Table 2. Stepwise treatment of the particulate system, assayed by the particulate method. For all species the first-order initial decay rate was extrapolated to obtain the total light emission. *Pyrocystis lunula* alone showed a change in the initial decay rate in the presence of soluble luciferin, as well as a slow reaction that made the total light emission even larger than that reported here by approximately a factor of 10. After unbound luciferin was removed, the decay rate was the same as that of the starting material. Relative light emission is expressed as the percentage of activity at step 3 (CR, centrifuged and resuspended).

Extraction step	*Gonyaulax polyedra*		*Pyrocystis lunula*		*Pyrodinium bahamense*	
	10^9 photons	Percentage	10^7 photons	Percentage	10^9 photons	Percentage
1. Freshly extracted	135		162		1.1	
2. Luciferin added	279		783		880	
3. CR	119	100	352	100	505	100
4. Acid reacted; CR	<0.04	<0.04	0.4	0.1	0.3	<0.1
5. Luciferin added	4.8	4	97	28	189	38
6. CR	1.2	1.0	17	5	129	26
7. Acid reacted; CR	<0.03	<0.03	0.2	<0.1	0.2	<0.1
8. Luciferin added	0.5	0.4	5	1	67	13

total light emission in the particulate assay (Table 2). For *P. bahamense* this increase was by a factor of several hundred. In most *G. polyedra* extracts no increase was observed, although occasionally a twofold increase could be obtained.

In all three species the addition of exogenous luciferin to a particulate system that had been stimulated by acid and resuspended at *p*H 8.2 recharged the system, so that it again emitted a flash of light when the *p*H was lowered to 5.7. This process could be repeated several times with decreasing yields. The acid-stimulated particles to which exogenous luciferin was added were centrifuged at 31,000*g* for 10 minutes and resuspended to demonstrate the binding of luciferin to the particles.

The activity of the luciferin-recharged particles in the presence of soluble luciferin was always higher than the activity of these particles after centrifugation and resuspension in buffer free of luciferin (Table 2, steps 2, 3, 5 and 6). This may be due to loss of enzyme activity or release of bound luciferin during centrifugation and resuspension, or it may be that luciferase bound to particles can react with free luciferin in the particulate assay. The effect is most marked for *P. lunula*. The recharged *P. lunula* system in the presence of soluble luciferin showed a slower rate of light emission than the same system after centrifugation and resuspension in buffer free of luciferin.

Comparison of kinetics, profiles of activity as a function of *p*H, and emission spectra for "native" and recharged particulate systems offer evidence that recharging may be a physiological event. The kinetics of the rise and decay are identical within a species. All activity profiles between *p*H 4.2 and 6.7 are roughly parabolic with half-widths of approximately 0.6 *p*H unit. The *p*H optima for all recharged and native particles are identical. There are some differences in activity at suboptimum *p*H, which are not consistent among the species. However, the mean half-width of the profiles for the reconstituted particles differs from that of the native particles by less than 12 percent.

By a modification of the technique of simultaneous measurement of the light reaction by two phototubes, each with its own interference filter (*10*), we have been able to demonstrate that the relative shapes of the emission spectra of all the particulate systems, native and recharged, are essentially identical. The bioluminescence emission spectrum of the native particulate system is the same as that of mechanically stimulated cells (*3*) and the in vivo spectra of the three species are identical (*8*).

Our experiments do not settle the question of whether in vivo bioluminescence is due to soluble or particulate components. However, the demonstration, in additional species, of particulate systems which are labile, contain dissociable luciferin and luciferase, and can be made to recycle by the addition of luciferin strengthens the argument that the particulate system is the physiological one (*11*).

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12. Supported by AEC Division of Biology and Medicine contract AT(11-1)3277. Contribution No. 682 of the McCollum-Pratt Institute and Department of Biology of the Johns Hopkins University. C.W.F. is supported by NIH predoctoral training grant GM-57 to the Department of Biology.

