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Symbioses in *Amphipholis squamata* (Echinodermata, Ophiuroidea, Amphiuridae): geographical variation of infestation and effect of symbionts on the host's light production

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Abstract

Populations of the polychromatic and bioluminescent species *Amphipholis squamata* from eight locations were examined for internal and external symbionts. At three locations (two in the United Kingdom and one in Papua New Guinea), no symbionts were present, while four species were recovered from the remaining locations: *Cancerilla tubulata* and *Parachordeumium amphiurae* (copepods), *Rhopalura ophiocomae* (orthonectid) and an undescribed species of rhabdocoel turbellarian. No ophiuroid individual hosted more than one symbiont species, despite the presence of two or more within a population. Symbiont presence and prevalence varied with location, and with colour variety, but with no apparent pattern or trends. Light-production characteristics of the host were affected by the presence of all symbionts except *C. tubulata*. These effects, however, did not vary between colour varieties or between geographical locations, but were specific to the symbiont species: the presence of *P. amphiurae* resulted in enhanced intensity of light production, while that of *R. ophiocomae* and the turbellarian species resulted in reduced intensity. The kinetics of light production (time until maximum output) were altered only by the presence of the turbellarian. Changes in the light-production characteristics are discussed in relation to morphological, energetical and physiological effects of the symbioses.

Keywords: *Amphipholis squamata*; Echinoderm symbioses; Geographical distribution; Host luminescence; Ophiuroid symbionts; Physiological reaction

1. Introduction

Echinoderms act as hosts to a wide variety of symbionts, separately or simultaneously, regardless of the nature of the host–symbiont relationship [1, 2]. One echinoderm species may host a parasite, a commensal, or both, and most symbionts do not appear to be mutually exclusive [1, 3]. Echinoderm symbionts vary in degree of host specificity from monoxenous symbionts, such as copepods, to polyxenous symbionts, such as ciliates. Multiple hosts, however, are always of the same echinoderm class [2].

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The cosmopolitan ophiuroid *Amphipholis squamata* has a wide range of tolerance towards symbionts, with as many as 15 different organisms reported in association with individuals. These consist of copepods (seven species), ciliates (four species), polychaetes (two species), orthonectids (one species) and a turbellarian (one species, first mentioned here). The associations have been reported from individuals of various seas and oceans in accordance with the wide distribution of this ophiuroid [1, 4]. Symbiont life-cycles and the fine morphological interface of the host–symbiont relationship have been investigated for several species [2, 5]. Impacts of infestation on the ophiuroid population structure have also been examined, notably by investigating the influence of symbiont infestations on the ophiuroid life-cycle, and its brooding and regenerative abilities [6–10]. Effects on the ophiuroid physiology or metabolism, however, have been little studied as indicated by the fact that only one symbiont species has been assessed for its effect on the energetical activity of the ophiuroid [7].

Amphipholis squamata is a polychromatic species with various colour varieties present in distinct numbers and proportions at different locations [11, 12]. Individuals have long been reported to produce light [13, 14], with light-production characteristics varying between colour varieties, and between brooding and non-brooding individuals [12]. Other biological features, such as concentration of neuropeptides, types of neuroreceptors on photocytes, life-cycle, reproductive pattern and genotype, are known to differ between individuals of different varieties [15], and also between individuals from widely distant locations ([16]; D.R. Murray, Macrogeographic genetic variation in the cosmopolitan brooding brittle star, *Amphipholis squamata*. Master thesis, Mount Allison University, 1989). We aimed to determine whether symbioses in *A. squamata* varied with host colour variety or geographic location of the population, and what effect the different symbionts had on the host light-production characteristics.

2. Materials and methods

Individuals of *A. squamata* (Delle Chiaje, 1828) were collected between March 1993 and June 1996 from eight different locations in various geographical regions (Fig. 1), i.e. in France (Langrune-sur-Mer and Roscoff), the U.K. (Wembury, Prawle Point, Millport and New Haven), New Zealand (Portobello) and Papua New Guinea (Laing Island). All locations were intertidal (from mid- to upper-intertidal, except for Prawle Point where specimens were collected from tidal pools), and individuals were collected at low tide by hand using fine forceps (the ophiuroid is small, adult specimens having a disc diameter of approx. 3 mm with arms approx. 15 mm long). The habitats consisted of muddy or detritic sand under boulders or mussels beds, except for Prawle Point were the ophiuroid was found amongst algae *Corallina officinalis*. All individuals found at each search were collected, regardless of microhabitat, size or colour variety.

