THE ECOLOGY OF PROTOZOA IN CHINSTRAP PENGUIN GUANO

By H. G. Smith

Abstract. Observations on the protozoan fauna of the guano of chinstrap penguin colonies on Signy Island led to the formation of a hypothesis describing the influence of the penguin population on the guano fauna through their effect on the physical and chemical properties of the guano. In one colony, the numbers of three guano-specific species of Protozoa (*Tetramitus rostratus* Perty, *Philaster* sp. Fabre-Domergue and *Vorticella microstoma* Ehrenberg) in an experimental area from which penguins were excluded by a fence, and in a control area, were determined on 16 occasions over 15 months, together with observations on guano properties. Seasonal variation and differences between the exclosure and control area were examined in an attempt to distinguish the influences of climate and the penguin population on the Protozoa of the guano. The guano within the exclosure had a lower pH than that of the control area and became overgrown by the alga *Prasiola crispa* Meneghini. No significant changes in the protozoan fauna of the exclosed guano had been observed by the end of the third summer since the erection of the exclosure.

On the coast of Signy Island, South Orkney Islands, there are several large areas occupied from late October to late April each year by colonies of breeding and later moulting chinstrap penguins (*Pygoscelis antarctica*). Chicks are present in January and February. Penguins are completely absent in winter. The annual cycle of the chinstrap penguin on Signy Island has been described in detail by Conroy and others (in press). The penguins deposit acid excreta (pH *c.* 5·9) and feathers on the ground which, as a result of physical agitation by the penguins' feet and nest-building activities, become intimately mixed with previously deposited material and mineral matter derived from the substratum. They decompose into "guano" described by Holdgate and others (1967) as a black reducing mud, alkaline, containing high concentrations of extractable potassium, phosphorus and nitrogen.

A survey of the terrestrial Protozoa of Signy Island in 1968–69 showed that the guano of chinstrap penguin colonies was inhabited by a small group of protozoan species, three of which occurred only in penguin colonies and other areas heavily contaminated by marine birds and mammals: shag nesting clonies and elephant-seal wallows. The three species specific to such areas are:

Tetramitus rostratus Perty (Mastigophora : Polymastigida)
Philaster sp. Fabre-Domergue (Ciliata : Hymenostomatida)
Vorticella microstoma Ehrenberg (Ciliata : Peritrichida).

T. rostratus is known as a "coprozoic" or "coprophilic" species (Bunting, 1926; Brent, 1954). *V. microstoma* has been described as an excellent indicator of "polysaprobic" conditions (Kolkwitz and Marsson, 1909) and it is very common in activated sludge when the effluent is of inferior quality (Curds, 1969). *Philaster* sp. has not been identified with a described species; it has the characters of *Philaster armata* Kahl but is much smaller.

Penguin guano then is a habitat for a group of Protozoa adapted to alkaline (pH = 6·5–8·0), eutrophic mobile conditions, with low organic content (loss on ignition = 30-45 per cent), where the major habitat-determining factor is the biotic influence of penguins. It is in marked contrast to the acid (pH = 3·5–4·5), oligotrophic stable conditions of the moss peats of Signy Island, with high organic content (loss on ignition = 93–98 per cent), where the major habitat-determining influence is climate. Testacida, which are abundant in moss peats, are entirely absent from penguin guano.

Areas on Signy Island which were apparently once penguin colonies or elephant-seal wallows, but from which animals had been absent for an unknown number of years, were observed to be overgrown by *Prasiola crispa* Meneghini—a green alga described by Prescott (1954) as typical of arcto-alpine habitats and common on soil rich in nitrogen. These areas contained the three guano-specific Protozoa species described above and also some of the species usually associated with acid habitats (Smith, 1973*a*):

Phryganella acropodia (Hertwig and Lesser) Hopkinson (Rhizopoda : Testacida)
Oxtricha fallax Stein (Ciliata : Hypotrichida)
Uroleptus sp. Ehrenberg (Ciliata : Hypotrichida).

Material from *Prasiola*-covered areas had a pH *c.* 5·6 and loss on ignition was 50–65 per cent. These areas may thus be considered intermediate between alkaline guano and acid moss peat as habitats for Protozoa.

A parallel phenomenon was observed in the nematode fauna by V. W. Spaull (personal communication); *Caenorhabditis* sp. (Osche) Dougherty was with rare exceptions the only nematode observed in chinstrap penguin guano and it did not occur outside areas contaminated by animals. Areas which had become overgrown by *Prasiola* also contained *Panagrolaimus* sp. Fuchs, a species commonly occurring in animal-enriched acid habitats.

In view of their distinct habitat preference, the three guano-specific species of Protozoa were selected as useful species for intensive ecological study.

Model

In order to provide a conceptual framework in which to carry out the study, a hypothetical model of the ecological relationships of the protozoan fauna was constructed. This viewed the Protozoa and the components of their environment as a web of dependent and independent variables. The model is shown in Fig. 1; the direction of causality linking two variables is indicated by an arrow. The model did not aim to represent the total environment of the Protozoa—in particular, the abundance of bacterial food for Protozoa in the guano was not considered. It attempted to describe specifically the biotic influence of the penguins on the protozoan fauna through their effect on some measurable properties of the guano—pH, moisture (per cent dry weight) and loss on ignition (per cent dry weight). It was considered that the significant effects of penguins were the supply of nutrients through the deposition of penguin litter (excreta and moult features), which supports the guano fauna and micro-flora, and physical agitation of the guano (trampling and nest building) which prevents the growth of vegetation. It was then predicted that, if penguins cease to occupy an area where guano had accumulated, the effects of their removal would be: a drop in guano pH, overgrowth of the guano by *Prasiola crispa*, increase in organic content (higher loss on ignition) and colonization of the guano by more acid-tolerant Protozoa. There would be a lag between the attainment of suitable conditions for other Protozoa species and the colonization by them of the guano.

