Marine Biology 53, 69–77 (1979)

MARINE BIOLOGY

Luminescence from Non-Bioluminescent Tissues in Oceanic Cephalopods

R.E. Young[1], C.F.E. Roper[2], K. Mangold[3], G. Leisman[4] and F.G. Hochberg, Jr.[5]

[1]Department of Oceanography, University of Hawaii at Manoa; Honolulu, Hawaii, USA,

[2]Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution; Washington, D.C., USA,

[3]Laboratoire Arago; Banyuls-sur-Mer, France,

[4]Scripps Institution of Oceanography; La Jolla, California, USA and

[5]Santa Barbara Museum of Natural History; Santa Barbara, California, USA

Abstract

Several tissues (e.g. kidney, blood, digestive gland) in oceanic cephalopods which do not exhibit *in vivo* bioluminescence, luminesce when homogenized in the presence of air or when simply exposed to air in a vial (blood). The source of the luminescence appears to be a luciferin: treatment of kidney homogenates and blood with a photophore extract presumably containing luciferase resulted in a 20-fold increase in light production. Luminescence was also found in the renal fluid, which may be the source of luminescent clouds produced by squids. The variability in luminescence found in some tissues of cephalopods appeared to be related to feeding. Luminescence was also detected in the digestive glands of midwater octopods.

Introduction

Cephalopods are known for the spectacular nature of their bioluminescent displays and for the structural and functional variety of their luminescent organs. Luminescence in cephalopods is either intrinsic, coming from specialized tissues of the animal or extrinsic, coming from symbiotic luminous bacteria.

The chemicals involved in intrinsic luminescence defied careful analysis for many years, because the methods of extraction failed to produce an active substrate (e.g. Girsch *et al.*, 1976). Recently, Goto *et al.* (1974) and Inoue *et al.* (1975, 1976) isolated a luciferin and an oxyluciferin from the branchial photophores of the oceanic squid *Watasenia scintillans*. A stable luciferin was extracted only under oxygen-free conditions. Attempts to extract luciferin from the digestive gland were unsuccessful, although a preluciferin and a dehydropreluciferin were obtained (Inoue *et al.*, 1976, 1977).

During a previous search for bioluminescent tissues in cephalopods, we observed chemiluminescence on treating with H_2O_2 several organs and tissues not known to exhibit bioluminescence. While H_2O_2 is adequate for a preliminary survey and is commonly used to stimulate luminescence from bioluminescent organs (see Herring, 1976, 1977) it can provide ambiguous results when dealing with low-level light emission: H_2O_2 can stimulate chemiluminescence in the absence of bioluminescent compounds (Stauff *et al.*, 1973). In the present study, we used a technique that offered little chance of activating non-bioluminescent substances.

Our objectives in this study were twofold. We examined a squid, *Symplectoteuthis oualaniensis*, in detail to determine the source of chemiluminescence, and we surveyed a variety of other species to determine whether or not these tissues are chemiluminescent in all bioluminescent cephalopods. In the survey we concentrated primarily on the digestive gland although, when possible, other tissues were examined.

Cephalopods were classified into 3 groups according to their bioluminescent capabilities: (I) species with intrinsic luminescence; (II) species with extrinsic (bacterial) luminescence; (III) species without luminescence. Species with intrinsic luminescence are restricted to the oceanic squids in the suborder Teuthoidea: Oegopsida. Bacterial luminescence presumably occurs in *Spirula spirula*, some sepiolids (order Sepioidea), and in some

0025-3162/79/0053/0069/$01.80

loliginids (suborder Myopsida). Photophores have not been found and luminescence is not known to occur in any of the Octopoda. In addition, sepiids, various other sepioids, and some squids (teuthoids) lack photophores (see review by Herring, 1977).

The survey included species in all 3 categories. A total of 19 species was examined. With the exception of *Grimalditeuthis bomplandi*, all species examined here in the category of species with intrinsic bioluminescence have been confirmed to bioluminesce from observations of animals in aquaria (personal observations).

Materials and Methods

Most of the research reported here was conducted during a cruise aboard the University of Hawaii's research ship, R.V. "Kana Keoki", off leeward Oahu, Hawaii, in June 1978.

Specimens of the bioluminescent squid, *Symplectoteuthis oualaniensis* (130 to 220 mm mantle length), were captured at night at the surface with dipnets or squid jigs near a night light. These squid were maintained either in large covered tubs on deck with frequent water changes, or in large aquaria in a portable laboratory which had temperature-controlled running sea water (see Young and Roper, 1977). Individuals kept for periods up to 36 h were isolated in separate aquaria to prevent cannibalism; those kept on deck were used within a few hours of capture. Most other species were captured in a modified 3 m Isaacs-Kidd Mid-Water Trawl and either analyzed immediately or kept alive in a shipboard aquarium system until needed. Specimens of *Euprymna scolopes*, however, were dipnetted or seined in neritic waters at night. A total of 18 species of oceanic cephalopods and 1 species of neritic cephalopod were examined. The choice of species and numbers of individuals examined were governed primarily by which species were captured in the trawl and at the night-light stations.

