remained on the right side; on the left side, part of the maxilla and the zygomatic bone were also present as were two premolars and three molars.

Observations made on a wild pig carcass consumed within a few days by other pigs also indicate the great efficiency of pigs as scavengers and help explain the paucity of animal remains on the forest floor. Thus, without discussing such factors as the extreme acidity of the soil or smaller scavengers (for example, monitor lizards) it becomes clear that the possiblility that anything more than an occasional skull fragment or rib end would survive on this particular tropical rain forest floor for a great enough length of time to be buried and become fossilized seems minimal. Most pongids today still live in tropical rain forests where, presumably, their evolution occurred. Pigs are generally found in these forests. Direct observation of the scavenging of an orangutan carcass in an Asian forest corroborates evidence from Africa and suggests that the scavenging of wild pigs may play an important role in the destruction of pongid remains.

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References and Notes

1. G. Schaller, *The Mountain Gorilla: Ecology and Behavior* (Univ. of Chicago Press, Chicago, 1968), p. 398.
2. D. Fossey, *Natl. Geogr.* **140**, 576 (1971).
3. J. Van Lawick-Goodall, *Anim. Behav. Monogr.* **1**, 170 (1968); G. Teleki, *J. Hum. Evol.* **5**, 562 (1976).
4. G. Teleki, *Folia Primatol.* **20**, 81 (1973).
5. T. Ransom, thesis, University of California, Berkeley (1971).
6. Funding for the orangutan research was provided by the Wilkie Brothers Foundation, the L. S. B. Leakey Foundation, the National Geographic Society, the New York Zoological Society, the Herz Foundation, the Jane and Justin Dart Foundation, and the Van Tienhoven Foundation of Holland, to whom I am very grateful. I thank R. Brindamour for his help, without which the long-term research would not have been possible. The late L. S. B. Leakey was instrumental in enabling me to begin this research. The Indonesian Institute of Sciences and the Nature Conservation and Wildlife Management Department served as sponsors.

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Bioluminescence: Dual Mechanism in a Planktonic Tunicate Produces Brilliant Surface Display

Abstract. *Luminescent flashes emanate spontaneously and on mechanical stimulation from the bodies of* Oikopleura dioica *(Urochordata, Larvacea); flashes also emanate, on mechanical stimulation only, from both their occupied and discarded mucous houses. The luminescence is intrinsic to the animals and their houses. Field observations suggest that, because of this dual method of light production, larvaceans may contribute substantially to surface coastal displays of marine bioluminescence.*

Brilliant displays of bioluminescence, usually attributed to dinoflagellates, have long been observed in the surface waters of the sea, especially near coasts (*1, 2*). I report here that in some cases larvaceans (Urochordata), and especially *Oikopleura dioica* Fol 1872, contribute significantly to such displays and that the luminescence is produced not only by the organisms themselves but also by the mucous "houses" that they produce (*3*).

The importance of larvaceans in marine planktonic ecosystems has been recognized (*4, 5, 5a*). These organisms are widely distributed and, among zooplankton, their abundances may be second only to, or even exceed, those of copepods, especially in coastal waters (*6–8*). Recent reports of larvacean luminescence (*1, 9*) derive ultimately from a single brief report (*6*) of observations on *O. albicans* (*10*). No reports deal with mechanisms of light production or with field ecology of luminescence in these animals.

Laboratory observations confirmed that both *O. dioica* and its houses luminesce. Freshly collected animals, removed from their houses and transferred to Millipore-filtered seawater, flashed repeatedly with and without mechanical stimulation (Fig. 1, A and B). The flashes, with intensities up to 2×10^9 quanta per second per animal (*11*), emanated from the animals' trunks and had a minimum rise time of 18.0 ± 3.8 msec, a half-decay time of 18.5 ± 4.3 msec, and a total duration of 138 ± 33 msec (mean ± the standard deviation of six flashes) (*12*). Upon mechanical stimulation, discarded houses could be made to flash repeatedly in the absence of the animals, even up to 4 hours after the animals had left them (Fig. 1, C and D). No spontaneous flashes were observed from the houses.