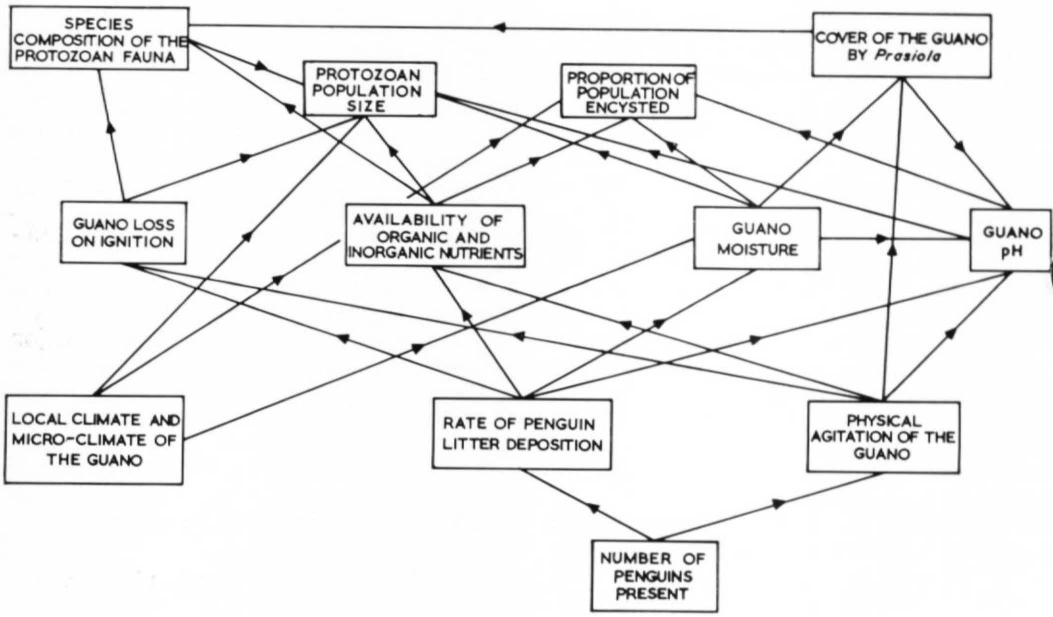

Fig. 1. Hypothetical model of the ecological relationships of Protozoa inhabiting chinstrap penguin guano.

However, since there is presumably a continual influx of wind-blown cysts of Protozoa species from other habitats in the vicinity, it was expected that, once suitable conditions for the development of these species existed in the guano, their presence would be detected the same season. It was not known at what speed these changes would occur. Studies on an Adélie penguin colony on Inexpressible Island (Campbell and Claridge, 1966) indicated that the abandonment of nesting sites by penguins resulted in chemical decomposition of the colony "soil" and leaching of carbon, nitrogen and phosphorus from the site, but they gave no indication of the time scale of these processes.

Penguin Exclosure Experiment

To test the hypothesis, a study of the guano-specific species of Protozoa (*Tetramitus rostratus*, *Philaster* sp. and *Vorticella microstoma*) was carried out between December 1969 and April 1971 at a site within the chinstrap penguin colony at North Point, Signy Island (Fig. 2). This site consisted of an experimental area, from which penguins had been artificially excluded since September 1968, and an adjacent control area. On 16 occasions at approximately monthly intervals the following measurements were made on a sample of guano, consisting of material

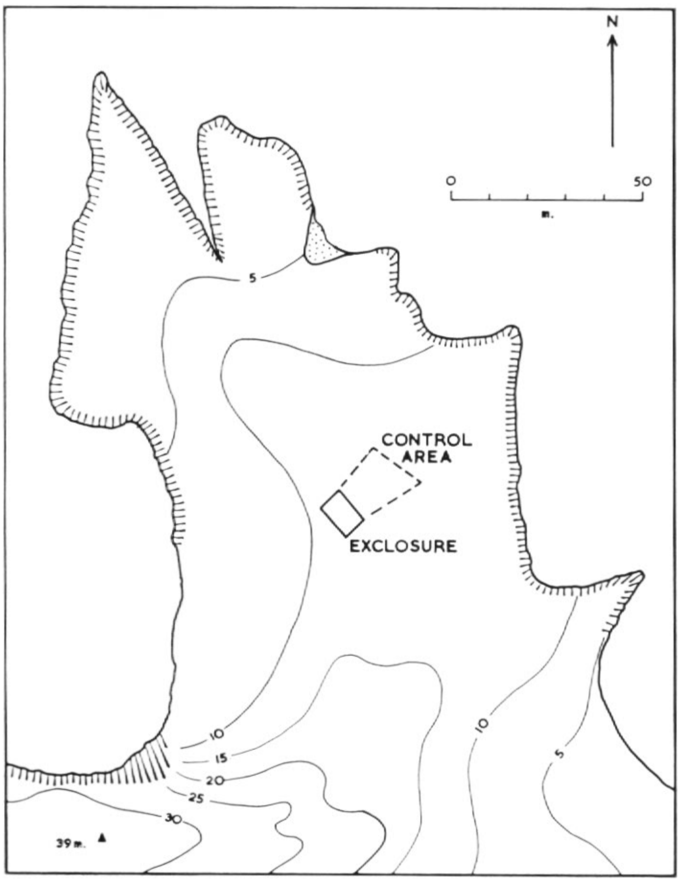

Fig. 2. Sketch map of the chinstrap penguin colony at North Point, Signy Island, showing the position of the experimental site. The contours are in metres.

from the 0–5 cm. horizon, taken from six points at random, both within the exclosure and from six points at random, both within the exclosure and from the control site:

Total numbers each of *T. rostratus*, *Philaster* sp. and *V. microstoma*.
Numbers of each of these species in an encysted state.
Guano pH.
Moisture (per cent dry weight) of guano.
Loss on ignition (per cent dry weight) of guano.