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Abandoned Larvacean Houses: A Unique Food Source in the Pelagic Environment

Abstract. *Observations made by using conventional scuba techniques reveal that abandoned larvacean houses serve as food sources for marine, planktonic copepods. Techniques have been developed for photographing these houses in the field for the first time. The abundance of larvacean houses in the open ocean indicates that they are important in pelagic food webs and as a source of particulate organic matter.*

Larvaceans, pelagic tunicates of the class Appendicularia, are one of the commonest elements of marine zooplankton. As a major metazoan link between the nannoplankton—tiny, one-celled phytoplankters—and the larger planktonic and neritic animals of the ocean, they are central to many marine food chains. Larvaceans filter out nannoplankton through a unique feeding structure, the "house," secreted around the animal by the oikoplast epithelium of the body (Fig. 1). When the incurrent filters of the house clog with algae and detrital particles the animal abandons the house and proceeds to build a

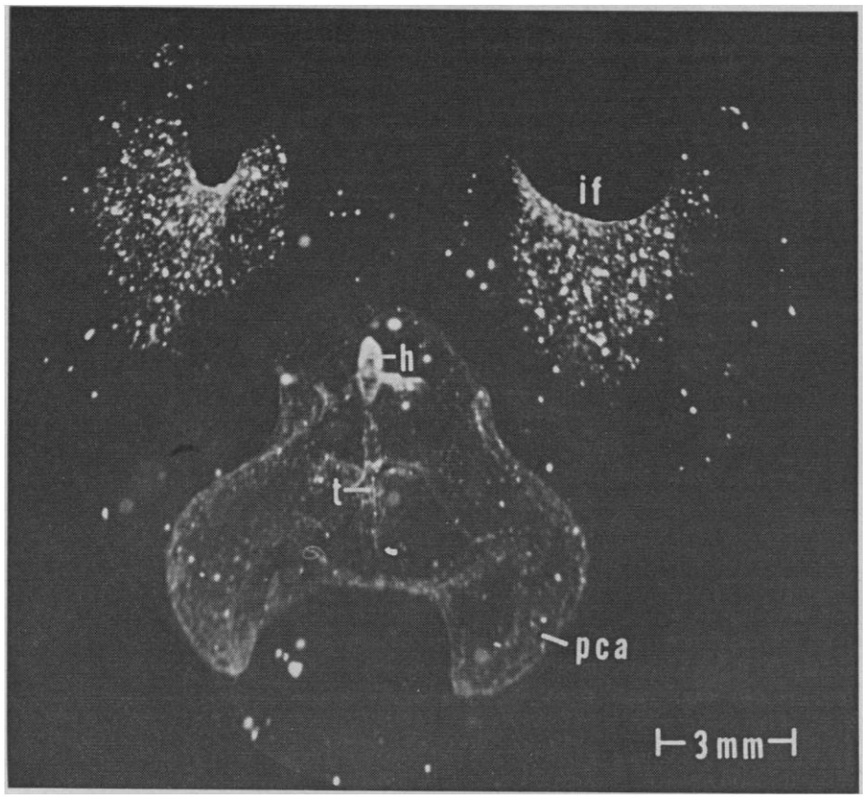


Fig. 1. Head-on view of *Megalocercus abyssorum* in its house. The larvacean's sinusoidal tail movement draws water through the coarse-meshed incurrent filters (*if*), where large particles are removed, and into the inner particle-collecting apparatus (*pca*), where small particles, predominantly nannoplankton, are caught and consumed. Water exits through a chamber near the animal's tail (*t*). The head is denoted by *h*. The photograph was taken in the field with Nikonos II underwater camera having a 44-mm extension tube and backlighted with a Giddings Sea Star I strobe light; Tri-X film at 1/60 second and *f*16 was used. This represents the first such picture ever published of a larvacean in its natural habitat.

new one (*1*). *Oikopleura rufescens*, for example, builds a new house every 4 hours (*1*), and *Kowaleweskia tenuis* builds one every 2 hours (*2*). The ecological significance of these numerous abandoned houses in the pelagic environment has never been considered previously. In addition, the use of organic aggregates as a food source by zooplankters has generated much speculation (*3–5*). I report here direct field and laboratory observations of copepods feeding on the clogged filters of abandoned larvacean houses in the open ocean.