Bacterial, intracellular, and extracellular luminescence has been recognized in metazoans (*1*). Lohmann (*6*) believed that the light in *O. albicans* emanated from a glandular secretion released into the house by the paired buccal glands. The flashes of the animal's trunk are compatible with this explanation, since light could arise from a secretion into the thin layer of mucus comprising the rudimentary house. However, the ability of discarded houses to luminesce repeatedly by means of short flashes and in the absence of the animal suggests membrane-associated sources of light (*13*). Possibilities include (i) luminescent organisms adhering to or living on or in the houses and (ii) *Oikopleura* cells or cell membranes included in the house material itself. Alternatively, a luminous secretion may be sequestered in the house material and reexposed to a necessary factor in the surrounding seawater when the house is mechanically stimulated (*14*).

Bacteria and phytoplankton do occur on and in occupied and discarded larvacean houses (*4, 9*), but there is strong evidence against their being the sources of the light. First, new houses built by *O. dioica* in Millipore-filtered seawater free of these organisms still luminesced in the absence of the animals (*15*). Second, phase-contrast microscopic observations of freshly collected houses did not reveal dinoflagellates on or in the houses. Third, known in situ bacterial luminescence is continuous (*1, 16*). Finally, bacterial luciferase could not be detected in discarded houses (*17*).

Thus, it seems clear that luminescence in both *O. dioica* and its houses is endogenous. That houses can flash apart from the animals suggests mediation of the luminescent response by *Oikopleura* cells or cell membranes left in the houses. The houses of larvaceans are usually assumed to be noncellular (*6, 9*). However, there is evidence (*18*) that small granules ("Häutungskörper"), visible in intricate, species-specific patterns in the rudimentary houses of certain Oikopleuridae, are degenerate cells or nuclei deposited from the animal's trunk epithelium into the house material during its secretion. The significance of these structures is unknown, but it may be that functional cell membranes remain in the expanded house.

Field observations of *O. dioica* suggest that this dual luminescence in larvaceans may contribute significantly to brilliant surface displays of marine bioluminescence. Such displays are usually attributed to high concentrations of dinoflagellates, although often the evidence for their involvement is indirect (*2*). Rarely have the responsible organisms been identified (*2*), and then usually only in specific luminescent bays (*7, 19*) or during conspicuous blooms.

An unusually brilliant display of sur-

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face luminescence, caused almost entirely by flashing of the houses and the bodies of *O. dioica*, occurred in June 1977 at Puerto Escondido, Baja California Sur, Mexico (*20*). Agitation of the water in the bay produced a dense array of vivid, relatively long flashes of light from sources estimated at 5 mm or more. While I was floating motionless in the channel or while I was viewing undisturbed bayshore water I observed only shorter, spontaneous flashes from about 1-mm sources and at much lower concentrations than the stimulated flashes (*21*).

Examination of the water with a diver's light revealed numerous occupied and empty houses of *O. dioica* (*22*), ranging in size from about 2 to 6 mm, at densities visually estimated at about 10^4 per cubic meter. There was little other conspicuous zooplankton except nonluminous copepods, and analysis of plankton samples (Table 1) suggested that *O. dioica* was the only luminous species present in sufficient numbers to account for the intense, stimulated light in the bay.

Several observations confirmed that *O. dioica* was the major source of luminescence at Puerto Escondido. (i) The stimulated flashes and the houses were of comparable sizes. (ii) The stimulated flashes, visible in dim illumination, could be discerned to come from houses. (iii) Sudden in situ illumination of a large, stimulated flash usually revealed either an occupied or a discarded house. (iv) Sudden illumination of a small, spontaneous flash usually revealed an *O. dioica*, either in its house or freely swimming. (v) Phytoplankton cells, 0 to 35 μm in diameter, were not luminescent (*23*). (vi) Although there were cells of potentially luminescent dinoflagellates in both net and bottle samples (Table 1), their sizes and numbers were insufficient, and their flashing properties would have been unsuitable, to account for my observations.