During the winter months the depth of snow and ice covering the site was measured on each sampling occasion. During the summer months the numbers of penguins, at each stage of the breeding cycle, were counted. Both adults and chicks were counted, and the adults classified as breeding (nesting, incubating or nursing), moulting or post-moulting. While the colony was snow-free in the second summer (November 1970–March 1971), the per cent cover of the guano in the exclosure by macroscopic thalli of *Prasiola* and the rate of litter production per penguin per day in the control area were measured at fortnightly intervals.

No measurements were made of the local climate of the site, nor of the micro-climate of the guano. Throughout the period of the study meteorological observations were made at the British Antarctic Survey station at Factory Cove, Signy Island, 4·3 km. away.

The Experimental Site

The penguin exclosure was erected in September 1968 by V. M. Spaull in the course of a study on the nematode fauna in penguin guano. A control site was staked out immediately adjacent to it (Fig. 2). It was erected before the arrival of the first penguins of the 1968–69 season when the colony was still under snow. No test was made at this time for homogeneity between the guano in the exclosure and that of the control area. The exclosure had an area of 68 $m.^2$ and the control an area of 198 $m.^2$; it was hoped that these areas would be large enough to include sufficient within-site variation to minimize variation between the exclosure and the control. The size of the exclosure was limited by the availability of fencing materials. The exclosure and control occupy flat ground in the middle of the chinstrap penguin colony; they have apparently the same local climate and drainage, and have snow cover of even depth in winter.

Field Methods

The penguin exclosure was a fence 80 cm. high of chicken wire supported by wooden stakes held in stone-filled oil drums, and reinforced at the corners with guy ropes. It was kept repaired as necessary. The fence was observed to have been breached by 30 moulting penguins on 25 February 1970; they were removed. Moulting penguins do not appear in great numbers until after the beginning of March (Conroy and others, in press), so the breach must have occurred only a few days before it was observed. The influence of the intruders was therefore considered to have been minimal.

Guano sampling was done with a trowel in summer and with an ice-axe in winter. Samples were transported for laboratory examination in polythene containers.

Prasiola cover was measured by the point-quadrat method (Goodall, 1952); the quadrat frame was a 1 m. length of wood bearing five 15 cm. nails at random intervals. The nail points were sharpened to 100 μm. diameter. 400 points throughout the area within the exclosure were examined by this method and scored as positive or negative for *Prasiola*.

The rates of litter deposition per penguin per day were obtained from pairs of penguins placed in a cage, lined with a polythene sheet, for 96 hr. The sheet was weighed at the beginning and end of the 96 hr. period to determine the fresh weight of litter deposited; a sample of the material was taken for dry-weight determination.

Laboratory Methods

Protozoa in the guano samples were enumerated by a modification of Singh's (1955) dilution-culture method. A suspension of fresh guano in 0·5 per cent sterile saline was used to prepare a series of doubling dilutions of guano from 1/5 to 1/80. Cultures were established by inoculating 0·10 ml. of each dilution into polypropylene rings (eight replicates at each level) on a soil-extract agar base with *Aerobacter aerogenes* (NCIB strain 418) as food organism. Cultures were kept moist with 0·5 per cent saline, incubated at 12° C for 14 days, then inspected for the development of Protozoa. Each of the three species was scored as present or absent in each ring culture. Note was also taken of any species appearing in the cultures of the guano from the exclosure which had been hitherto unobserved. A replicate enumeration procedure was performed on each sample using guano material treated with 2 per cent HCl for 12 hr. in order to kill off active cells but leave cysts viable. Numbers of Protozoa per gram of guano fresh weight were calculated from their frequency in the ring cultures by Fisher's method (Fisher and Yates, 1963):

$$Qi = \frac{\text{antilog}\left(\frac{Xi}{n}\log a - K\right)}{dv},$$

where Qi = Number of species i per gram of guano fresh weight; Xi = Frequency of species i in ring cultures irrespective of dilution level; n = Number of replicates at each level; a = Serial dilution factor; d = Dilution at highest level; v = Volume of inoculum in ml.; K is obtained from Fisher and Yates (1963, table $VIII_2$). 95 per cent confidence limits were attached to Xi/n using as the mean value of its variance log $2/n$ log a. Separate upper and lower limits for each value of Qi could then be calculated.

pH's of samples were determined electrometrically; moisture by oven drying at 100° C for 48 hr.; loss on ignition by ashing in a muffle furnace at 450–500° C for 10 hr.

The quantity of penguin litter (excreta and feathers) deposited on the control area was calculated as:

$$T = \frac{n(\mathrm{b})f(\mathrm{b}) + n(\mathrm{m})f(\mathrm{m}) + n(\mathrm{p\text{-}m})f(\mathrm{p\text{-}m}) + chf(\mathrm{ch})}{A},$$

where T = Quantity of litter deposited per m.^{2} per day; n(b) = Number of breeding adults present on the site; n(m) = Number of moulting adults present on the site; n(p-m) = Number of post-moulting adults present on the site; ch = Number of chicks present on the site; f(b) = Quantity of litter deposited per breeding adult per day; f(m) = Quantity of litter deposited per moulting adult per day; f(p-m) = Quantity of litter deposited per post-moulting adult per day; f(ch) = Quantity of litter deposited per chick per day; A = Area of control site in m.^{2}.

There is apparently no diurnal rhythm in the behaviour of breeding chinstrap penguins (Conroy and others, in press), so a count of penguins present on the control area at any time of day may be considered representative of the 24 hr.

The per cent cover of the guano in the exclosure by *Prasiola* is calculated from the point quadrat results as:

$$\frac{\text{Number of points positive for } \textit{Prasiola}}{\text{Number of points examined}} \times 100 \text{ per cent.}$$

It was estimated that, since the nails of the point quadrat were sharpened to 100 μm. diameter, the error owing to the finite size of the "point" was less than 1 per cent. The significance of increase or decrease in cover between any pair of dates on which measurements were taken could be determined from the χ^2 value calculated from a 2 × 2 contingency table.

RESULTS

The depth of snow and ice over the site and monthly air temperatures for the meteorological screen at Factory Cove are plotted in Fig. 3.