Field observations were made at depths of 10 to 15 m in the Florida Current 6 to 10 km west of Bimini, Bahamas, by using conventional scuba techniques (*6*). Abandoned houses were identified with species according to their shape and size and the presence of filtering apparatus. Houses of *O. longicauda*, *O. fusiformis*, and *Megalocercus abyssorum* were most abundant. The densities of the houses, obtained by direct visual counts inside a hand-held grid (10 by 20 by 20 cm), varied from 44.4 to 623.0 houses per cubic meter. Up to 33 percent of the abondoned larvacean houses observed on 15 dives had one to five *Oncaea mediterranea* (Copepoda: Cyclopoida) resting on the incurrent filters or darting about on the inner apparatus, where particles are collected (Table 1). I also observed *Microsetella norvegica* (Copepoda: Harpacticoida), *Paracalanus aculeatus* (Copepoda: Calanoida), *Conchoecia rotundata* (Ostracoda), and euphausiid larvae resting on houses, and *O. mediterranea* resting on the mucus feeding web of *Gleba cordata* (*7*) and on unidentified particulate organic matter as well.

Laboratory experiments show that *O. mediterranea* actually feeds on larvacean houses. Two hundred freshly caught, adult, female copepods were starved for 18 hours in filtered seawater (0.45-μm Millipore filter) containing 25 mg of streptomycin G per liter and then divided among 40 small watch dishes, five animals per dish. Twenty dishes were set aside as a control. One house of *M. abyssorum*, collected by hand and washed, was added to each of the remaining 20 dishes. Six hours later the number of fecal pellets produced by each group was counted. The 100 experimental *O. mediterranea* fed on larvacean houses, producing an average of 4.73 fecal pellets per copepod (10 to 41 pellets per dish), whereas control animals produced 0.02 fecal pellet per copepod (0 to 1 pellet per dish). Continuous observation revealed that the copepods were highly active and spent little time (averaging 4.6 percent of the 6-hour experimental period) actually resting on houses. Thus, the occasional low frequency of copepods resting on houses in the field probably reflects this motility as well as the population size of *O. mediterranea* on a particular day.

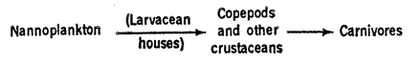
Table 1. Frequency of occurrence of copepods (predominantly *Oncaea mediterranea*) feeding on abandoned larvacean houses in the Florida Current. House densities were obtained by direct visual counts at depths of 10 to 15 m. A Plexiglas rod grid (10 by 20 by 20 cm, or 1/250 m^3) held before the diver's faceplate served to delineate volume. One hundred such grids were counted on each dive; *N*, number of houses.

Date	Abandoned larvacean houses (N/m^3)	Houses with copepods (%)
14 October 1971	114.0	13.2
19 October 1971	44.4	16.7
12 November 1971	58.9	14.3
17 November 1971	268.8	4.3
20 November 1971	74.5	5.2
24 November 1971	478.7	2.5
26 November 1971	496.0	6.5
12 January 1972	115.4	13.3
20 January 1972	603.4	10.1
8 February 1972	387.8	16.8
16 February 1972	623.0	0
23 February 1972	275.2	0
24 February 1972	399.9	9.3
30 March 1972	540.3	33.3
6 April 1972	466.5	25.7
Mean	329.8	10.7

Abandoned house filters contain naked dinoflagellates, coccolithophores, unidentified organic particles, and a few diatoms, silicoflagellates, and tintinnids. An examination of the copepod fecal pellets revealed coccolith fragments and unidentified organic matter. While feeding, *O. mediterranea* rests on the surface of the house and uses its first and second maxilla and maxillipeds to scoop particle-laden mucus toward the mouth. No specific food selectivity was observed, although copepods tend to prefer the inner particle-collecting apparatus, which contains smaller particles than the outer incurrent filters.

The implications of this study are fourfold. First, suspension-feeding, adult, marine copepods are unable to

utilize most nannoplankton since they have difficulty filtering particles smaller than 5 to 10 μm in diameter (*5, 8*). This discovery of the use of concentrated nannoplankton on abandoned larvacean houses by pelagic copepods, as either a major or a supplementary food source, reveals avenues in the pelagic food web, of the form:



The abundance of these abandoned houses and the variety of crustacean species observed on them suggest that this food chain may be significant. Second, strictly herbivorous, planktonic copepods are not limited to suspension feeding or direct capture of large phytoplankters (*9*) as the only modes of obtaining food. Abandoned larvacean houses, mucus feeding webs of pelagic gastropods (*7*), and organic aggregates (*3*) provide innumerable microsurfaces that are essentially benthic in nature and suitable for benthic feeding methods. Third, the abundance of abandoned houses suggests that larvaceans may be a major source of the particulate organic matter in the sea. As they decay, the houses form organic aggregates and may provide free surfaces for the absorption of dissolved organic matter, a process already documented for surfaces produced by bubbles and Langmuir circulations (*3, 10*). Last, since larvacean houses are invariably destroyed, disintegrated, or filtered through most conventional plankton nets, direct observations must be made of many planktonic forms if their biology is to be correctly understood.

