Reports and Letters

Quantitative Underwater Study of Benthic Communities Inhabiting Kelp Beds off California

By use of a Cousteau-Gagnan Aqualung and a heavy-duty diving suit, the sea bottom off La Jolla, Calif., was explored during the spring of 1955 in an attempt to assess the standing crop of the benthic animals and plants that live in association with the giant kelp, *Macrocystis pyrifera*. This method of investigation insures free movement under water and enables one to make more extensive observations of the submerged communities than one can by dredging or by diving with a helmet (*1*).

The visibility at the bottom is largely limited by the depth, the density of overlying kelp, the turbulence, and the amount of suspended matter in the water. Thus, on clear days at a depth of 20 m, the visibility ranges between 1 and 10 m.

Cross sections of the sublittoral region, which extends more than 400 m from the low-tide level to the outer edge of the kelp beds, were surveyed. The organisms dwelling on the bottom were analyzed quantitatively by the quadrant method. The quadrants used enclosed an area of ¼ m²; for the larger kelp as well as for animals dispersed over wider areas, the number of organisms was counted along lines of 2- or 10-m length in different directions.

The results of this survey reveal a zonal distribution of the chief submerged plants and animals, which seem to be controlled chiefly by light and temperature.

Thus, on the exposed rocks below mean low-water spring tide, a community dominated by the sea grass *Phyllospadix scouleri* flourishes best under surf conditions and extends down to a depth of about 7 m, covering 80 to 100 percent of the rock surface. Epiphytes on *Phyllospadix* consist largely of *Porphyra naiadum* var. *australis, Melobesia mediocris,* and *Ectocarpus granulosus*; *Callithammion californicum* and *Ceramium pacificum* occur as secondary epiphytes. This community is poor in animals, and—apart from *Membranipora, Caprella,* hydroids, and a few small snails—the gross weight obtained in the quadrants is owing mainly to the sea grass and amounts to an average value of 3634 g/m².

Table 1. Standing crop (average fresh weight) of the coralline community at different depths below mean sea level.

Depth (m)	Plants (g/m²)	Animals (g/m²)	Total wt. (g/m²)
1.5	4667	125	4792
7	2490	329	2819
15	1972	392	2364
22	606	377.2	983.2

A *Pterygophora-Eisenia* community flourishes where the sea grass ceases to grow, at first as widely scattered individuals intermingled with *Egregia laevigiata, Cystoseira osmundacea, Codium fragile, Dictyota binghami* and *Dictyopteris zonarioides*; it reaches a climax in deeper water between 10 and 20 m, where the two main Laminariales, *Pterygophora* and *Eisenia* form, together with *Laminaria farlowii,* a "forest" under the large *Macrocystis* plants.

Macrocystis pyrifera forms extensive beds ranging in width between 200 and 500 m in a depth of water from 7 to 25 m. Individuals become more crowded in the middle of the beds, with holdfasts 1 to 2 m apart. At the outer edge, *Macrocystis* becomes replaced by scattered individuals of the elk kelp, *Pelagophycus porra,* in a depth of water of 20 to 25 or 30 m.

Where *Macrocystis* grows on outcropping rocks or on boulders, it meets with competition from sea urchins, which clear whole areas of kelp; this is demonstrated by the patchy appearance of the beds at the surface. Survival of kelp plants depends largely on the enormous production of juvenils, which grow on a variety of substrata, including other algae and sea grass.

The standing crop of the *Macrocystis* plants in these beds is estimated to be between 25 and 40 tons/acre, and average annual yield is estimated to be about 4 to 6 tons/acre (fresh wt.). This was judged by observations at the bottom and on the surface, as well as by studies of aerial photographs, by weighing representative samples, and by laboratory experiments on the growth rate of kelp. Under favorable conditions, the latter ranges between 3 and 5 cm per day.