Numbers of the three Protozoa species per gram of guano fresh weight are plotted in Figs. 4, 5 and 6. Owing to the large estimation errors inherent in the dilution-culture technique, no significance can be attached to the month-to-month variation in mean numbers during the winter. Significant results are the summer peaks in the numbers of all three species, and that the total populations over-winter in an encysted state. There appear to be no consistent differences in the numbers of Protozoa between the exclosure and control area. No evidence was obtained of the guano in the exclosure becoming colonized by different species.

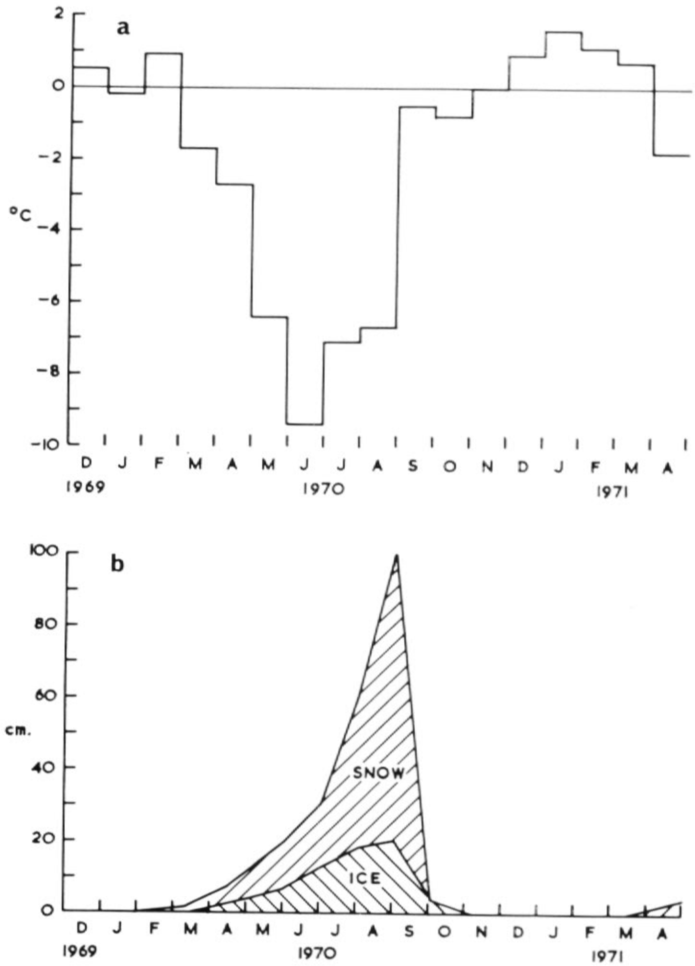

Fig. 3. a. Air temperature; monthly means at Factory Cove, Signy Island.
b. Snow and ice depth; experimental site, chinstrap penguin rookery, North Point, Signy Island.

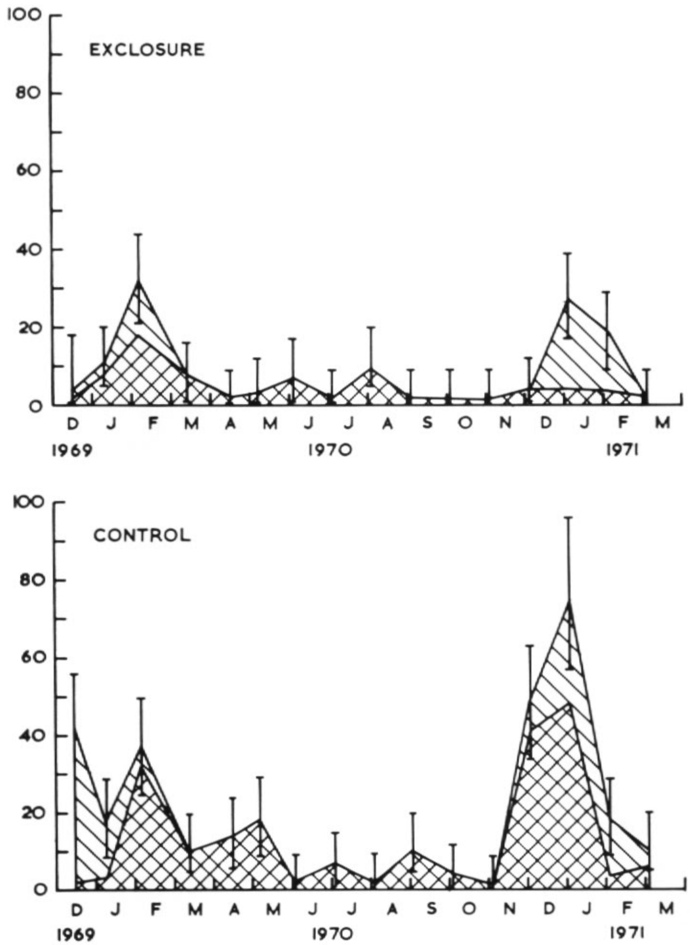

Fig. 4. Mean numbers of *Tetramitus rostratus* per gram guano fresh weight with 95 per cent confidence limits. Cross-hatched areas are the encysted fraction of the population; diagonally hatched areas are the active fraction.

