{13}/13!$	3.089×10^{-2}	3.12	4.951×10^{-2}	9.4279	3.02
14	6	$k.m^{14}/14!$	4.331×10^{-2}	4.374	5.941×10^{-2}	18.6157	4.256
15	5	$k.m^{15}/15!$	5.663×10^{-2}	5.720	4.951×10^{-2}	32.1545	5.62
16	4	$k.m^{16}/16!$	6.944×10^{-2}	7.013	3.960×10^{-2}	48.6283	6.93
17	4	$k.m^{17}/17!$	8.017×10^{-2}	8.097	3.960×10^{-2}	64.9217	8.02
18	3	$k.m^{18}/18!$	8.738×10^{-2}	8.825	2.970×10^{-2}	77.3573	8.77
19	3	$k.m^{19}/19!$	9.024×10^{-2}	9.114	2.970×10^{-2}	82.5245	9.05
20	4	$k.m^{20}/20!$	8.852×10^{-2}	8.941	3.960×10^{-2}	79.2349	8.86
21	3	$k.m^{21}/21!$	8.270×10^{-2}	8.353	2.970×10^{-2}	69.2773	8.29
22	4	$k.m^{22}/22!$	7.375×10^{-2}	7.449	3.960×10^{-2}	54.8992	7.37
23	1	$k.m^{23}/23!$	6.290×10^{-2}	6.353	9.901×10^{-3}	40.2349	6.33
24	2	$k.m^{24}/24!$	5.124×10^{-2}	5.175	1.980×10^{-2}	26.5761	5.14
25	3	$k.m^{25}/25!$	4.036×10^{-2}	4.076.	2.970×10^{-2}	16.3725.	4.02
35	16	$k.m^{35}/35!$	–	–	–	–	–
45	8	$k.m^{45}/45!$	–	–	–	–	–
55	10	$k.m^{55}/55!$	–	–	–	–	–

Inspecting the 6^{th} column of $(O-E)^2$ we see that the deviation of observed values from expected ones are quite large for over 50 per cent of the cases observed. We can safely state that the distribution of _Anthopleura elegantissima_ at leasts tends towards a clumped population.

We must pass our results through a chi-square test to check it's accuracy or the level of confidence. The chi-square test has the following equation:-

$$\text{Chi-square} = \sum \frac{(\text{Observed} - \text{Expected})^2}{\text{Expected}} = 1978.75$$

A chi-square graph can have cut-off values at the two extremes but in our case it is necessary to include quadrat samples with no _Anthopleura_ elegantissima in them. Therefore the top extreme values cannot be discarded. The chi-square distribution will have $\nu = 135-1 = 134$ degrees of freedom.

We have a percentage value of greater than 99.5% which is accurate enough for us to reject the null hypothesis that the _Anthopleura elegantissima_ is randomly distributed and to accept the alternate hypothesis that the _Anthopleura_ are clumped.

With 134 degrees of freedom 1978.75 is a figure way beyond the 99.5% level even though we did not add the values of the last three frequencies to the chi-square, ~~Yet~~ due to the fact that at 35! (thirty-five factorial) the computer overflowed. Yet the %age accuracy of the results is enough to reject the null-hypothesis.

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2\. ## PLOTLESS TECHNIQUES :-

Point-to-plant distance ratio.

(Devised by Holgate, 1965)

ONE-TAILED HYPOTHESIS :

NULL HYPOTHESIS H_0 : *Anthopleura elegantissima* has a random distribution in the area under study.

ALTERNATE HYPOTHESIS H_a : *Anthopleura elegantissima* does not have a random distribution in the area under study.

Points are chosen randomly.

P_1 = Distance from the random point to the first nearest *Anthopleura elegantissima*.

P_2 = Distance from the random point to the second nearest *Anthopleura elegantissima*.

