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Ecological Studies of Symbiosis in *Convoluta roscoffensis*

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ABSTRACT: The size of the *Convoluta roscoffensis* population on a beach in Herm, Channel Islands is subject to wide seasonal variation, being very low in early summer and high in autumn and winter. Of the ecological factors measured, only light – expressed as photosynthetically active radiation – showed a correlation with population size. Low worm numbers, and also low numbers of algae and chlorophyll *a* content per worm, were associated with high light intensities, and *vice versa*. Worm spacing within colonies, however, remained relatively constant, with a mean value of 9×10^5 worms m^{-2}. Estimates of photosynthetic cover (chlorophyll biomass) of 320 mg chlorophyll *a* m^{-2}, and annual primary productivity for 1977, of 872.9 g carbon fixed m^{-2} of colony, approach estimates for coral reefs; they were remarkably high for the potentially disturbed environment of the intertidal sandy beach.

INTRODUCTION

The intertidal flatworm *Convoluta roscoffensis* Graff is symbiotic with the unicellular green alga *Platymonas convolutae* Parke et Manton. Adult worms do not ingest food, and this algal-animal association can grow completely autotrophically in the light in seawater containing nitrate and phosphate (Holligan and Gooday, 1975). Each worm is 2 to 4 mm long and contains 2 to 7×10^4 endosymbiotic algal cells (Doonan, 1979). Colonies of *C. roscoffensis* are found on sandy beaches, mainly in north-west France and the Channel Islands (Keeble, 1910). When the tide goes out, the worms emerge from beneath the sand to lie in rivulets of beach run-off water; exposure to sunlight allows their algae to photosynthesise.

Most of our knowledge of *Convoluta roscoffensis* comes from laboratory observations concerned with cytological and biochemical aspects of symbiosis; published field studies (Gamble and Keeble, 1903; Keeble and Gamble, 1907; Guérin, 1960; Fraenkel, 1961) have been largely descriptive. Here we present the results of a quantitative study of *C. roscoffensis* in its natural habitat over an extended period of time. The study was made in order to assess the relative productivity and dynamics of this symbiotic system: what is the importance of the symbiosis to the ecology of the partners involved, and how does the resulting productivity compare with that of non-symbiotic systems? Summaries of some of our results have been presented by Gooday and Doonan (1980).

MATERIALS AND METHODS

We have studied Shell Beach, Herm, Channel Islands with its large, conspicuous population of *Convoluta roscoffensis*. The beach was uniform and sandy, making core-sampling easier than on a pebble beach. Within the study site, an area of 20 m by 20 m was chosen in which seasonal changes in the *C. roscoffensis* population could be measured. The site was visited 6 times, from September 1976 to May 1978. The beach profile was measured according to the method of Emery (1961), and an analysis of sand particle size was carried out in the 20 m × 20 m grid. To determine levels of dissolved nutrients in the beach run-off water, samples from above and below colonies of *C. roscoffensis* were collected and immediately vacuum filtered through a Millipore Sterifil System, using Millipore filters (pore size 0.45 μm). Samples intended for nitrate, total nitrogen and total phosphorus analysis were poured into polythene bottles and frozen immediately in dry ice. Samples intended for inorganic phosphate analysis were poured into glass bottles containing a few ml of chloroform, and kept cool in subdued light. All samples were analysed within 1 wk of collection, at the Plymouth Laboratory of the Marine Biological

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Association of the U.K. Salinity was measured by the method of Harvey (1955); pH was recorded *in situ* using a portable pH meter.

Inorganic phosphate content was determined as described in Strickland and Parsons (1972); total soluble phosphorus, by the same method on samples irradiated overnight with ultraviolet light (Hanovia Lamp, UVS 500) according to Armstrong and Tibbits (1968). Levels of nitrate were measured by the method of Wood et al. (1967); total nitrogen, by the same method on u.v. irradiated samples.