Tissues were prepared in the following manner. An incision was made along the ventral midline of the mantle and the funnel-locking cartilages were separated from the mantle. In the case of *Symplectoteuthis oualaniensis*, a blood sample was taken with a syringe from either the cephalic vein or one of the efferent branchial vessels. The optic lobe of the brain was removed through a dorsal incision in the head to reduce the possibility of contamination. Pieces of tissue from other organs generally were removed in the following sequence: renal fluid, funnel retractor muscle, gill filaments, branchial gland, nidamental gland, ovary or eggs, testis and spermatophores, kidney, digestive duct appendages ("pancreas"), digestive gland ("liver"), and stomach contents. Observations were recorded on the stage of digestion in the caecum and stomach. Samples of tissue were rinsed several times in Millipore-filtered sea water (1.2 µm pore size) to avoid contamination by the blood or fluids of other tissues. Each sample of tissue was placed in a clean 1 ml tissue homogenizer with 0.5 ml filtered sea water and homogenized. The contents were poured into a scintillation vial that was placed into an SAI model 2000 ATP photometer. (Use of any manufacturer's name does not imply endorsement of the product.) The ATP photometer at its most sensitive setting could detect light emission from an empty vial (probably phosphorescence) and from a variety of substances including animals that had been in formalin for 8 h or more. To avoid confusing our results with this type of low-level light emission, we set the photometer at a setting of 5.0 on a 0 to 10 sensitivity scale. Intensities were recorded on a Hewlett Packard strip-chart recorder on the 0 to 10 V scale. The limit of sensitivity at this setting is 0.01 V. This value is equivalent to 1.5×10^6 quanta sec^{-1} or approximately equivalent to 6.2×10^{-7} µW cm^2. The calibration was made using the standard of Hastings and Weber (1963).

Since the primary purpose of this study was to determine the presence or absence of luminous activity in various organs and tissues, and not to ascertain relative intensity of light produced per gram of tissue, measurements to determine the quantity of tissue examined were made on only a few samples. In most cases we simply attempted to take samples of equal size, then homogenized them in equal volumes of water. Our accuracy of maintaining a constant sample size is indicated by the weighed samples: the dry weights of nearly 85% of the 62 weighed samples were within a factor of 1.5 of the mean value. The results are so clear for most tissues that this degree of variability in the quantity of tissues examined is of little consequence. To obtain dry weights the vials containing samples were weighed, cleaned and weighed again in the laboratory ashore to give the dry weight of the sample. The dry weights can be used only to approximate the intensity of light emitted per milligram of tissue, since the sample may absorb light depending on its degree of pigmentation. This is true

particularly of the digestive gland, digestive duct appendages, and blood.

Most tests were made on individuals that were alive at the time of examination. We defined as alive all undamaged specimens in which pumping action was evident in the systemic and branchial hearts.

The extraction of *Symplectoteuthis oualaniensis* luciferase was based on procedures given by Shimomura *et al.* (1961). Individual photophores, dissected from the patch of photophores on the anterodorsal mantle, were homogenized in 2 ml of filtered (1.2 µm pore size) sea water. Approximately 0.5 g dry Whatman DE 32 (DEAE cellulose) were added to the supernatant, and the mixture was stirred. After standing for 5 to 10 min at room temperature, the supernatant was removed and discarded. Then approximately 2 ml of buffer (500 mM NaCl, 10 mM potassium phosphate, pH 6.2) were added to the cellulose residue; the mixture was stirred thoroughly and allowed to stand for 5 to 10 min. The supernatant was removed with a Pasteur pipet and used as the luciferase extract.

The agar medium used to grow bacteria from the gills and digestive gland contained 5 g peptone, 3 g yeast extract, 3 ml glycerol, and 13 g agar per liter of 75% sea water (see Nealson, 1978).

In this study we re-examined most of the organs that emitted light with H_2O_2. Since no suitable control tissue was available from a nonluminous squid, we used optic lobe and muscle tissue for comparison.

Results

Symplectoteuthis oualaniensis

Symplectoteuthis oualaniensis has numerous photophores embedded in the muscular tissue of the head, arms, mantle and fins (Roper, 1963). In addition, two photophores are located on the ventral surface of the intestine. None of the other tissues or organs examined in this species are known to be bioluminescent.

