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DIETARY MAINTENANCE OF BIOLUMINESCENCE IN A DEEP-SEA MYSID

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Dietary acquisition of the specific constituents of bioluminescent systems, frequently suggested for various midwater organisms (Haneda, Johnson & Shimomura, 1966; Shimomura, Inoue, Johnson & Haneda, 1980; McCapra & Hart, 1980), has been demonstrated experimentally only in the batrachoidid fish, *Porichthys notatus*. The luciferins and luciferases of *Porichthys* and the ostracod *Vargula* (= *Cypridina*) *hilgendorffii* cross-react, suggesting structural similarity (Cormier, Crane & Nakano, 1967; Tsuji, Haneda, Lynch & Sugiyama, 1971). Normally, non-luminescent *Porichthys* from Puget Sound luminesce after injection of *V. hilgendorffii* luciferin or feeding with dried *V. hilgendorffii* (Tsuji, Barnes & Case, 1972; Barnes, Case & Tsuji, 1973) or *V. tsujii* (Warner & Case, 1980). Specific dietary maintenance of bioluminescence has also been suggested for bioluminescent crustaceans. Shimomura *et al.* (1980) found large amounts of luciferin in the digestive tracts of various shrimps, and suggested that their luminescence had dietary origins.

A major difficulty in studying dietary bioluminescence induction in marine organisms is finding experimental subjects that survive under strict dietary control for extended periods. The robust mysid shrimp, *Gnathophausia ingens* (Dohrn), has proved to be ideal for such studies. Relatively abundant off the coast of Southern California, it survives in the laboratory for up to 2.5 years (Childress & Price, 1978). Mechanically stimulated *G. ingens* secrete bluish luminescent material (Fig. 1) from a gland opening on each second maxilla at the base of the exognaths (Illig, 1905). Laboratory maintained specimens usually became non-luminescent after 2 months, but some retained the ability to luminesce for up to 8 months. We have now demonstrated that *G. ingens* which have lost luminescence capacity on a diet restricted to tissues from non-bioluminescent animals rapidly regain this capacity after ingesting certain luminescent prey.

Specimens of *G. ingens*, trawled at depths of 600–800 m in San Clemente Basin (off the coast of southern California), during February and August, 1982, were maintained separately in one-quart containers of sea water at 5°C (Childress & Price, 1978). All were fed mackerel or salmon and tested for bioluminescence once a week. Most were no longer luminescent 4 months after capture, and all the animals used in dietary induction tests had not luminesced in response to mechanical stimulation for at least 1 month before experiments began.

Non-luminescent *G. ingens* were fed seven types of luminescent food items, namely: (1) euphausiid shrimps with photophores (live *Euphausia pacifica* and *Nematoscelis difficilis*); (2) a gonostomatid fish with photophores (live *Cyclothone acclinidens*); (3) a myctophid fish with photophores and caudal luminescent organs (pieces of freshly killed *Triphoturus mexicanus*); (4) a batrachoidid fish with photophores (pieces of freshly killed *Porichthys notatus*); (5) a luminescent coelenterate (frozen *Atolla* sp.); (6) a copepod possessing glands which release luminescent fluid (frozen *Gaussia princeps*); and (7) a shrimp possessing internal hepatic light organs (live *Sergestes similis*). They were fed every 2–3 days and tested for bioluminescence the day after each feeding, with the following results: 36 of 41 experimental animals regained luminescence capability after ingesting *Triphoturus*, *Cyclothone*, *Atolla*, *Sergestes* or *Gaussia* (Table 1). Three animals in these five groups never regained their luminescence, even though two of them received two different luminescent food items. Subsequent dissection showed that the luminescent organs were intact. Twenty-six specimens were fed euphausiid shrimp or *Porichthys*, and all remained non-luminescent. Eleven of these non-luminescent individuals were subsequently fed *Triphoturus*, and became luminescent within 2 days, demonstrating that they were potentially able to manufacture a luminescent secretion, but not from the euphausiid or *Porichthys* food items.