Instantaneous measurements of incident light in the field were made with a Weston Euro-Master exposure meter calibrated against a Lambda LI-190SR Quantum Sensor (T & J. Crump, Wickford, U.K.). Between visits to Herm incident light was computed from data collected at the meteorological station at Jersey Airport, and converted (multiplication by 0.5) to PAR (photosynthetically active radiation). By using values of mean daily PAR and daily lengths of tidal exposure for 1977 (Doonan, 1979) it was possible to calculate the portion of total PAR available to *Convoluta roscoffensis* when exposed by the tide.

To estimate their primary productivity, the amount of carbon fixed by the photosynthetic activity of the symbiotic algae inside *Convoluta roscoffensis* was measured, at the Plymouth Laboratory, by adapting the ^{14}C method (Strickland and Parsons, 1972), i.e. by treating the worms as 'units of phytoplankton'. Measurements were made within 5 d of collection. During this time the worms were maintained in ambient conditions, with frequent changes of seawater, and no alterations in algae or animals were observed. The method, described in detail by Doonan (1979), involved incubating groups of 20 worms (3 to 4 mm in length) in Pyrex vials of 32.5 ml of seawater or Herm beach run-off water containing about 5 µCi of $NaH^{14}CO_3$ (Amersham) at ambient temperature. The vials were exposed to a range of light intensities (dark, 10%, 30%, 50%, 75% and 100% transmission; achieved by means of filters) for 3 to 4 h, during which time incident light was measured with a Lambda LI-192S Quantum Sensor attached to a chart recorder. Radioactivity of the samples was measured by liquid scintillation counting. Primary productivity was calculated as µg carbon fixed $worm^{-1}$ and also as mg carbon fixed (mg chlorophyll $a)^{-1} h^{-1}$ at light saturation, i.e. assimilation number, which required extraction of chlorophyll *a* from the algae inside *C. roscoffensis*. Groups of 10 worms were placed in 1 ml 90% acetone; extinctions of the extracts at 630 nm, 645 nm and 665 nm were measured, and chlorophyll contents calculated (Strickland and Parsons, 1972).

We determined population density both as number of worms per area of beach and as number per area of worm colony. The former method involved random sampling from the 20 m by 20 m grid; the latter, sampling from colonies which were then photographed so that their area could be calculated. In both cases, 5 cm cores of sand and worms were taken with a Perspex corer (internal diameter 2.5 cm). Sampling of the grid involved dividing it into 100 numbered 2 m squares, and sampling each of these at coordinates (measured in cm) chosen from random number tables. Methods of separation and counting of worms are described by Doonan (1979).

Estimations of the number of algae inside *Convoluta roscoffensis* were made, and the values used in context both of seasonal changes in algal numbers and of photosynthetic rate in relation to body length and algal numbers. *Platymonas convolutae* were released from their hosts for counting as follows: 25 worms of similar length were placed into a 3 ml Teflon homogeniser and washed several times with filtered seawater. All the seawater was pipetted off and 0.75 ml of filtered seawater added. The worms were gently homogenised by hand to give a uniformly green suspension (10 to 15 s). Counts of algae were made on a haemocytometer. This technique was the most reproducible of several tried (e.g. digestion with trypsin, bubbling with CO_2), giving the most uniform disruption of the worms with the least disruption of the released algae. The reliability of the method can be gauged by the standard deviations in Table 3.

RESULTS

In the study area, *Convoluta roscoffensis* occurred about midway between mean high water and mean low water of neap tide, at approximately the point at which the angle of the beach changed (Fig. 1). About 10 m up the beach from this point, the beach run-off water emerged. The sand was chiefly weathered granite and shell fragments. It was coarse on the landward edge of the sampling grid with about 90% of particles between 1 and 3 mm in diameter, and fine at the seaward edge where about 60% of particles were less than 0.3 mm in diameter. The temperature of the beach run-off water (and the corresponding sea and air temperatures) showed a two-fold seasonal variation, but solar energy, expressed as mean daily PAR, exhibited a larger fluctuation (Table 1). It was calculated that in 1977 the total PAR on the study area was 7,821 E m^{-2}, and that *C. roscoffensis* was exposed to 4,883 E m^{-2} of this total.