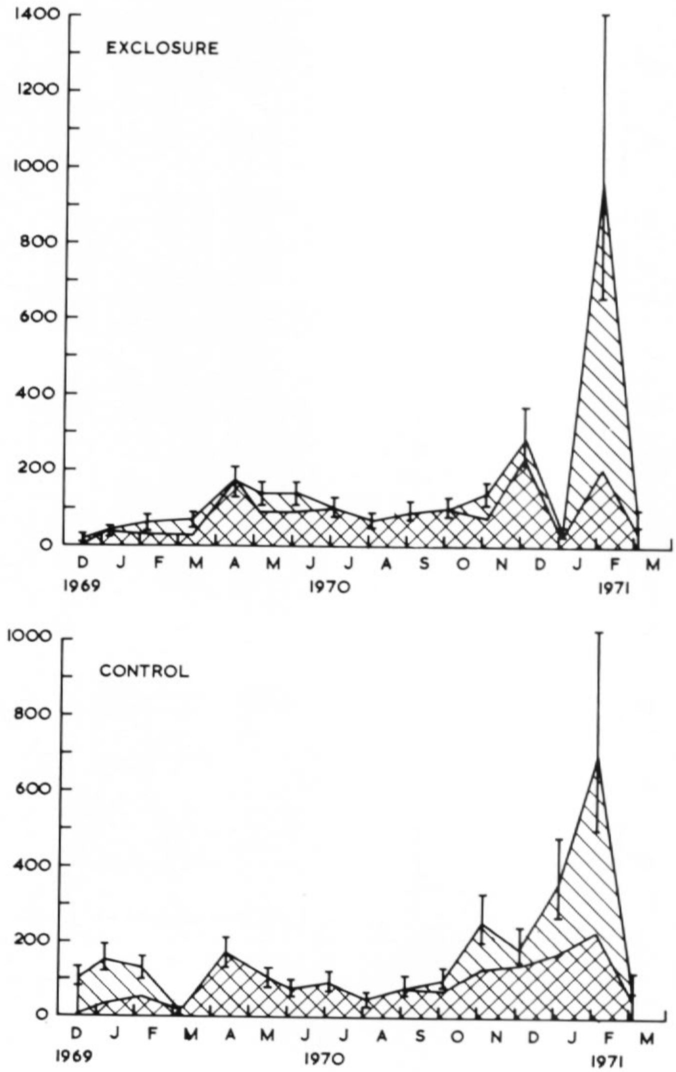

Fig. 5. Mean numbers of *Philaster* sp. per gram guano fresh weight with 95 per cent confidence limits. Cross-hatched areas are the encysted fraction of the population; diagonally hatched areas are the active fraction.

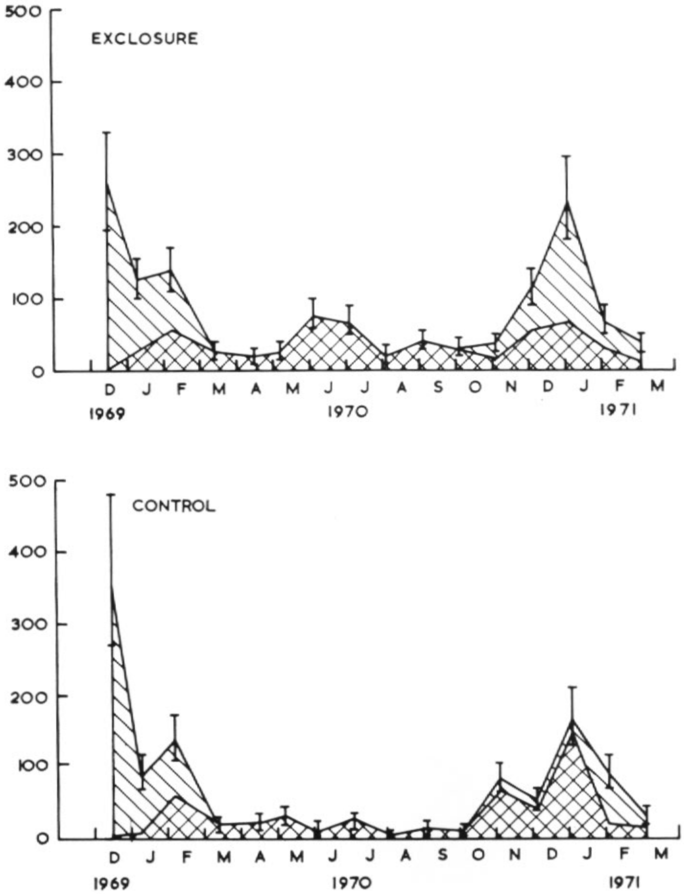

Fig. 6. Mean numbers of *Vorticella microstoma* per gram guano fresh weight with 95 per cent confidence limits. Cross-hatched areas are the encysted fraction of the population; diagonally hatched areas are the active fraction.

The pH, per cent moisture and per cent loss on ignition of the guano, together with the differences between the exclosure and control for each of these properties are plotted in Figs. 7, 8 and 9. The generalized "distance" between the exclosure and control, based on these properties, in the form of Mahalanobis' D^2 statistic, is plotted in Fig. 10. The values of D^2 for two samples was calculated using the following formula (Rao, 1952):

$$(N_c+N_e-2)W_{ij} = \sum_{k=1}^{N_c} (X_{ick}-\overline{X}_{ic})(X_{jck}-\overline{X}_{jc}) + \sum_{k=1}^{N_e} (X_{iek}-\overline{X}_{ie})(X_{jek}-\overline{X}_{je}),$$

where N_c and N_e are sample sizes from the control and exclosure, each characterized by p variates, X_{ick} and X_{iek} are values for the i^{th} character of the k^{th} replicate from the control and exclosure with means $\overline{X}_{ic}$ and $\overline{X}_{ie}$.

Then $$D^2 = \sum_{i=1}^{p} \sum_{j=1}^{p} (W^{ij})(\overline{X}_{ic}-\overline{X}_{ie})(\overline{X}_{jc}-\overline{X}_{je}),$$

where W^{ij} is the inverse of the pooled covariance matrix W_{ij}.

The significance of the distance between the control and exclosure was determined by Hotelling's T^2 test (Morrison, 1967):

$$T^2 = \frac{N_c N_e}{N_c + N_e} \cdot D^2.$$

Then the statistic $\frac{(N_c + N_e - p - 1)}{p(N_c + N_e - 2)} \cdot T^2$ was used as a variance ratio with p and $(N_c + N_e - 1 - p)$ degrees of freedom. The values of T^2 and their significance level are shown on Fig. 10.

The numbers of penguins in the control area on each day that a count was made are given in Table I on p. 47.