At least three types of marine bioluminescent systems are known: coelenterate, dinoflagellate and *Cypridina*. They differ from one another in the chemical structure of their luciferins and luciferases, and by weak or absent *in vitro* cross-reactions between the components of the different systems. Prey representing these three systems were utilized in this investigation. While the luminescent system of the myctophid *Triphoturus* has not been analysed, Inoue, Okada, Kakoi & Goto (1977) and Inoue, Kakoi, Okada & Goto (1979) present evidence for coelenterate-type luciferin in one other myctophid, and Shimomura *et al.* (1980) confirm its presence in two myctophid species. Although some degree of cross-reactivity with *Cypridina* luciferin may occur with impure extracts (Tsuji & Haneda, 1971), it is likely that the bioluminescent system of myctophids utilizes coelenterate-type luciferin (Cormier,

Table 1. *Bioluminescent responses of Gnathophausia ingens to selected food items*

Food items	Responses to food items	Minimum-maximum time to onset of luminescence (days)	Responses to <i>Triphoturus mexicanus</i> †
<i>Euphausia pacifica</i> and <i>Nematoscelis difficilis</i>	0/14*	—	7/7
<i>Cyclothone acclinidens</i>	2/2	3–4	
<i>Triphoturus mexicanus</i>	11/12	1–4	
<i>Porichthys notatus</i>	0/12	—	4/4
<i>Atolla</i> sp.	5/6	2–14	1/1
<i>Sergestes similis</i>	11/12	1–8	0/1
<i>Gaussia princeps</i>	7/9	1–13	1/2

* Number responding/number tested.

† Only animals that had not given bioluminescent responses to food items were fed *Triphoturus mexicanus*.

1978). *Sergestes similis* probably possesses coelenterate-type luciferin since it is present in two other *Sergestes* species (Shimomura *et al.* 1980). The luminescent system of the copepod, *Gaussia princeps*, has not been characterized, but the luciferin of *Pleuromamma pisecki*, a copepod belonging to the same family, has been categorized as coelenterate-type (McCapra & Hart, 1980), supporting the possibility of coelenterate-type luciferin in *Gaussia*. Euphausiids apparently possess a different luminescent system. Even though aqueous extracts of the luminescent system of the euphausiid, *Euphausia similis*, give luminescent cross-reactions with crude *Cypridina* luciferase (Tsuji *et al.* 1971), other studies report that euphausiids have a photoprotein system unlike the luciferin/luciferase system of *Cypridina* (Shimomura & Johnson, 1967, 1968), and more recently, euphausiid fluorescent substance has been shown to be biochemically similar to dinoflagellate luciferin (Dunlap, Hastings & Shimomura, 1980). The luminescent system of *Porichthys notatus* is of the *Cypridina* type (Cormier *et al.* 1967; Tsuji *et al.* 1971, 1975). The luminescent system of *Gnathophausia longispina* is characterized as coelenterate-type (Shimomura *et al.* 1980) and the luciferin of *G. ingens* is related to the coelenterate-type (Cormier, 1978). It is apparent from the present study that *G. ingens* is able to utilize the components of coelenterate-type luminescent systems in myctophids and sergestids, but not the dinoflagellate-type system found in euphausiids, or the *Cypridina*-type system of *Porichthys*. Although the luminescent systems of *Cyclothone* and *Atolla* have not been analysed, this study strongly suggests that they possess coelenterate-type luminescence, since *Gnathophausia* can utilize their systems. Since the luminescent system of *Gnathophausia* appears specific for coelenterate-type luciferin/luciferase, it may be useful as a bioassay for animals possessing coelenterate-type luminescence. Studies are in progress to characterize more fully the specificity of the *Gnathophausia* luminescent system.

Luminescent emission spectra were determined for *Triphoturus*, freshly trawled *G. ingens*, and *G. ingens* with luminescent capability restored after ingesting *Triphoturus*. Spectra taken with an optical multichannel analyser (Princeton Applied Research OMA II) were standardized and digitally smoothed (Widder, Latz & Case, 1983). The *Triphoturus* spectrum (caudal organ) differs substantially from that of *G. ingens*, with the spectral peak shifted 12 nm towards the shorter wavelengths, and with a markedly more narrow full width at half maximum intensity (FWHM), 62 nm compared to 83 nm for *Gnathophausia* (Fig. 2A). Natural and *Triphoturus*-induced *G. ingens* spectra have identical λ_{\max} at 481 nm and the same FWHM (Fig. 2B).

The differences in luminescence spectra between *G. ingens* and *Triphoturus* argue that some aspect of the chemistry of the two luminescent mechanisms may differ. A similar situation exists in the *Porichthys-Cypridina in vivo* interaction in which it is known that the enzyme originates with *Porichthys* and the *Porichthys* emission is bimodal (485 and 507 nm) while *Cypridina* luciferin and luciferase emit light with a single peak at 459 nm (Reynolds, Botos & Barba, 1972; Tsuji *et al.* 1975). The variation in emission spectra may be caused by other factors, however, such as the reaction environment, which is intracellular in *Triphoturus* and extracellular in *G. ingens*.