The numbers of *O. dioica* in the samples are an underestimate of the density of stimulated flashes in the water column because even empty houses can flash, and each *O. dioica* builds and abandons several houses per day (*22*). An average occupancy of only one in nine houses and total house densities of up to 1130 per cubic meter were reported for six species of Oikopleuridae in the Gulf of California near Puerto Escondido (*4*). If we assume like durability and turnover rates for houses of *O. dioica*, then my estimated *O. dioica* densities of 2720 to 16,000 per cubic meter (Table 1) represent a total house (and potential flash) concentration of about 10^4 to 10^5 per cubic meter at Puerto Escondido.

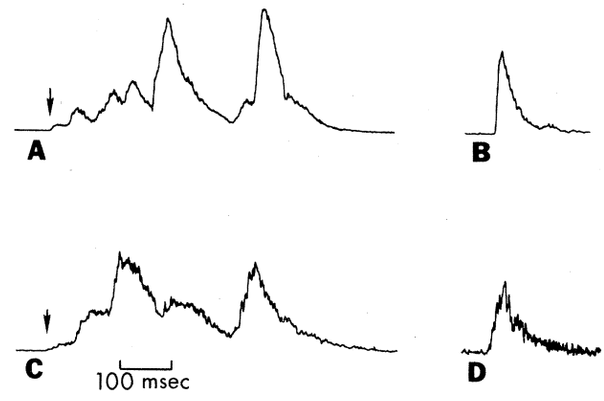

Fig. 1. Luminescent flashes of *Oikopleura dioica* recorded from the animal (A and B) and from the discarded house (C and D). Flashes A, C, and D resulted from a mechanical stimulus (arrow) consisting of a rapid injection of seawater into the specimen chamber (*12*). The isolated house flash (D) occurred within 1 second after the stimulus. Isolated, nonstimulated flashes (B) occurred in the animal 1 to 5 seconds or more after a stimulus. The intensity is represented by arbitrary units.

In other locations larvaceans often approach or exceed the densities reported here (*7, 24, 25*). Even in tropical bays where luminescence is clearly associated with abundant dinoflagellates, larvaceans may have a heretofore unrecognized significance. Larvaceans have been reported in only one study (*7*) (as algal grazers) in the plankton, and their densities were 7000 to 11,000 per cubic meter, enough to account for a significant portion of the observed luminescence.

Larvacean luminescence is probably widespread geographically and seasonally among several species (*26*), yet larvaceans have been largely overlooked as sources of light in studies of marine bioluminescence. This is presumably because (i) their luminescence is not widely known, (ii) the animals themselves are small relative to their houses and light flashes, (iii) their houses are almost transparent, difficult to recognize in plankton samples, and previously not known to provide luminescence, and (iv) many may pass through standard plankton nets and are overlooked in plankton samples (*27, 5a*). However, my results show that, because of their widespread distribution and abundance and their unique dual method of producing light from not only the animals themselves, but also from multiple, discarded houses, larvaceans may often contribute substantially to marine bioluminescence.

Table 1. Calculated densities of plankton at Puerto Escondido channel. Estimates are based on a single net tow and two 0.5-liter water samples taken at 2200 to 2300 hours, 15 June 1977. The net was towed from the pier by hand through a 28-m horizontal path at a depth of 1 to 2 m. It had a mouth diameter of 11 cm and mesh size of 75 μm, and it filtered 0.266 m^3 of water. Differences between the two columns probably resulted from small sample sizes and clogging of the net by *O. dioica* houses. "Other invertebrates" included small numbers of hydromedusae, chaetognaths, and larval ctenophores, mollusks, and polychaetes.