Fifteen specimens were examined, but only a few samples of tissues were weighed (Table 1). Three tissues, kidney, blood and digestive gland, usually gave relatively high luminescence when homogenized, although activity in all of these was low or undetectable in a few individuals. The digestive duct appendages and gills occasionally displayed moderate luminescent activity, while the branchial gland, nidamental gland, ovary, testis and spermatophores had consistently very low or no detectable activity. Renal fluid was measured on two squid, and in each the luminescent activity was high, similar to that of the blood.

The general pattern of luminescence from the tissue was a long, slow increase in the light intensity, often requiring up to 20 to 25 min to reach maximum intensity. Frequently the intensity of the luminescence was increased by shaking the vial.

Two organs, the kidneys and gills, contain large amounts of blood, the volume of which can vary depending on the state of contraction of the organ. Weighed samples showed that the luminescent activity of the kidney may be greater than the luminescent activity of the blood (Table 1). Unweighed samples from another specimen showed high luminescent activity in the kidneys when the luminescent activity of the blood was at the limit of detection. In each specimen, luminescent activity of the gill was comparable to or lower than that of the blood.

Blood stored in a syringe in the refrigerator for 36 h still produced light when placed into a vial. Even when the hemocyanin of the blood was deep blue, indicating a high degree of oxygenation, the luciferin reacted only when we put the blood into the vial, where it was exposed to abundant O_2.

Since the digestive gland (via the duct of the digestive gland and diges-

Table 1. *Symplectoteuthis oualaniensis*. Luminescence of tissues. Values are relative intensity as volts per 100 mg dry weight x 10. ND: no data

Specimen no.	Tissue									
	Kidney	Blood	Digestive gland	Digestive duct appendages	Gill filaments	Branchial gland	Nidamental gland	Eggs/ ovary	Stomach contents	Optic lobe
1	30.8	10.4	20.2	2.4	10.8	0.6	0.4	0.2	0.0	0.0
2	60.8	40.2	54.0	4.0	23.6	1.0	0.0	0.0	0.0	0.4
3	17.2	ND	79.0	ND	ND	ND	ND	ND	ND	ND
4[a]	72.9	ND	95.4	20.7	7.4	ND	4.3	9.4	ND	11.2

[a]This squid lay moribund for several hours.

tive tract), kidneys (via the renal sac and renal pores) and gills are in contact with the sea water in the mantle cavity, we considered the possibility that low-level luminescent activity in these organs came from luminescent bacteria. Samples of gill and digestive gland were streaked onto agar media. Considerable bacterial growth resulted from the gill sample, but all the colonies were non-luminescent. No bacteria were cultured from the sample of the digestive gland. In addition, homogenized samples of digestive gland and kidney were passed through a 0.2 µm Gelman membrane filter to exclude bacteria. The