The lowermost stratum of vegetation is occupied by a coralline community that forms an extensive cover under the larger kelps. *Corallina chilensis, C. gracilis, Bossea orbigniana, B. gardneri, Lithothrix aspergillus,* and *Lithothamnion* spp. are the chief calcified algae inhabiting this substratum. The quantity and quality of organisms inhabiting this community were found to vary with depth as follows: (i) the percentage of cover by the dominant alga decreases with depth, hence the production per unit area decreases; (ii) *Bossea* preponderates over the *Corallina* beyond a depth of 15 m. These modifications are accompanied by modification in the fauna that live in association with the corallines. For example, the sea fans, red and pink abalones, the sea star *Henricia leviuscula,* together with the holothurian *Stichopus parvimensis* and the acorn barnacle *Balanus tintinnabulum,* become noticeable at 20 to 25 m; some of these appear for the first time at such depths. Table 1 gives a comparison of production of the coralline community at different depths.

It has also been found that, while the total weight of plants decreases with depth, that of the animals tends to increase. On the basis of the foregoing survey, an average figure for the standing crop of the organisms that inhabit the rocky bottom of the kelp beds, including *Macrocystis* itself, would approach 9.4 kg/m² of sea bottom, or approximately 38 tons/acre. These figures do not include the pelagic forms.

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Notes

1. Contribution from the Scripps Institution of Oceanography, new series, No. 824.

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Metabolite of Phenobarbital in Human Urine

A metabolic product of phenobarbital was recently isolated from the urine of a dog that had received daily doses of the drug for three weeks. It was identified as the *p*-hydroxy derivative and was subsequently synthesized by Butler (*1*). We have found this metabolite in the urine of two human beings who died after barbiturate ingestion. It was possible to determine the concentrations of the free and conjugated forms.

Ultraviolet spectrophotometry was used for initial quantitation (*2*); this was followed by paper chromatography for the identification of the original drug and for

its separation from the metabolite (*3*).

In case No. 1, death was attributed to acute barbiturate intoxication and positional asphyxia. A half-filled bottle of phenobarbital tablets was found near the body.

The entire sample of urine (390 ml) was buffered at *pH* 7 and exhaustively extracted with ether. The ether extracts were combined, washed with 0.2*N* HCl, dried with powdered Na_2SO_4, and shaken with 0.05*N* NaOH until all the barbiturate was extracted. The ultraviolet absorbencies were recorded at *p*H 9.5 and *p*H 2. The barbiturate was then reextracted with fresh ether from acid solution, the volume was reduced, and the ether solution was applied to 6 sheets of paper for chromatography (*3*).

For a more controlled preliminary study, two 2-ml samples of the original urine were extracted in parallel by the afore-mentioned procedure. In order to locate and study the zones after chromatography, we used the following procedure: initial scanning of one strip by densitometry (*4*) *pinpointed the* R_f values; the strip was eluted section by section with 0.05*N* NaOH, and the ultraviolet absorption spectra were recorded to determine which of the substances present possessed spectra characteristic of the 5,5-disubstituted barbiturates. At the same time, quantitation was accomplished. The other strip was split vertically in two, and the silver acetate and potassium permanganate tests were applied (*3*).

Two barbiturates were found. One was located at R_f 0.50 and was presumably phenobarbital. It gave a positive result with the silver acetate reagent and a negative result with potassium permanganate. The other barbiturate was located at R_f 0.29 and gave positive results with both reagents. The results obtained when known and comparable amounts of both phenobarbital and *p*-hydroxyphenobarbital (*5*) were carried through the same procedure coincided in every respect.

The phenobarbital and its metabolite were removed from the 6 sheets of paper. Each substance was recrystallized from hot water. Mixed melting-point determinations with the known pure compounds proved the presence of phenobarbital and *p*-hydroxyphenobarbital in the urine.

The concentration of the metabolite was calculated from the absorptivity of the *p*-hydroxyphenobarbital. The concentration of phenobarbital was 5.8 mg/100 ml of urine. The concentration of *p*-hydroxyphenobarbital was 11 mg/100 ml.

The 390 ml of urine that had been extracted was then hydrolyzed acording to Butler's method (*1*); after hydrolysis, the procedure was continued as outlined here.

The concentration of *p*-hydroxyphenobarbital was found to be 9.2 mg/100 ml of hydrolyzed urine. The compound was recrystallized and mixed melting-point determinations confirmed the identification.

A barbiturate concentration of 7.2 mg/100 ml of blood was found by ultraviolet spectrophotometry (*2*). The results of a paper chromatographic study as outlined for urine showed that the only barbiturate detectable in extracts from 25 ml of blood was phenobarbital.