Organism	Density (number/m^3)	
	Net tow	Water samples
Diatoms	150	
Dinoflagellates		
Ceratium sp.	300	
Dinophysis sp.	300	
Peridinium sp.	200	
Unidentified	700	
Copepoda		
Acartia sp.	42,000	220,000
Other Calanoida	500	
Cyclopoida	4,000	24,000
Nauplii	8,300	16,000
Cladocera	700	4,700
Other crustaceans	100	
Oikopleura dioica	2,720	16,000
Other invertebrates	800	
Fish embryos	50	

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References and Notes

1. E. N. Harvey, *Bioluminescence* (Academic Press, New York, 1952).
2. P. B. Tett and M. G. Kelly, *Oceanogr. Mar. Biol. Annu. Rev.* **11**, 89 (1973).
3. For accounts of larvacean biology, including the ecology of larvacean houses, see A. L. Alldredge [*Sci. Am.* **235** (No. 1), 94 (1976)] and (*8*). Larvaceans are much smaller (about 0.6 to 1 mm in trunk length for adult *O. diocia*) than the houses they occupy (at least 5 mm).
4. A. L. Alldredge, *Limnol. Oceanogr.* **21**, 14 (1976).
5. Larvaceans are known prey for fish and planktonic invertebrates [A. L. Alldredge, *Mar. Biol.* **38**, 29 (1976) and references therein; E. S. Hobson, *U.S. Natl. Mar. Fish. Serv. Fish. Bull.* **72**, 915 (1974); J. E. Bailey, B. L. Wing, C. R. Mattson, *ibid.* **73**, 846 (1975)]. A. L. Alldredge, *Science* **177**, 885 (1972).

5a. E. S. Hobson and J. R. Chess, *U.S. Natl. Mar. Fish. Serv. Fish. Bull.* **74**, 567 (1976).

6. H. Lohmann, *Schr. Naturwiss. Ver. Schleswig-Holstein* **11**, 347 (1899).
7. H. H. Seliger *et al.*, *Limnol. Oceanogr.* **16**, 608 (1971).
8. R. Fenaux, *Les Appendiculaires des Mers d'Europe et du Bassin Méditerranéen* (Masson, Paris, 1967).
9. H. Lohmann, in *Handbuch der Zoologie*, W. Kükenthal and T. Krumbach, Eds. (De Gruyter, Berlin, 1933–1934), vol. 5, part 2, p. 1.
10. E. H. Giglioli [*Atti Accad. Sci. Torino, Cl. Sci. Fis. Mat. Nat.* **5**, 485 (1870)] reported in a vague account that vivid red, blue, and green light emanated from the tail of an unidentified larvacean. Neither Lohmann (*6*) nor I have seen light in the tail in three species of *Oikopleura*, although colorful iridescence occurs in the caudal muscles.