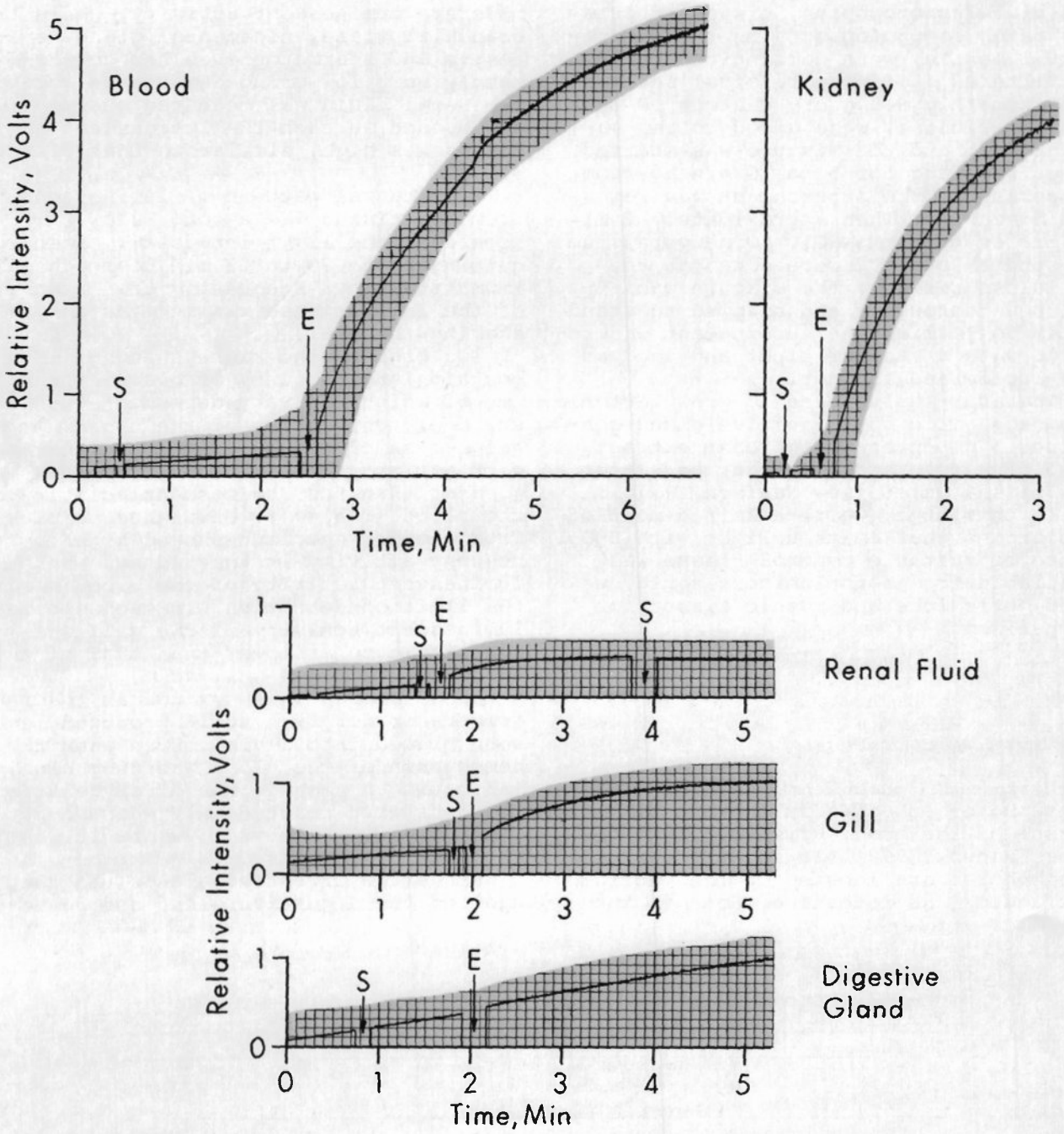

Fig. 1. *Symplectoteuthis oualaniensis*. Luminescence from homogenates of various tissues or body fluids before and after adding squid luciferase. S: Vial shaken but nothing added; E: enzyme added. All tissues were from same individual and were treated with 20 µl luciferase extract from the same batch. Instrument settings were standard, except for kidney, where reduced settings lowered values by a factor of 2.5

filtrate of both samples retained luminescent activity.

To demonstrate that the luminescence we recorded probably came from squid luciferin, squid luciferase was extracted from a dense patch of photophores embedded in the anterodorsal surface of the mantle. Treatment of 0.5 ml of squid blood with 20 µl of luciferase extract produced an immediate response: an abrupt increase occurred in the rate at which the light intensity increased (Fig. 1). In one test, a sample with extract added was producing light over 20 times as intense as the control (sample of blood without the extract) within 2 min of adding the extract. This reaction was demonstrated many times with different samples of squid blood and luciferase extract, usually with the same immediate response. However, the magnitude of the luminescent response varied, depending on the concentration and age of the extract. A similar, immediate, intense response occurred when kidney tissue was treated with the extract (Fig. 1). Treatment of the renal fluid and gill homogenate with the extract resulted in a mild luminescent response, while no response was detected with similar treatment of the digestive gland (Fig. 1). Twenty microliters of extract added to buffer alone produced no detectable luminescence.

Species Survey

The species of cephalopods examined during the survey and the category of their luminescence are presented in Table 2. The relative luminescent activity of tissues and organs of the species surveyed is given in Table 3.

Species with Intrinsic Bioluminescence

High luminescent activity frequently was recorded in the digestive gland, digestive duct appendages and blood of species with intrinsic photophores. High or moderate activity occasionally occurred in the kidney and gill. The muscle tissue, optic lobe and the reproductive and accessory reproductive organs usually exhibited no detectable luminous activity, but very low values sometimes occurred in these organs when luminous activity in the digestive gland was very high.

The highest intensities of luminescence from the digestive gland were recorded in *Pyroteuthis addolux, Pterygioteuthis microlampas, P. giardi,* and *Enoploteuthis* sp. (Table 3). These species are small squids in the family Enoploteuthidae. All other species with intrinsic bioluminescence that we examined, except *Grimalditeuthis bomplandi*, showed luminous activity in the digestive gland, although usually it was low. *G. bomplandi* develops photophores only as an adult, and the specimen we examined was a juvenile.