The beach run-off water was relatively rich in nitrogen and phosphorus (Table 1). The nitrogen was chiefly as nitrate, accounting for 54% of the total nitrogen in comparison to 40%, 3% and 3% for

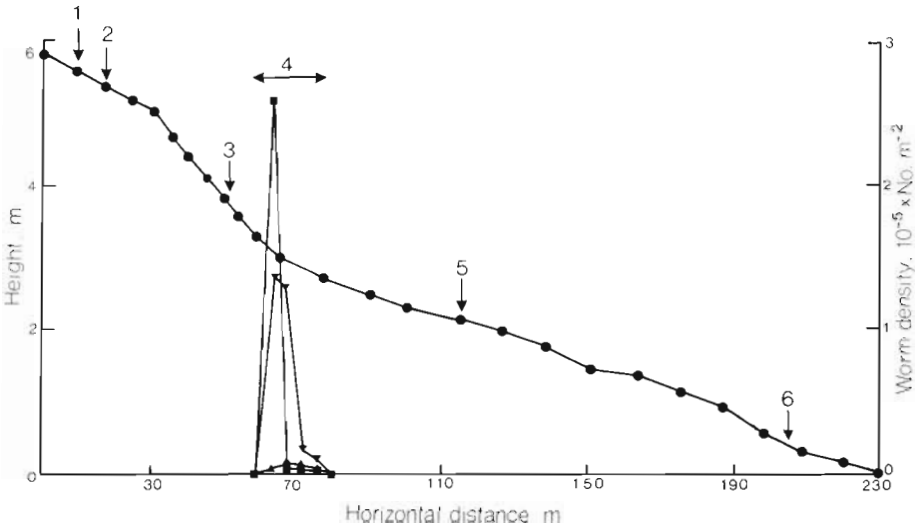

Fig. 1. Profile of beach study area and site and population densities of *Convoluta roscoffensis*. Circles: height; squares: worm densities on beach, September 1977; ▼ March 1978; ▲ May 1978. 1 Mean high water spring; 2 mean high water neap; 3 site of run-off emergence; 4 site of sampling grid; 5 mean low water neap; 6 mean low water spring

organic nitrogen, ammonia, and urea respectively (average values for 5 samples at each of the collections). The phosphorus was chiefly present as inorganic phosphate, accounting for 90% of the total phosphorus. Nitrate revealed the most distinct seasonal variation; it was higher in summer. In contrast to Holligan and Gooday (1975), these studies showed no systematic differences in nutrient levels in water samples collected above and below colonies during single periods of tidal exposure. Salinity and pH values of these samples, averaging at 34.8‰S and 7.5 respectively, were close to those of seawater, indicating that this run-off water was seawater from the previous high tide.

The population size of *Convoluta roscoffensis* in the study grid showed a wide seasonal variation, being very low in early summer and high in autumn and winter (Table 1, Fig. 1). This variation was reflected in the size of colonies rather than the density of animals within an individual colony (i.e. 'worm-spacing') which remained relatively constant (Table 1) with a mean value of $9.0 \pm 1.6 \times 10^5$ worms m^{-2} (a total of 65 estimations over the period of study). Biomass, expressed as g chlorophyll *a* m^{-2} of colony, however, did show a pattern of fluctuation similar to that of population size with low summer values (Table 1). Since worm density varied little, this fluctuation was the result of changes in chlorophyll *a* content per worm; more detailed measurements indicated that both algal numbers per worm, and chlorophyll *a* content per alga were higher in autumn and winter than in summer (Gooday and Doonan, 1980).