11. The measurement was made by N. Langerman at Scripps Institution of Oceanography, La Jolla, Calif., on 25 and 26 July 1977 with the methods of K. H. Nealson, T. Platt, and J. W. Hastings [*J. Bacteriol.* **104**, 313 (1970)].
12. Data were calculated from recordings similar to those in Fig. 1B and made by J. Morin at the University of California, Los Angeles, on 29 August 1977. Bioluminescence was produced by mechanical stimulation of an animal or house placed in a vial in a lighttight chamber over a side window photomultiplier tube (RCA 1P21). The specimens were stimulated by injecting 1 to 3 ml of seawater into the vial through a small hole in the chamber lid. The output voltage from the photomultiplier tube was proportional to light intensity (in arbitrary units) and was displayed on a chart recorder (Clevite Brush). The response time of the circuit and the recorder were well above the recorded flash frequencies.
13. J. Morin, personal communication.
14. J. Case, personal communication.
15. Animals freshly collected at Long Beach, Calif., were immediately removed from their houses and washed in two changes of Millipore-filtered (0.45 μm) seawater. They were then allowed to build new houses, which they discarded after a 30- to 60-minute occupancy. Invariably these isolated, "clean" houses flashed repeatedly upon mechanical stimulation, and microscopic examination revealed almost no adherent or filtered particulate matter.
16. J. W. Hastings and K. H. Nealson, *Annu. Rev. Microbiol.* **31**, 549 (1977).
17. N. Langerman and M. Haygood performed the bacterial luciferase assay at Scripps Institution of Oceanography, La Jolla, Calif., on 25 and 26 July 1977 with the methods of J. W. Hastings, W. H. Riley, and J. Massa [*J. Biol. Chem.* **240**, 1473 (1965)].
18. W. F. Körner, *Z. Morphol. Oekol. Tiere* **41**, 1 (1952).
19. H. H. Seliger and W. D. McElroy, *J. Mar. Res.* **26**, 244 (1968).
20. Puerto Escondido is a small, enclosed bay (0.7 by 0.3 km, maximum depth 14 m) connected on its south side to the Gulf of California by a narrow channel. I made in situ observations in shallow water along the southwest side of the bay and in the channel shortly before or after high tide on clear, moonless nights between 2100 and 2400 hours on 14 to 16 June 1977. Water temperature and salinity were 25°C and 36 parts per thousand, respectively.
21. Although I have no in situ measurements of intensity, several colleagues at the site confirmed my subjective impression that the light was at least as brilliant as that of temperate coastal displays caused by dinoflagellates, whose intensity is established (*2*). Subjective, visual estimates by me and several colleagues of the instantaneous density of flashes during extreme agitation are of the order of 10^4 to 10^5 per cubic meter; we estimated the instantaneous density of spontaneous flashes to be about 10^2 to 10^3 per cubic meter.
22. *Oikopleura dioica* secretes and discards four to seven houses per day at 13°C [G.-A. Paffenhöfer, *Mar. Biol.* **22**, 183 (1973)].
23. Seawater filtered through a 35-μm screen did not luminesce with or without agitation.
24. G.-A. Paffenhöfer, in *Tenth European Marine Biology Symposium*, G. Persoone and E. Jaspers, Eds. (Universa Press, Wetteren, Belgium, 1976), vol. 2, p. 437; T. Wyatt, *Mar. Biol.* **22**, 137 (1973); H. Seki, *La Mer (Bull. Soc. Franco-Jpn. Oceanogr.)* **11**, 153 (1973); D. Binet, *Doc. Scient. Centre Rech. Oceanogr. Abidjan* **7**, 45 (1976); T. M. Tundusi, *Bol. Inst. Oceanogr. Sao Paulo* **19**, 131 (1970).
25. C. P. Galt, thesis, University of Washington, Seattle (1970).
26. Luminescence has been observed by me and my colleagues in *O. dioica* at Long Beach and La Jolla, Calif., and at Friday Harbor, Wash.; in *O. labradoriensis* at Friday Harbor; by Lohmann (*6*) in *O. albicans* at Messina, Italy; it was reported in *O. vanhoeffeni* in the Okhotsk Sea [N. Tarasov, *Marine Luminescence* (Academy of Science U.S.S.R., Moscow, 1956) (in Russian)]. Several colleagues observed luminescence with similar properties (abundant, long, intense flashes from relatively large sources) at Puerto Escondido during the 8-day period before this study, during August 1977, and in June 1976 (A. Miller, personal communication).
27. A. Bückmann, *Mar. Biol.* **21**, 349 (1973). Juveniles of *O. dioica*, often the most abundant age class (*25*) and 40 percent of my larvacean catch, have a body width of only 60 to 80 μm, and so could pass through most plankton nets. The houses of such animals are about 0.5 mm in diameter and would produce a light flash similar to that of *Noctiluca* [R. Eckert, *Science* **147**, 1140 (1965)].
28. Supported in part by a grant-in-aid from the Office of Graduate Studies and Research, California State University, Long Beach. I thank Lt. A. Encinas, Long Beach Marine Department, for access to collecting sites; A. Miller and his subtidal ecology class for introducing me to Puerto Escondido and for corroborating certain field observations; J. Ford, E. Horvath, S. Keck, and P. McIlvaine for collections; P. Brehm, G. Brown, M. Haygood, and N. Langerman for discussions and technical assistance; T. McCoppin and D. Nelson for translations; P. Anderson, J. Case, A. Herrera, and T. Pietsch for criticism of the manuscript, and J. Morin and L. Stuart for discussion, laboratory and field assistance, and criticism.