Considerable variation occurred between individuals in the intensity of the luminescence produced by the most active tissues examined. For example, the most intense response of the digestive gland in *Pyroteuthis addolux* was 1225 times greater than the weakest response. With the exception of *Symplectoteuthis oualaniensis*, the luminous activity of the digestive duct appendages also exhibited great variability, but values were either very high (2 samples) or very low (5 samples). The two squid with high values also exhibited high luminous activity in the digestive glands; both individuals had recently fed and absorption was underway. The stomach (and contents) showed moderate to very high light intensities. The 5 squid with low luminous values for the digestive duct appendages, had completed digestion and the stomach was empty or contained only indigestible debris. The digestive gland in these 5 individuals gave moderate to low luminescence. Most specimens of *S. oualaniensis* examined appeared to have completed digestion and showed consistently low activity in the digestive duct appendages. (One specimen of *S. oualaniensis* held in captivity for 36 h gave moderate luminescence from the digestive duct appendages, but it had been moribund for at least 3 h. This starved and dying squid displayed high luminescent values in all tissues that normally exhibit activity and low values in the tissues that normally exhibit no detectable activity.) In squid where the digestive duct appendages were not examined for luminescence, observations of the state of digestion indicate that, in general, individuals that had fed recently had higher luminescent activity in the digestive gland than in individuals in which digestion had been completed.

Species with Bacterial Luminescence

Two species were examined that have bacterial photophores (*Euprymna scolopes* and *Heteroteuthis hawaiiensis*). Dilly and Herring (1978), however, doubt the bacterial nature of the photophore of a related species, *H. dispar*, but their decision largely rested on the histological examination of inadequately fixed material. The bacterial nature of the photophores

Table 2. Species examined, and luminescence categories classified as: I, intrinsic bioluminescence; II, bacterial bioluminescence; III, not known to be bioluminescent

Species examined	No. of specimens	Category of luminescence	Mantle length (mm)
Order Sepioidea			
Family Sepiolidae			
Euprymna scolopes	5	II	20-22
Heteroteuthis hawaiiensis	1	II	ca. 15
Order Teuthoidea; Suborder Oegopsida			
Family Ommastrephidae			
Symplectoteuthis oualaniensis	14	I	130-220
Family Enoploteuthidae			
Pterygioteuthis micropampas	8	I	11-18
Pterygioteuthis giardi	2	I	15, 21
Pyroteuthis addolux	6	I	18-30
Abraliopsis sp. A	3	I	19-30
Abraliopsis sp. B	4	I	13-20
Enoploteuthis sp.	2	I	16, 17
Family Onychoteuthidae			
Onychoteuthis compacta	4	I	16-30
Family Octopoteuthidae			
Octopoteuthis nielseni	1	I	25
Family Histioteuthidae			
Histioteuthis dofleini	1	I	110
Family Ctenopterygiidae			
Ctenopteryx siculus	1	I	15
Family Mastigoteuthidae			
Mastigoteuthis inermis	2	III	32, 45
Family Grimalditeuthidae			
Grimalditeuthis bomplandi	1	I	70
Family Cranchiidae			
Liocranchia reinhardti	2	I	43, 150
Megalocranchia fisheri	1	I	65
Order Octopoda			
Family Bolitaenidae			
Japatella diaphana	4	III	16-110
Eledonella pygmaea	6	III	15-28

of the two species examined here has been recently confirmed (G. Leisman and K. Nealson, unpublished data).

Because of the possibility of contamination of the samples from luminous bacteria, we examined only a few organs: the digestive gland and arm in *Euprymna scolopes* and the digestive gland and funnel retractor muscle in *Heteroteuthis hawaiiensis*. The luminous activities of the digestive glands and the other tissues were not detectable at the normal settings on the ATP photometer (i.e., activities were less than 0.01 V). However, at a higher sensitivity of the photometer, it was possible to detect very weak luminescence in the digestive gland and the other tissues. The maximum values in *E. scolopes* were comparable to 0.001 V for the digestive gland and 0.0007 V for the arm muscle. The activity of the digestive gland in *H. hawaiiensis* was comparable to 0.004 V at the normal settings, while the funnel retractor muscle was comparable to 0.0002 V.

Species without Photophores or Bacterial Luminescence

Three species that lack photophores and bacterial luminescence were examined. The squid *Mastigoteuthis inermis* has no known photophores, although they occur in many of its congeners. No luminescence was recorded in any of the tissues examined at the normal settings of our instrument. The tissue was not examined at the more sensitive setting.

Two pelagic octopods, *Japatella diaphana* and *Eledonella pygmaea* were examined. In most specimens of both species, there was luminescent activity in the digestive gland homogenates. The range of intensity varied from undetectable to 1.2 V. Other tissues examined had no detectable activity (see Table 3).