The rates of litter deposition per penguin per day were:

$$f(\text{b}) = 5\cdot67 \pm 0\cdot44 \text{ g.}$$
$$f(\text{m}) = 24\cdot14 \pm 0\cdot64 \text{ g.}$$
$$f(\text{p-m}) = 14\cdot79 \text{ g.}$$
$$f(\text{ch}) = 10\cdot64 \pm 2\cdot31 \text{ g.}$$

The figures are means with 95 per cent confidence limits, calculated from replicate measurements with caged penguins. There were no replicate estimates of f(p-m); since the numbers of post-moulting penguins was never more than five (Table I), any inaccuracy in the value of f(p-m) has a negligible effect.

The rate of deposition of penguin litter on the control area and the cover of guano in the exclosure by *Prasiola* are plotted in Fig. 11. The exclosed guano was observed in both summers to be extensively covered with *Prasiola*, but during the extremely dry weather in December 1970 and January 1971 there was considerable die-back; the thalli became dry and lost green pigment. They recovered rapidly with the return of moist conditions in February.

Discussion

The results suggest that variation in the quantities measured in this study can be attributed to three causes:

i. The influence of physical factors—particularly the annual cycle of ambient temperatures.
ii. The instantaneous influence of the presence or absence of penguins.
iii. The long-term influence of the presence or absence of penguins.

Clearly, seasonal climate is responsible for the summer peaks in the numbers of all three Protozoa species. It is interesting to note that there were high numbers in early January 1971 (and a large proportion of these active) when the guano was extremely dry. This contrasts with observations on the Testacida population of a moss peat on Signy Island which showed a large decline in numbers during this period (Smith, 1973*b*).

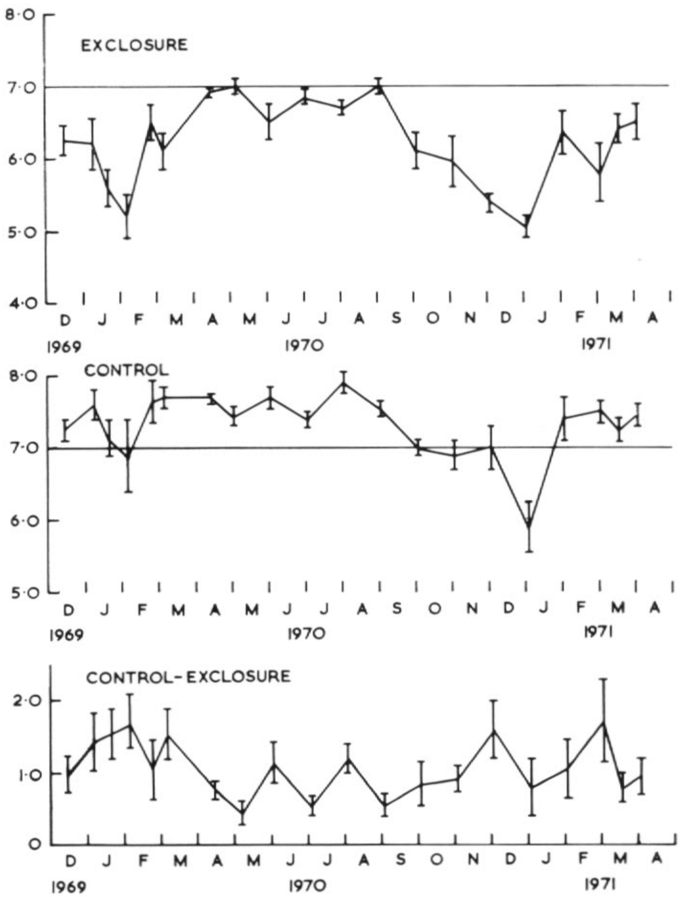

Fig. 7. Mean pH of guano with 95 per cent confidence limits.

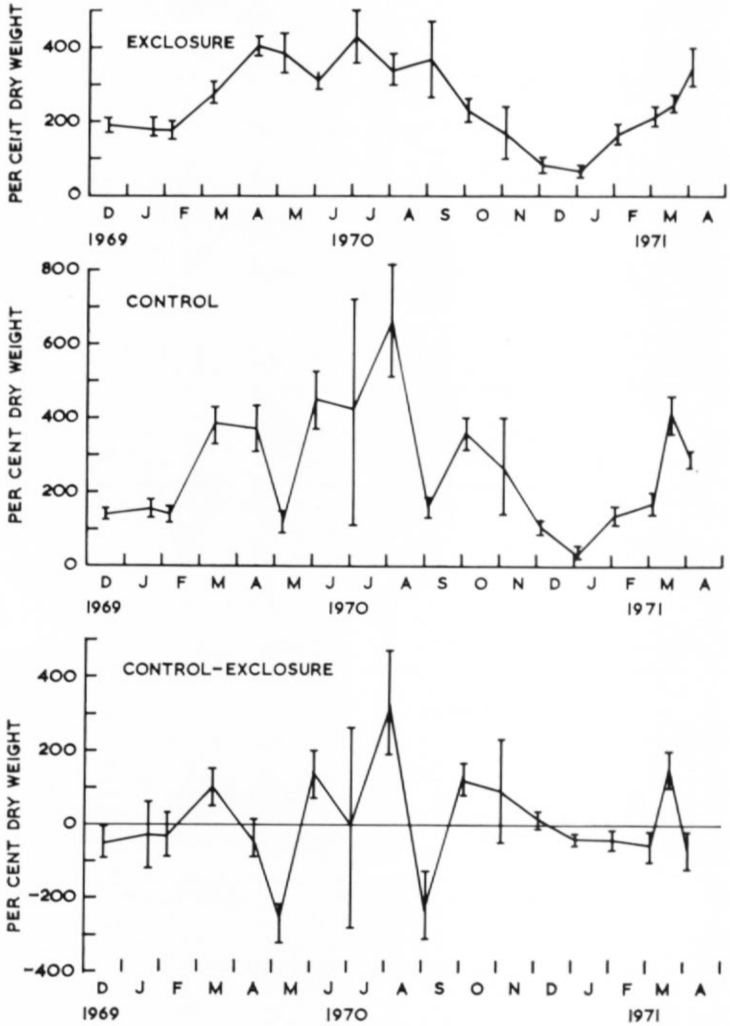

Fig. 8. Mean guano moisture per cent dry weight with 95 per cent confidence limits.