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Fluorescence-Immunocytochemistry: Simultaneous Localization of Catecholamines and Gonadotropin-Releasing Hormone

Abstract. *Gonadotropin-releasing hormone and dopamine were identified simultaneously in the same block of tissue from the median eminence of the rat brain. Two distinct bands of dopamine terminals were found in the lateral median eminence: an inner band which overlapped the gonadotropin-releasing hormone terminals and an outer band which appeared juxtaposed to portal capillaries.*

The concept that monoamines are involved in the secretion of gonadotropins from the anterior pituitary gland was proposed initially more than 30 years ago (*1*). Data now suggest that both catecholamines and indoleamines participate in the release of luteinizing hormone and follicle-stimulating hormone from the anterior pituitary (*2, 3*). Direct morphological studies of monoamines and releasing hormones have been facilitated by the development of specific histofluorescence (*4*) and immunocytochemical (*5*) techniques. However, certain technical limitations have prevented the direct morphological study of both monoamines and peptide hormones within a single tissue block. Here we describe the correlative distribution of the catecholamines norepinephrine (NE) and dopamine and the hypothalamic peptide gonadotropin-releasing hormone (GnRH) in the median eminence of the rat brain. The distributions were determined by means of a fluorescence-immunonocytochemical technique that allows the simultaneous visualization of monoamines and neuropeptides within adjacent tissue sections.

Six adult male albino Sprague-Dawley rats (200 to 300 g) were killed by decapitation. The calvaria was removed, the brain was excised, and the diencephalon was dissected. Each tissue block was submerged in Freon-22 cooled to −100°C by liquid nitrogen and freeze-dried in a

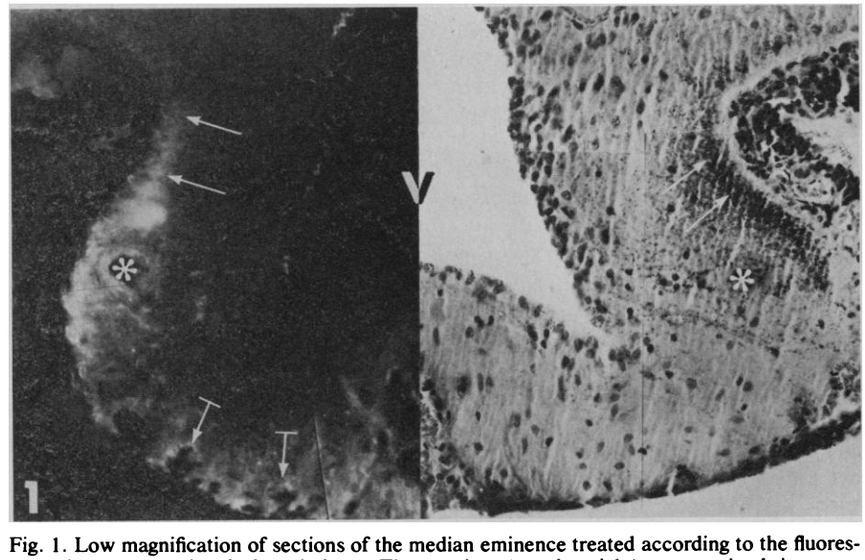

Fig. 1. Low magnification of sections of the median eminence treated according to the fluorescence-immunocytochemical technique. The section (on the right) was stained immunocytochemically and was turned over so that it could be compared with the exactly contiguous surface of the adjacent tissue section (on the left) that was treated for monoamine histofluorescence. Catecholamine and GnRH terminals (arrow) were found in the lateral regions of the median eminence. Dopamine terminals were also identified in the contact zone of the ventral and medial regions of the median eminence (arrows with bar) where only a few GnRH fibers were seen. The blood vessels in each section are identical and provide useful antomical landmarks (asterisks); *V*, third ventricle (×187).

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