Photosynthetic carbon fixation rates were measured for worms 3 to 4 mm long collected at different times; they varied little in relation to the amount of light energy received. For example, at 1.0 E m^{-2}, fixation ranged between 1.1 and 1.7 µg C $worm^{-1}$, over a temperature range of 10° to 22° (Table 2).

In June 1977, measurements made at light of up to 20 E m^{-2} revealed above about 10 E m^{-2} a rapid decrease in carbon fixation (i.e. photoinhibition occurred) giving, for example, a value of 0.34 ± 0.12 µg C fixed $worm^{-1}$ at 19 E m^{-2} (19°).

In most experiments, light saturation occurred

Table 1. *Convoluta roscoffensis*. Seasonal variation in enviromental factors and the population on Herm, Channel Islands

Factor	Date					
	1976	1977			1978	
	Sep	Feb	May	Sep	Mar	May
Mean run-off temp. (°C)	16.3±1.4	9.3±1.0	16.5±3.0	17.0±1.7	9.2±2.2	13.2±1.8
Mean daily PAR (E m^{-2})	18.0	11.0	54.0	14.0	18.0	42.0
Total nitrogen in run-off water (µg atoms N l^{-1})	12.0±2.6	16.0±1.6	8.6±0.2	14.3±1.8	34.8±8.1	14.6±5.0
Total phosphorus in run-off water (µg atoms P l^{-1})	0.8±0.1	0.4±0	0.5±0.1	0.8±0.1	0.8±0.2	0.9±0.1
Density: 10^{-3} × No. worms m^{-2} on beach	N.D.	182.8	4.6	256.1	121.1	1.7
10^{-5} × No. worms m^{-2} of colony	11.1±2.6	8.5±2.0	N.D.	10.8±1.8	8.5±2.5	7.5±1.9
Biomass in colony (g chlorophyll *a* m^{-2})	0.25±0.05	0.25±0.06	N.D.	0.32±0.05	0.17±0.05	0.09±0.02

Values, except for PAR and worm density, quoted with standard deviations (n = 5)

Table 2. *Convoluta roscoffensis*. Primary productivity of worms with body length of 3 to 4 mm

	1976	1977			1978	
	22 Sep	8 Feb	8 Jun	5 Oct	9 Mar	1 Jun
µg C fixed worm^{-1} at 1 E m^{-2}	1.1	1.1	1.2	1.4	1.5	1.2
Max. photosynthetic rate (µg C worm^{-1} h^{-1})	0.66	0.45	0.28	0.33	0.49	0.40
Assimilation No. (mg C [mg chl. *a*]$^{-1}$h^{-1})	2.9	N.D.	2.8	1.1	2.5	N.D.
Temperature (°C)	22	11	18	14	13	18

Table 3. *Convoluta roscoffensis*. Photosynthetic rate in relation to body length and algal numbers

Date	Body length (mm)	10^{-3} × mean no. algae worm^{-1} (n = 15)	Ratio algal no. in 3–4 mm / algal no. in 2–3 mm	Ratio C fixation in 3–4 mm / C fixation in 2–3 mm
05. 10. 77	3–4	69.9 ± 5.4	1.37	1.54
	2–3	51.0 ± 9.2		
10. 03. 78	3–4	44.3 ± 7.5	1.43	1.25
	2–3	30.9 ± 3.1		

between 1 and 2 E m^{-2}; the mean maximum photosynthetic rate ranged from 0.21 to 0.96 µg C worm^{-1} h^{-1}, giving assimilation numbers from 1.1 to 3.0 mg C (mg chlorophyll *a*)$^{-1}$ h^{-1} (Table 2). Fixation of carbon in the dark was very low, so that at saturating light levels fixation in the light ranged from 73 to 518 times that in the dark.

Worms with body length of 3 to 4 mm contained about 1.4 times as many algae as worms 2 to 3 mm long, and fixed a correspondingly greater amount of carbon over the same period of time (Table 3).