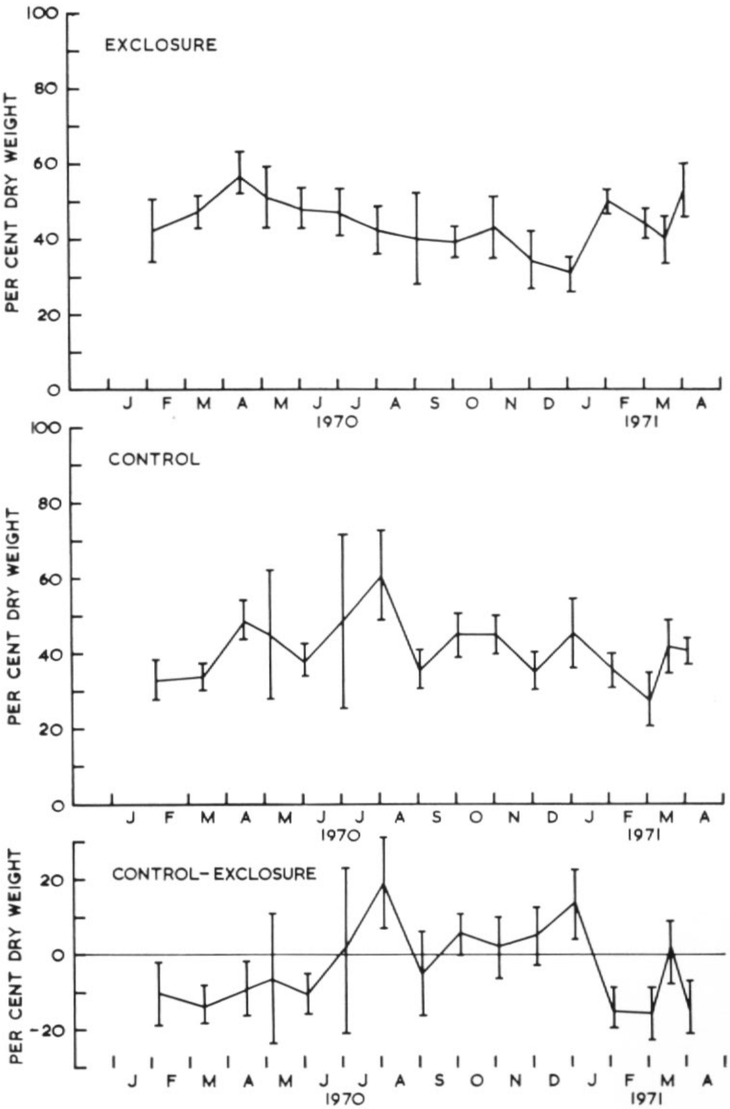

Fig. 9. Mean guano loss on ignition per cent dry weight with 95 per cent confidence limits.

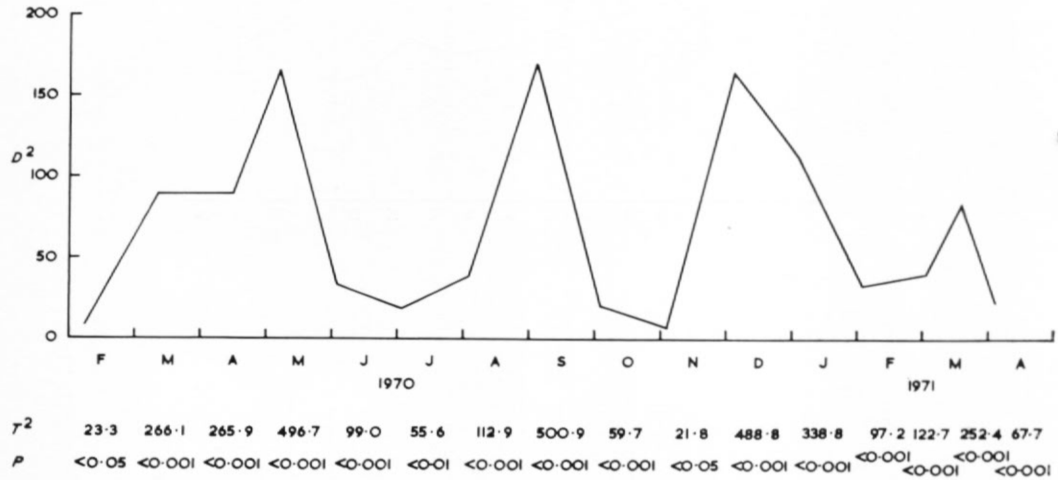

Fig. 10. Mahalanobis' D^2 statistic; the generalized distance between the exclosure and the control area is based on the pH, moisture and loss on ignition of the guano. Figures below the graph give Hotelling's T^2 statistic and significance level.