Discussion

Several new findings are presented in this report. The first is that various squid tissues emit light after homogena-

Table 3. Relative intensity of luminescence (volts x 10) of cephalopod tissues. (A) Unweighed samples; (B) weighed samples (relative intensity per 33 mg dry weight). Values of weighed samples would be comparable to values of unweighed samples if the latter had an average weight. Numbers in parentheses indicate number of specimens examined; zero indicates intensity was less than 0.01 V. ND: no data

Species (category)	Tissue												
	Arm or funnel rectractor muscle (A)	Optic lobe (A)	Digestive gland (A)	Digestive gland (B)	Digestive duct appendages (A)	Kidney (A)	Gill (A)	Testis (A)	Spermatophores (A)	Ovary (A)	Nidamental gland (A)	Stomach (A)	Blood (A)
Euprymna scolopes (II)	0(1)	ND	0(5)[c]	0(1)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Heteroteuthis hawaiiensis (II)	0(1)	ND	0(1)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Symplectoteuthis oualaniensis (I)	0.1-101[a](3)	0-2.0(2)	0-24.5(10)	6.6-31.9(4)	0-5.8(7)	1.5-25(11)	0-6(8)	ND	ND	0-1.2(4)	0-0.8(6)	0-0.8(4)	0.4-21.4(9)
Pterygioteuthis microlampas (I)	0(3)	0-0.2(2)	0-140(6)	10.1(1)	0-22.7(2)	ND	0-0.2(2)	0(2)	0-0.3(2)	0-2.2(2)	0-2.8(2)	3.5(1)	ND
Pterygioteuthis giardi (I)	0-0.1(3)	0(1)	13-146(4)	93.7-222.1(3)	0(1)	8(1)	1.3(1)	0.3	0.2	0(1)	0(1)	0(1)	ND
Pyroteuthis addolux (I)	0(5)	0.7-1.7(2)	0.2-245(6)	0.3-60.9(3)	0.8-80(2)	0.2-3.5(2)	0.5-10.5(3)	0-0.3(2)	0-0.2(2)	3(1)	ND	0.8-1213(3)	ND
Abraliopsis sp. A (I)	0(2)	0(1)	0.2-5.2(3)	0.2(1)	0(2)	ND	0(2)	0(2)	0(2)	ND	ND	0(1)	ND
Abraliopsis sp. B (I)	0(4)	ND	0-26(4)	0-29.9	ND	ND	ND	ND	0.1(1)	0(1)	0(1)	ND	ND
Enoploteuthis sp. (I)	0(2)	ND	0.9-76(2)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Onychoteuthis compacta (I)	0(4)	ND	0-13(4)	0-16.1(2)	ND	ND	0(1)	ND	ND	ND	ND	ND	ND
Octopoteuthis nielseni (I)	0(1)	ND	0.2(1)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Histioteuthis dofleini (I)	0(1)	ND	1.3(1)	3.0(1)	ND	ND	ND	0(1)	0(1)	ND	ND	ND	ND
Ctenopteryx siculus (I)	0(1)	ND	2.5(1)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Mastigoteuthis inermis (III)	0(2)	ND	0(2)	ND	ND	ND	0(1)	ND	ND	ND	ND	ND	ND
Grimalditeuthis bomplandi (I)	0[b](1)	ND	0(1)	0	ND	ND	ND	ND	ND	ND	ND	ND	ND
Liocranchia reinhardti (I)	0(2)	ND	0-0.4(2)	0-0.3(2)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Megalochranchia fisheri (I)	0(1)	ND	0.3(1)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Japatella diaphana (III)	0(5)	ND	0-0.3(4)	0.1-0.5(3)	ND	ND	0(1)	ND	ND	ND	ND	ND	ND
Eledonella pygmaea (III)	0(5)	0(1)	0-12(6)	1.2(1)	ND	ND	0(2)	ND	ND	ND	ND	0(1)	ND

[a] Photopore probably present.

[b] Neck muscle.

[c] Three measurements made on a different instrument.

tion. Secondly, a substance, presumably squid luciferase, is easily extracted and reacts strongly with kidney homogenates and blood to emit light. To our knowledge, this is the first demonstration of a reaction, *in vitro*, apparently between luciferase and luciferin from squid. Thirdly, light emission from the renal fluid was detected. The fourth new finding is that chemiluminescence was observed in the digestive gland of all bioluminescent cephalopods with intrinsic bioluminescence but not in those with bacterial bioluminescence; there appears to be a relationship between this chemiluminescence and recent feeding. Finally, chemiluminescence was also found in the digestive gland of midwater octopods not known to be bioluminescent.

The simple homogenization technique employed indicates that the chemiluminescence that we observed emanates from a naturally luminescent compound or compounds. Addition of an extract containing squid luciferase to kidney or blood resulted in an immediate, strong increase in luminescent intensity, suggesting that the light emitter in these tissues is a squid luciferin (*sensu* Inoue *et al.*, 1976). The weak reaction of renal fluid and gill tissue and the lack of a reaction of the digestive gland upon addition of the luciferase-containing extract could be interpreted in sev-

eral ways. However, because the different nature of the tissues may have greatly altered the experimental conditions (i.e., the low pH of the renal fluid may inactivate the luciferase, and the digestive gland homogenate has a variety of digestive enzymes which may interfere with the reactions), the significance of the variability of the reactions is unclear.