DATA :-

SAMPLING POINTS.	P_1 (in cm)	P_2 (in cm)	$(P_1)^2$ (in cm^2)	$(P_2)^2$ (in cm^2)	$(P_1)^2/(P_2)^2$
1	16·0	18·0	256	324	0·790
2	8·5	12·0	72·25	144	0·502
3	2·3	7·0	5·29	49	0·108
4	10·5	11·0	110·25	121	0·911
5	4·3	6·0	18·49	36	0·514
6	3·0	4·0	9	16	0·563
7	2·7	4·5	7·29	20·25	0·36
8	2·0	2·3	4·0	5·29	0·756
9	2·2	6·0	4·84	36	0·134
10	2·0	3·5	4·0	12·25	0·327
11	2·4	3·9	5·76	15·21	0·379
12	3·2	4·4	10·24	19·36	0·529
13	1·5	3·8.	2·25	14·44.	0·156.

SAMPLING POINTS	P_1 (in cm)	P_2 (in cm)	$(P_1)^2$ (in cm^2)	$(P_2)^2$ (in cm^2)	$(P_1)^2/(P_2)^2$
14	1·9	2·8	3·61	7·84	0·460
15	12·0	15·5	144	240·25	0·599
16	3·2	3·5	10·24	12·25	0·836
17	2·1	2·8	4·41	7·84	0·563
18	2·3	2·9	5·29	8·41	0·629
19	1·4	2·6	1·96	6·76	0·290
20	2·0	3·6	4·0	12·96	0·386
21	3·8	6·0	14·44	36	0·401
22	3·0	4·0	9	16	0·563
23	2·5	3·0	6·25	9	0·694
24	2·6	3·1	6·76	9·61	0·703
25	4·2	4·7	17·64	22·09	0·799
26	3·0	4·3	9·0	18·49	0·487
TOTAL			740·26	1220·3	13·439.

CO-EFFICIENT OF AGGREGATION $A = \Sigma \left[\frac{(P_1)^2}{(P_2)^2}\right] / n$

where n = total number of sampling random points.
$n = 26$.

Co-efficient of Aggregation = $A = \frac{13 \cdot 439}{26} = 0 \cdot 517$.

Co-efficient of Aggregation = 0·500 Random Distribution
Co-efficient of Aggregation < 0·500. Uniform Distribution.
Co-efficient of Aggregation > 0·500 Clumped Distribution.

From the results obtained we see that the co-efficient of Aggregation A is greater than 0·500. It is equal to a value of 0·517 and hence the results show that Anthopleura elegantissima has a clumped distribution.

A statistical test of the deviation of the co-efficient is shown by a z-test which will give us the level of confidence as well as the level of freedom. That is to say it will give us the percentage accusacy of the results which would decide wether to accept or reject the null hypothesis which is being tested by the z-test designed for randomness.

$$z\text{-test} = \frac{0.500 - A}{\frac{0.2887}{\sqrt{n}}}$$

where n = number of sampling points = 26.
A = index of Aggregation.

0.2887 is the standard deviation of A values for a random population.

$$\therefore z\text{-value} = \frac{0.500 - 0.517}{\frac{0.2887}{\sqrt{26}}} = \frac{-0.017}{0.0566}$$

$$z\text{-value} = -0.300.$$

Standard z-values are positive only but since the standard graph of z-values is symmetrical, -0.300 will have the same percentage accusacy as $+0.300$. At a level of freedom of $\nu = n-1 = 25$, we have a level of confidence between 40% and 50%. This is much below the critical confidence level established (95%) and leads us to reject the hypothesis that Anthopleura elegantissima is randomly distributed. The z-test is done on the assumption that the Anthopleura are randomly distributed, (a fact obvious since we used 0.2887 as the standard deviation of aggregate values for a random population).