An estimate of annual primary production of *Convoluta roscoffensis* for 1977 was made using values of daily carbon fixation per worm and worm density in the field at different seasons. The value for carbon fixed was 872.9 g m^{-2} of colony.

DISCUSSION

Light was the only environmental factor that correlated with seasonal fluctuations in the *Convoluta roscoffensis* population, i.e. numbers of worms, numbers of algae and, less marked, chlorophyll *a* content per worm. Low worm numbers, low algal numbers and low chlorophyll levels were associated with high light intensities. Light (PAR) and worm density on the beach were negatively correlated with a coefficient of 0.90. The chlorophyll content of many algae is inversely proportional to the amount of light received during growth (Meeks, 1974), and the symbiotic algae *Chlorella* sp. inside *Hydra viridis* have been observed to bleach under high light intensity (Pardy, 1976). Pardy suggests that decreases in algal chlorophyll levels may be the result of both a cell-regulated process and a process of photo-destruction.

Three aspects in the behaviour of *Convoluta roscoffensis* likely to affect light utilization for photosynthesis are (1) rapid secretion of mucus, which Fraenkel (1961) suggests might allow the worms to float above the sand and so be exposed to light reflected from the sand; (2) burrowing back down into the sand after a few hours in bright sunshine (perhaps to avoid photoinhibition?); and (3) continual motion, which must affect shading each other. An estimate, made using photographs, of the area of an adult worm as viewed from above was 0.50 ± 0.06 mm^2 (14 measurements). This gives mean values of % cover of worms on the sand in colonies of 34 to 56 (using values in Table 1). Thus, although there is sufficient space for all worms to be fully exposed to the light, they move ceaselessly, and hence shade each other to some extent.

The photosynthetic cover (chlorophyll biomass) of colonies of *Convoluta roscoffensis*, of up to 320 mg chlorophyll *a* m^{-2}, is similar to that of coral reefs, or of epipsammic foraminiferans with algal symbionts, and of an intertidal epilithic community of algae (reviewed by Raven, 1981). The highest theoretical value for free-living phytoplankton in the euphotic zone is 200–300 mg chlorophyll *a* m^{-2} (Talling et al., 1973), while Anderson (1967) suggests that the maximum useable chlorophyll cover in natural communities is 600 mg m^{-2}. Raven (1981) concludes that photosynthetic cover is usually higher in less disturbed environments. The intertidal zone can be a very disturbed environment, but for *C. roscoffensis*, motility overcomes this by re-establishing the temporary stability of the colony between each tide.

Comparison of the value of annual primary production of *Convoluta roscoffensis* of close to 1 kg C m^{-2} with other photosynthetic systems shows that it is higher than values for phytoplankton, approaching values for coral reefs (Ryther, 1969; Taylor, 1973; Gooday and Doonan, 1980; Muscatine, 1981). This is a remarkable achievement for organisms living in such a potentially disturbed environment as is an intertidal sandy beach. Assimilation numbers of 1.1–3.0 mg C (mg chlorophyll *a*)$^{-1}$ h^{-1} for *C. roscoffensis* are similar to values found for free-living plankton and for algae on a sandy beach (reviewed by Raven et al., 1979; Gooday and Doonan, 1980). The annual primary production of the symbiotic algae inside *C. roscoffensis* is higher than values for free-living phytoplankton (Gooday and Doonan, 1980; reviewed by Muscatine, 1981).

These high levels of photosynthetic activity in *Convoluta roscoffensis* may be attributed to: (1) its gregarious habit and ability to re-form colonies after disruption by the tide; (2) the dense packing of algal symbionts inside the worm (estimated density 10^8 algae cells ml^{-1} of host tissue, assuming values of 4×10^4 algae in a worm volume of 0.4 µl); and (3) the intimate cellular interrelationships which allow algae to be maintained within the host in peak photosynthetic condition, while exporting a large proportion (37–58%; Smith, 1978) of their photosynthate to the animal tissue.

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