The chemiluminescence observed could result from a substance (presumably squid luciferin) reacting with molecular oxygen either spontaneously or via interaction with an enzyme (luciferase). We consider the presence of an enzyme unlikely, especially in the blood, although we did not test for this. In either case, some mechanism must exist *in vivo* for preventing the reaction. For example, oxygenated blood could be stored for many hours in syringes, yet when exposed to air in the vials the blood emitted light which reached extinction in less than 1 h.

The occurrence of luminescent activity in the kidneys and renal fluid is intriguing. The kidneys are appendages of large veins covered by a transporting epithelium (Schipp and von Boletzky, 1975; Schipp *et al.*, 1975). The kidneys therefore contain large amounts of blood, but because the luminescent activity of kidneys can be much higher than that of the blood, it cannot be solely a result of the presence of blood. The high luminescent activity in both the kidneys and renal fluid indicates that some of the luminescent substrate is excreted. We present one possible explanation for this apparently anomalous function: the ejection of excreted substrate may produce a luminous cloud that could distract a potential predator. Both Cousteau (1954) and Church (1970) during dives in submersibles have observed squid to produce luminous clouds. Cousteau's brief description indicates a species similar to *Symplectoteuthis oualaniensis*, while Church thought the squid he observed was *Histioteuthis heteropsis*. On one occasion we have observed a captive squid, *Abralia trigonura*, to produce a very faint luminous cloud. These three fortuitous observations on distantly related species suggest that most oceanic squids may be able to produce luminous clouds similar to those produced by some midwater shrimps and fishes; but the source of the clouds in squid is unknown. [The cephalopods with luminescent clouds noted by Harvey (1952) have bacterial photophores.] Organs that might be responsible for luminous clouds, such as the ink sac or the funnel organ, produced no luminescence when treated with hydrogen peroxide during our preliminary studies. Based on the luminescent activity measured in the kidney and renal fluid, we now suggest that renal fluid, combined with substances (especially luciferase) from another source, perhaps the funnel organ, produces luminescent clouds. We plan to test this hypothesis in the near future.

The 14 species of cephalopods with known intrinsic luminescence all demonstrated luminescent activity in some of the tissues examined. High variability in the intensity of luminescence, however, occurred in a given organ from different specimens. Such variability was documented best in the digestive gland and the digestive duct appendages, in which the high luminescent activity appeared to occur in specimens that had fed just prior to capture. Inoue *et al.* (1976) isolated a precursor of luciferin ("preluciferin") from the digestive gland of the enoploteuthid squid *Watasenia scintillans*. The preluciferin from this squid was shown to be identical to the luciferin of some myctophid fish, an oplophorid shrimp, and possibly cnidarians (Inoue *et al.*, 1976, 1977). The strong possibility exists, therefore, that these cephalopods are obtaining preluciferin from their diet.

Several species were examined that lack both photophores and bacterial luminescence. *Mastigoteuthis inermis* is not known to have photophores, and we detected no luminous activity in any tissues or organs. However, only two specimens were examined and neither had fed just prior to capture. No species of the Octopoda is known to be bioluminescent. A moderate chemiluminescent activity, however, was found in the homogenized digestive gland of several specimens of the two species of midwater octopods we examined.

The chemiluminescent substance in the digestive gland may be preluciferin and not luciferin. In this case, chemiluminescence from the digestive glands could be a dietary by-product with no ultimate bioluminescent functions in these octopods or in the squids. On the other hand, if we are observing in the digestive gland luciferin that has been synthesized from preluciferin (regardless of dietary origin or not), as suggested by Inoue *et al.* (1976), our findings would indicate that the octopods have bioluminescent capabilities and that examination of the digestive gland will provide a simple assay for determining whether or not a given cephalopod species is bioluminescent.

The simple technique we used to analyze luminescent activity may provide

information on various aspects of the biology and physiology of these bioluminescent animals. For example, the high luminescent activity of the blood may indicate rapid resupply of luciferin to photophores, and therefore may be a measure of recent bioluminescent activity. Digestive-gland luminescent activity may provide a valuable tool for examining temporal feeding patterns. The presumed luciferin, a "luminescence-labeled" molecule, might provide information about secretion, excretion and transport mechanisms. In addition, the extraction method which seems to yield luciferase may allow complete reconstitution of the light-emitting reaction *in vitro* and become a valuable tool in studying this bioluminescent system.