Since the hypothesis is a one-tailed one, we reject the first (null hypothesis) and accept the 2nd one which states that Anthopleura are not randomly distributed

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DISCUSSION AND CONCLUSION

From the two-tailed and one-tailed hypothesis established and the Poisson and Plotless techniques carried out we can conclude that *Anthopleura elegantissima* are not randomly distributed but are in fact clumped in the area under study. The last distinction is very important and must be borne in mind. Knowing the kind of *Anthopleura elegantissima's* distribution pattern, we can identify the sub-species level. The *Anthopleura elegantissima* is of the clonal kind which is an intertidal species. The zonation of these *Anthopleura*, done as part of the preliminary studies suggests likewise and shows Anthopleura in the intertidal zone as well.

The Poisson as well as the Plotless technique, both seem to work well on this intertidal species. A quadrat of 0·25m x 0·25m is a good size for the anemone since it gave us a fair number of quadrats with no *Anthopleura elegantissima*. The same techniques, though not necessarily the same size are recommended for intertidal species such as Chitons, California mussels, or even goose-neck barnacles.

Intertidal *Anthopleura elegantissima* benefits greatly from a clumped distribution. It reproduces asexually by longitudinal fission which enhances the formation of close colonies since the two sister clones remain almost side by side unless a predator or an undesirable environmental factor causes it to move away. The clonal form, being lower mid-intertidal is exposed to sunlight and air much more than the solitary form in the sub-tidal zone. Hence the former's distribution is a very useful and important factor in reducing desication and water loss, since clumping reduces the surface area exposed to light. The sticking together in one unit makes the clonal Anthopleusa elegantissima more resistant and tolerant to the buffeting action of waves as well, commonly experienced by organisms of the intertidal as a result of going in and out of tides.

Since the organisms reproduce asexually the genotype is homogenous in a colony and a successful genotype's continuation is ensured. A very intriguing experiment done on nematocyte discharge by Stephen C. Ertman and Demorest Davenport, Dec 1981, showed that while A. elegantissima was not affected by the mucus of A xanthogrammica, yet the mucus of A. elegantissima had a significantly strong discharge effect upon A. xanthogrammica. This has important value for A elegantissima's colony since both the two different species of A. elegantissima and A xanthogrammica have a common biogeographical distribution though different zonations. Thus when an A. xanthogrammica approaches a colony of A. elegantissima, nematocyte discharge takes place, yet it is the A. xanthogrammica that responds by moving away. This has important clonal value since the colony is maintained and not disturbed by the stimulus.

The mathematical results point out to the fact that Anthopleusa elegantissima was of the clonal sub-species level at least in the area under study in the summer of '86, in which the observations were made. This is in agreement with the research and results of Lisbeth Francis, 1979, and the map constructed to show the biogeographical range of clonal and solitary Anthopleusa elegantissima.

The solitary form does not occur as far north up as Juan de Fuca straits. Also the research showed that the density of the anemone is not high enough to make Poisson techniques invalid for it, and that though the anemone is not totally sessile, it is sessile enough to make the point-to-plant ratio method successful.

The results of the research essay can be further confirmed by observing the Anthopleura on a daily basis during the months of June/July when asexual division of A elegantissima has the highest rate. Asexual longitudinal fission leaves behind division scars that are areas of smooth light-coloured tissue where the body wall has sealed after division. These scars are retained for up to normally three months (longer in the laboratory). Kenn P. Sebens, 1982. If these scars are visible on 90% or more than 90% we can confirm the results of this research essay that is the Anthopleura elegantissima is of the clonal kind since it reproduces asexually.

This research essay has been a valuable study in techniques, analysis and field biology. It served as a good introduction to statistical techniques being applied to practical purposes. It has also opened vistas of unlimited research. First of all, the same experiment should be repeated at time intervals or season intervals to see if the population structure or distribution changes drastically with time. Having the zonation of Anthopleura, correlations can be done of the zonation with other abiotic factors such as tidal height or the amount of light and exposure to light, air and heat. The correlation and mathematical relationship can be studied in greater depth.

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