TABLE I. THE NUMBERS OF BREEDING, MOULTING AND POST-MOULTING ADULTS AND NUMBERS OF CHICKS PRESENT ON THE CONTROL AREA ON 24 OCCASIONS. THE QUANTITY *ch* REFERS TO CHICKS INDEPENDENT OF THEIR NESTS; YOUNG NESTLINGS ARE EXCLUDED

Date	*n*(b)	*n*(m)	*n*(p–m)	*ch*
1969				
16 December	45	0	0	0
1970				
22 January	35	0	0	0
7 February	30	0	0	0
25 February	0	80	0	0
28 February	0	100	0	0
8 March	0	120	0	0
15 April	0	0	3	0
6 May	0	0	0	0
3 June	0	0	0	0
6 July	0	0	0	0
3 August	0	0	0	0
2 September	0	0	0	0
2 October	0	0	0	0
3 November	35	0	0	0
15 November	48	0	0	0
29 November	81	0	0	0
2 December	73	0	0	0
27 December	85	0	0	0
1971				
3 January	72	0	0	0
19 January	70	0	0	0
3 February	36	0	0	44
2 March	0	66	0	41
19 March	0	113	5	0
4 April	0	20	4	0

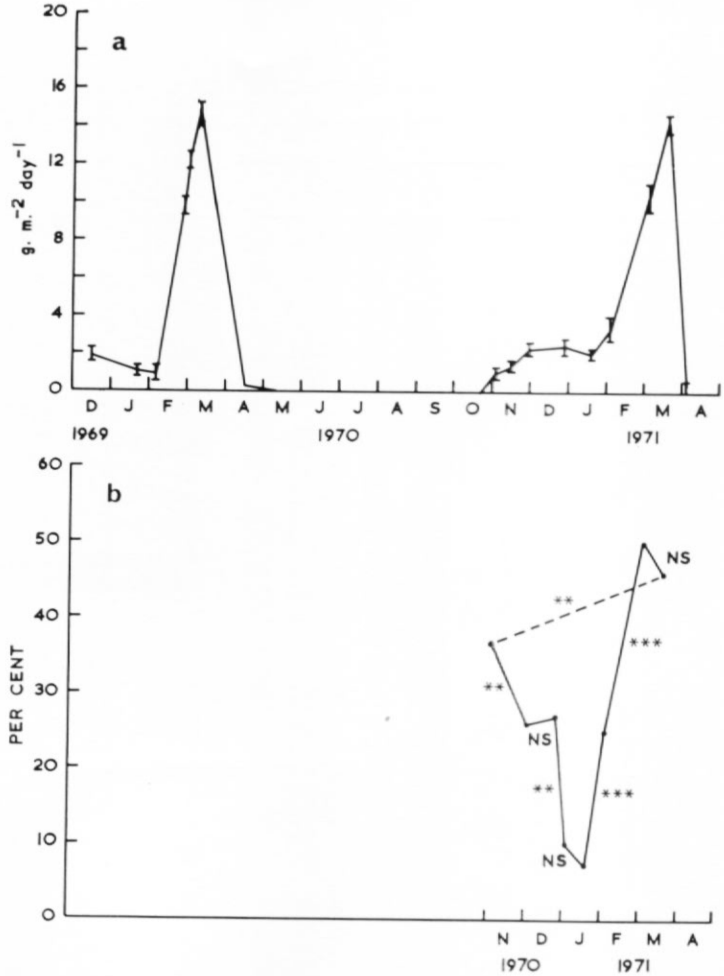

Fig. 11. a. Deposition of penguin litter on the control area, g./m.^{2} per day with 95 per cent confidence limits.
b. Cover of guano in the exclosure by *Prasiola crispa*.
** Significant change in cover ($P < 0 \cdot 01$).
*** Significant change in cover ($P < 0 \cdot 001$).
NS Change in cover not significant.

The influence of penguins is indicated by the differences between the exclosure and the control area. Variables which show a consistent difference are *Prasiola* cover and pH. Within season, variation in *Prasiola* cover of the guano in the exclosure is positively correlated with guano moisture (rank correlation $e = 0 \cdot 84$, $P < 0 \cdot 01$). This suggests that it is necessary for the physical agitation of the guano to be stopped for macroscopic thalli of *Prasiola* to grow, but that the extent to which it can maintain cover depends on an adequate supply of moisture. However, during January 1971 the guano in the exclosure, though very dry compared with its state during the rest of the year, was significantly more moist than the guano of the control (Fig. 8). It is possible that this is a second-order feed-back effect of the *Prasiola* cover on the guano, the cover conserving moisture in the guano by reducing evaporation from the surface.

Seasonal variations in guano pH both in the exclosure and in the control area are also positively correlated with moisture:

Rank correlations—exclosure $e = 0 \cdot 89$, $P < 0 \cdot 001$
—control $e = 0 \cdot 46$, $P < 0 \cdot 05$.

However, the differences in pH between the control and exclosure are not correlated with differences in moisture (rank correlation $e = 0 \cdot 19$, $P < 0 \cdot 5$). So the consistently lower pH in the exclosure guano indicates a long-term influence of the absence of penguins from the exclosure.

Other guano properties show no consistent difference between the exclosure and the control, but per cent loss on ignition does show a seasonal difference (Fig. 9), which suggests that the penguins have an instantaneous influence. Whilst litter is being deposited it has the effect of maintaining a higher concentration of mineral matter in the guano so that the control area has a lower per cent loss on ignition than that in the exclosure during the summer. It is also possible that the photosynthetic production by *Prasiola* is contributing organic matter to the guano in the exclosure. These influences do not operate in winter.

The graph of Mahalanobis' D^2 statistic (Fig. 10) indicates considerable month-to-month variation in the magnitude of the generalized "distance" between the exclosure and control area, based on the guano properties pH, moisture and loss on ignition, but there does not appear to be any regular seasonal variation or any trend towards increasing "distance" during the period of the study. However, the T^2 tests indicate that there was a significant difference between the exclosure and control throughout the study. It is therefore possible that a permanent divergence between the exclosure and control, in these guano properties, has arisen during the 3 years since the exclosure was erected.

The results do not provide any evidence that the protozoan fauna of the guano in the exclosure have responded to changed conditions. No different species were observed and the numbers of the guano-specific species were rarely significantly different from those in the control.

A study of the nematode fauna of the guano in the exclosure and control area from 1968 to 1970 gave similar results (personal communication from V. W. Spaull). Apart from a few isolated specimens of *Panagrolaimus*, *Caenorhabditis* was the only nematode observed in the guano over the period of 2 years.

Conclusion

The predictions made by the original hypothesis regarding a decrease in guano pH and overgrowth by *Prasiola* in the absence of penguins have been fulfilled. It appears, however, that changes in the physical and chemical properties of the guano must proceed further before changes are observed in the protozoan fauna of the guano.

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