Acknowledgements. We gratefully acknowledge the cooperation, support and assistance of the officers and crew of the R.V. "Kana Keoki" and the students of the University of Hawaii Department of Oceanography and Marine Option Program. S. Maynard coordinated all aspects of the preparation for the cruises, as well as conducting the sampling operations at sea. K. Nealson, Scripps Institution of Oceanography, loaned the ATP photometer and other laboratory equipment, and P. O'Brien weighed the samples. We appreciate review of the manuscript by D. Karl, K. Nealson, A. Bidder and S. Maynard and the comments of S. von Boletzky. This material is based on research supported by the National Science Foundation under Grant No. OCE76-81030 (R.E.Y.), the Smithsonian Institution (Fluid Research Fund) and National Museum of Natural History (C.F.E.R.). Hawaii Institute of Geophysics Contribution No. 957.

Literature Cited

Cousteau, J.: To the depths of the sea by bathyscaphe. Natn. geogr. Mag. *106*, 67-79 (1954)

Church, R.: Bioluminescence: the sea's living light. Oceans *3*(2), 20-29 (1970)

Dilly, P.N. and P.J. Herring: The light organ and ink sac of *Heteroteuthis dispar* (Mollusca: Cephalopoda). J. Zool., Lond. *18*, 47-59 (1978)

Girsch, W., P. Herring and F. McCapra: Structure and preliminary biochemical characterization of the bioluminescent system of *Ommastrephes pteropus* (Streenstrup) (Mollusca: Cephalopoda). J. mar. biol. Ass. U.K. *56*, 707-722 (1976)

Goto, T., H. Ilio, S. Inoue and H. Kakoi: Squid bioluminescence I. Structure of *Watasenia* oxyluciferin, a possible light-emitter in the bioluminescence of *Watasenia scintillans*. Tetrahedron Lett. *26*, 2321-2324 (1974)

Harvey, E.N.: Bioluminescence, 649 pp. New York and London: Academic Press 1952

Hastings, J. and G. Weber: Total quantum flux of isotropic sources. J. opt. Soc. Am. *53*, 1410-1415 (1963)

Herring, P.J.: Bioluminescence in decapod Crustacea. J. mar. biol. Ass. U.K. *56*, 1029-1046 (1976)

- Luminescence in cephalopods and fish. Symp. zool. Soc. Lond. *38*, 127-159 (1977)

Inoue, S., H. Kakoi and T. Goto: Squid bioluminescence III. Isolation and structure of *Watasenia* luciferin. Tetrahedron Lett. *34*, 2971-2974 (1976)

-, S. Sugiura, H. Kakoi, K. Hasizume, T. Goto and H. Ito: Squid bioluminescence II. Isolation from *Watasenia scintillans* and synthesis of 2-(p-hydroxybenzyl)-6-(p-hydroxyphenyl)-3, 7-dihydroimidaze [1,2-a] pyrazin-3-one. Chemy Lett. (Chem. Soc. Japan, Tokyo) *1975*(2), 141-144 (1975)

-, H. Taguchi, M. Murata, K. Kakoi and T. Goto: Squid bioluminescence IV. Isolation and structural elucidation of *Watasenia* dehydropreluciferin. Chemy Lett. (Chem. Soc. Japan, Tokyo) *1977*(3), 259-262 (1977)

Nealson, K.: Isolation, identification and manipulation of luminous bacteria. Meth. Enzym. *57*, 153-166 (1978)

Roper, C.F.E.: Observations on bioluminescence in *Ommastrephes pteropus* (Streenstrup, 1855), with notes on its occurrence in the family Ommastrephidae (Mollusca: Cephalopoda). Bull. mar. Sci. Gulf Caribb. *13*, 343-353 (1963)

Schipp, R. and S. von Boletzky: Morphology and function of the excretory organs in dibranchiate cephalopods. Fortschr. Zool. *23*, 89-111 (1975)

- - and G. Doel: Ultrastructural and cytochemical investigations on the renal appendages and their concrements in the dibranchiate cephalopods (Mollusca, Cephalopoda). Z. Morph. Tiere *81*, 279-304 (1975)

Shimomura, O., F.H. Johnson and Y. Saiga: Purification and properties of *Cypridina* luciferase. J. cell. comp. Physiol. *58*, 113-123 (1961)

Stauff, J., U. Sander and W. Jaeschke: Chemiluminescence of perhydroxyl-and-carbonate-radicals. *In*: Chemiluminescence and bioluminescence, pp 131-141. Ed. by M. Cormier, D. Hercules and J. Lee. New York: Plenum Press 1973

Young, R.E. and C.F.E. Roper: Intensity regulation of bioluminescence during countershading in living midwater animals. Fish. Bull. U.S. *75*, 239-252 (1977)

Dr. Richard E. Young
Department of Oceanography
University of Hawaii at Manoa
2525 Correa Road
Honolulu, Hawaii 96822
USA

Date of final manuscript acceptance: April 5, 1979. Communicated by N.D. Holland, La Jolla