Since some coelenterate-type bioluminescence is calcium dependent (Cormier, 1978; Shimomura, Johnson & Saiga, 1962), calcium might be expected to play a role

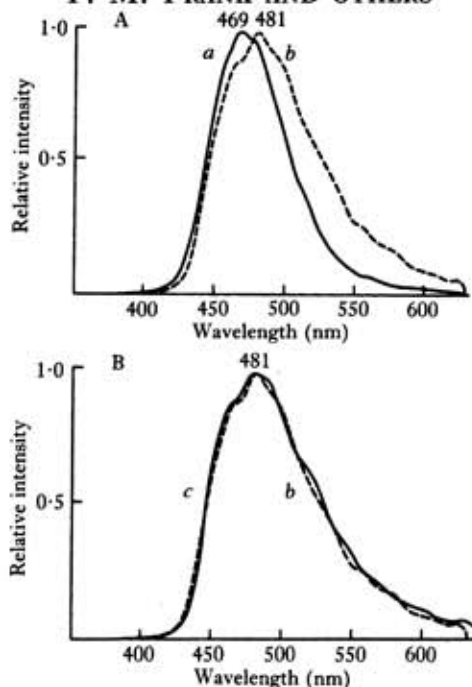


Fig. 2. (A) Emission spectra of caudal organ of *Triphoturus mexicanus* (a) and luminescent secretion of *Gnathophausia ingens* (b, dashed line). (B) Emission spectra of luminescent secretions of freshly trawled *G. ingens* (c) and *G. ingens* with luminescence restored after ingesting *T. mexicanus* (b, dashed line).

in the luminescence of *Gnathophausia*. However, preliminary experiments indicate that the *Gnathophausia* luminescent system is not dependent on external calcium, since luminescence appears normal when animals are maintained for 12 h in calcium-free, artificial sea water containing 10 mg l^{-1} EGTA and caused to secrete into the same or into distilled water.

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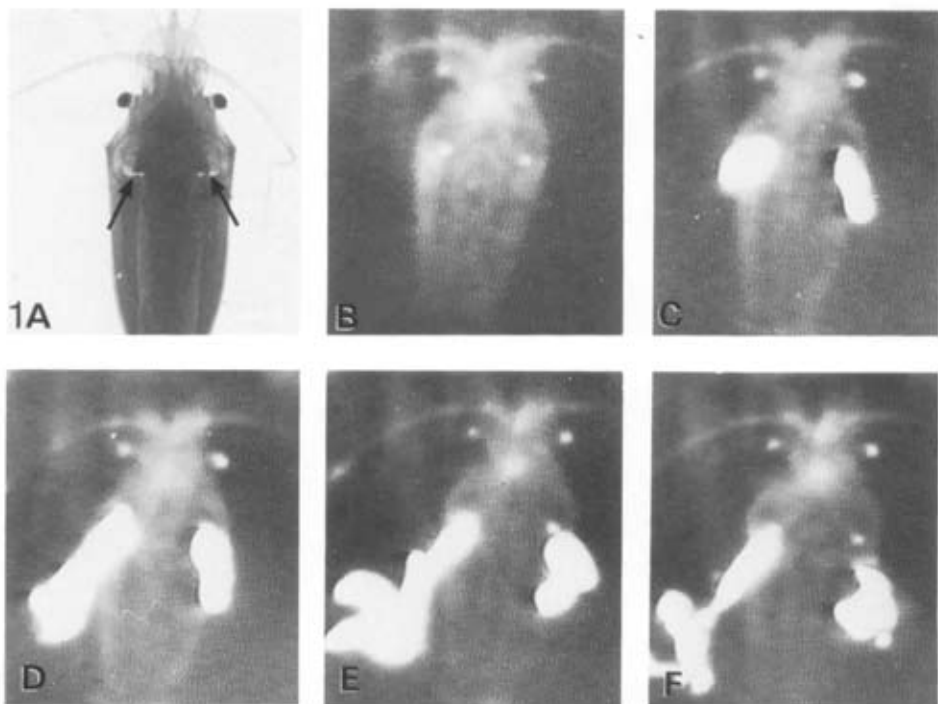


Fig. 1. Ventral aspect of *Gnathopausia ingens* showing (A) the position the gland openings on the 2nd maxillae (arrows) and (B-F) the sequence of *G. ingens* secreting luminescent fluid. B-F are image intensified-video photographs; time between frames, 0.5 s; photographs are actual size.