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Separation of Skin Reactive Intestinal Cancer Antigen from the Carcinoembryonic Antigen of Gold

Abstract. *Soluble fractions of human intestinal cancer and fetal intestinal cell membranes produced delayed hypersensitivity reactions in patients with intestinal cancer. These soluble fractions and perchloric acid extracts of intestinal cancer cells were fractionated by polacrylamide-gel electrophoresis. The Gold carcinoembryonic antigen was found in a region of the gels different from that of the skin reactive antigen.*

Delayed hypersensitivity reactions in patients with intestinal cancer have been elicited by a soluble fraction of membranes prepared from autologous and allogeneic tumor cells (*1, 2*). These reactions appeared to be specific since negative reactions were obtained with comparable soluble membrane fractions from normal cells. Skin reactive antigen was also detected in soluble cell membrane fractions from the intestines of 1- to 6-month fetuses.

The soluble fractions producing skin reactions were also shown to contain the carcinoembryonic antigen (CEA) of Gold (*1, 2*). Gold and Freedman (*3*) have shown that human intestinal cancers contain an antigen which is also found in embryonic entodermal tissues, during the first two trimesters of pregnancy. The CEA is a glycoprotein closely associated with the cell surface membrane (*4*).

A question raised by the previous studies (*1, 2*) was whether CEA was the same as the skin reactive antigen. One observation which cast some doubt as to whether these antigens were identical was that purified CEA produced negative skin reactions (*2*). Moreover, Lejtenyi *et al.* (*5*) have reported that purified CEA failed to induce blast transformation of the lymphocytes of patients with intestinal cancer. We have now separated active fractions by gel electrophoresis in order to ascertain the relation of skin reactive antigen to CEA.

Six patients (George Washington University Hospital) with rectal and colonic carcinomas were selected on the basis of positive tests with one or both recall (control) antigens—that is, mumps and SKSD [Varidase (Lederle), a product consisting of streptokinase (40 units) and streptodornase (10 units)]. All patients had definitive resection of localized tumor, were tested 1 to 2 weeks after operation, and were not receiving chemotherapy or other treatment. They were inoculated intradermally with 0.1 ml of the various gel fractions of sonicated cell membranes of allogeneic tumor and of fetal intestine, of CEA, or of partially purified CEA. Erythema and induration were measured at 24, 48, and 72 hours. A positive delayed reaction was defined as induration of 5 mm or greater at 48 hours. Only 20 to 30 percent of the membrane proteins were recovered in the soluble portions of the sonicated membranes. Membrane preparations, 50 to 100 μg of protein per 0.1 ml, produced delayed skin reactions; however, the pooled soluble sonicated membranes (prior to separation), of comparable or twice the protein concentration of membrane preparations, did not produce delayed skin reactions. The Sephadex fractions producing delayed skin reactivity at 5 to 25 μg of protein per 0.1 ml contained more than 50 percent of the protein recovered from the columns. The yields from the cancer extracts and from the fetal liver or intestinal material were similar. The elution patterns of the protein peaks containing the skin reactive antigen were virtually identical (ratio of elution volume to void volume of peaks was 2.4) from each tumor cell membrane preparation.

Partially purified CEA and purified CEA were obtained as follows (*6*). Homogenates of hepatic metastases of intestinal cancer were extracted with perchloric acid and separated by Sepharose 4B and Sephadex G-200, yielding partially purified CEA; the purified CEA was then obtained by preparative block electrophoresis. Both were tested within 1 month for skin reactivity. The skin reactive antigens from both the intestinal cancer and fetal cell membranes, as well as CEA and partially purified CEA, were further separated by gradient polyacrylamide-gel electrophoresis (*7, 8*). Immediately after it was used for skin testing, the partially purified CEA was subjected to electrophoresis; a similar preparation