A 30-g sample of homogenized liver was analyzed in the same manner as blood. A barbiturate concentration of 12 mg/100 g of liver was found. The chromatographic study showed that only phenobarbital was present in the liver extracts.

Only 25-ml of fluid stomach contents were available. This sample was analyzed by the same procedure that was used for blood and found to be negative for barbiturate.

In case No. 2, a bottle known to hold 75 1½ grain tablets of phenobarbital was found empty in a coat pocket of the deceased. A study of the urine and blood disclosed results similar to those obtained in case No. 1.

In the first case presented, 46 percent of the *p*-hydroxyphenobarbital was conjugated; in the second, 20 percent was in the conjugated form.

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References and Notes

1. T. C. Butler, *Science* 120, 494 (1954).
2. J. T. Walker, R. S. Fisher, J. J. McHugh, *Am. J. Clin. Pathol.* 18, 451 (1948).
3. E. J. Algeri and J. T. Walker, *ibid.* 22, 37 (1952).
4. A. J. McBay and E. J. Algeri, *ibid.* 24, 1139 (1954).
5. We wish to express our appreciation to Thomas C. Butler of the department of pharmacology, University of North Carolina School of Medicine, for his interest and cooperation in supplying us with the reference sample of *p*-hydroxyphenobarbital.

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Body Composition and Energetics in Obesity Induced in Mice by Adrenotropic Tumors

Some of the characteristics of a transplantable pituitary adrenocorticotropic tumor induced in mice that had been exposed to ionizing radiation have been described (*1*). The salient changes produced in the recipients are enlargement of the adrenals (and of no other endocrine organ,) leukopenia (notably lymphocytopenia), atrophy of the thymus and the spleen, polyuria rarely with glycosuria and hyperglycemia, obesity, and sensitivity to infection that usually kills the host while the tumor is still small. Some tumor-bearing mice reach weights in the neighborhood of 45 to 50 g as compared with a limit of about 30 g for controls of the same strain (LAF_1). In the course of successive passages, the "overweight" component of obesity became less marked, although obesity (as defined by a very much increased fat content) was still always present. This is particularly evident in the experiments presented here, where the mice, bearing small grafted tumors, had a normal weight but more than twice the normal fat content. In other words, they are "obese"—that is, grossly "overfat"—as determined by body composition.

This report (*2*) compares the energetics and body compositions of adrenocorticotropic tumor-bearing mice (ATO) with those of normal controls and of adrenalectomized tumor-bearing animals (Adrex-T) of similar age. It was noted earlier that obesity along with other secondary changes rapidly disappears if the adrenals of the tumor-bearing mice are removed. Therefore, the inclusion of this last type of animal provides a test of the effect of the tumor per se without the overproduction of adrenal hormones.

A total of 47 mice of the LAF_1 strain were used in the study of energetics. Sixteen of these mice had been grafted with tumors 10 weeks previously; at the time of the study, they weighed 27.0 ± 2.4 g. Fourteen animals of the same strain and age were grafted at the same time and immediately adrenalectomized; they weighed 27.4 ± 2.1 g when studied. The 17 control animals weighed 27.0 ± 2.5 g. Spontaneous activity was determined over a 3-week period using rotary (squirrel) cages as previously described (*3*); the groups were rotated among 16 cages so that each animal spent a total of 7 days under measurement. Food (ground Purina chow) intake was recorded for each animal during that period. Basal oxygen consumptions were determined as previously described (*4*). Results point to a positive energy balance in the ATO animals: spontaneous activity, expressed in number of revolutions per day, was found to be 6024 ± 3003 for the controls, 4336 ± 1997 for the Adrex-T mice, and 4555 ± 2439 for the ATO mice. The difference is significant ($p < 0.002$) between control and ATO animals, but it is not significant between the two groups of tumor-bearing mice. Food intakes were 4.8 ± 1.0 g/day for the controls, 5.1 ± 0.7 g/day for the Adrex-T mice, and 5.8 ± 0.9 g/day for the ATO mice. The difference between the controls and the ATO mice is highly significant ($p < 0.001$); between the Adrex-T and ATO mice, it is significant ($p < 0.002$). There was no difference in basal oxygen expen-