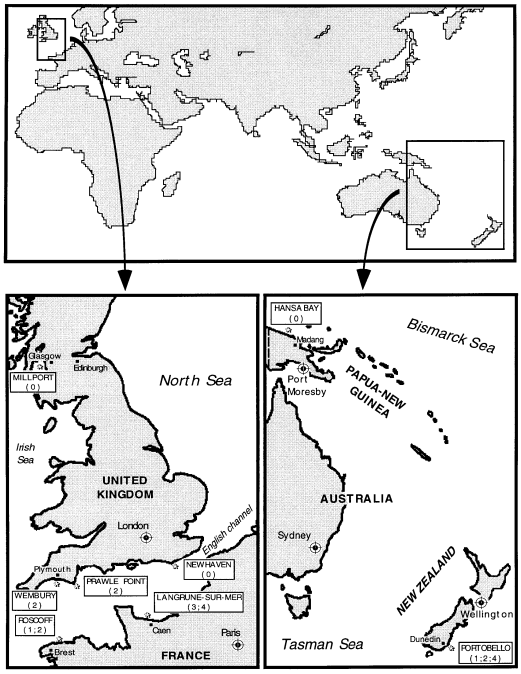
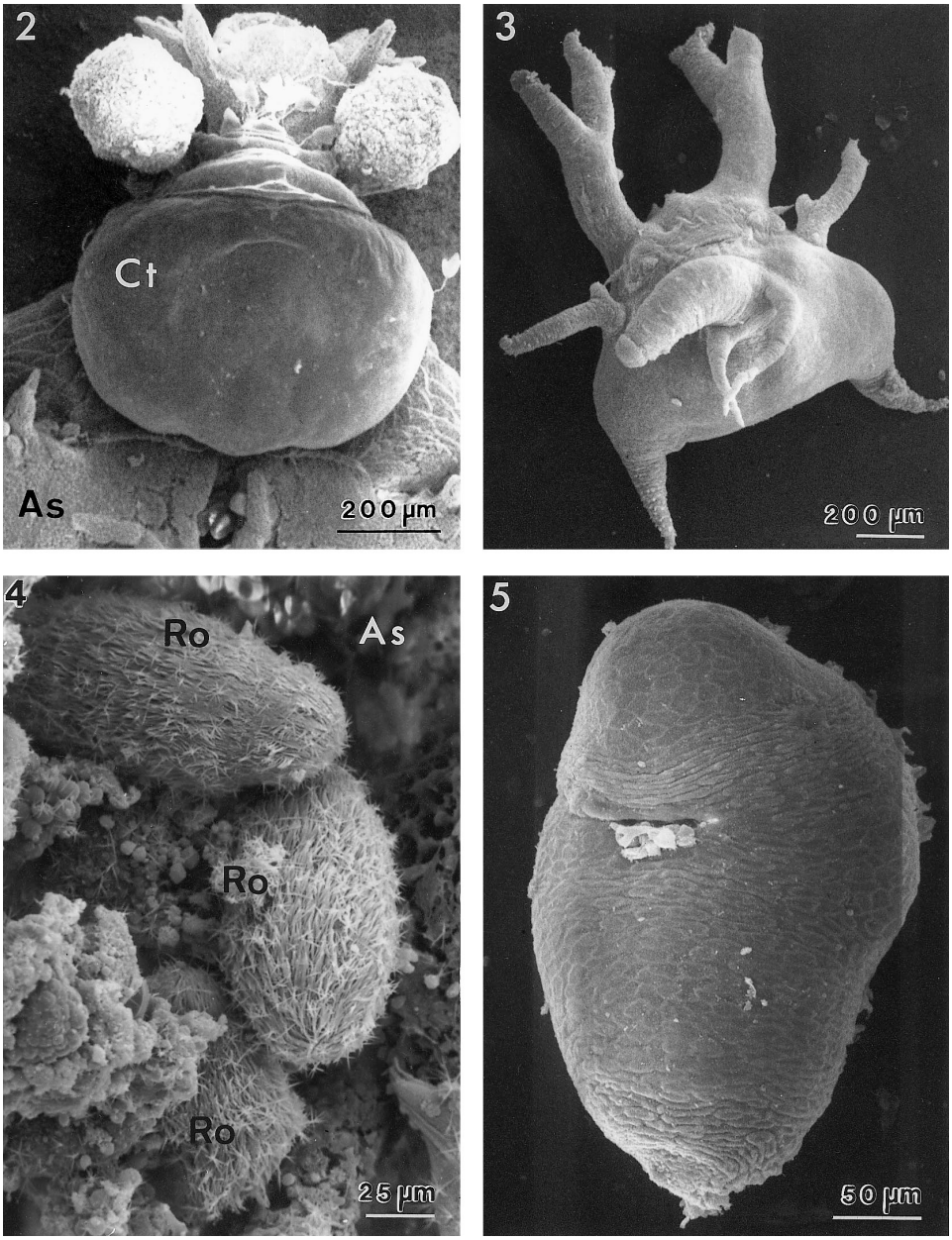


Fig. 1. *Amphipholis squamata*. The eight geographical locations that were investigated, and occurrence of the four symbionts considered: (1) *Cancerilla tubulata* (copepod); (2) *Parachordeumium amphiurae* (copepod); (3) *Rhopalura ophiocomae* (orthonectid); (4) the new species of rhabdocoel turbellarian; (0) Absence of symbionts.

2.1. Measurement of infestation

Collected individuals were transported to the laboratory in seawater, sorted according to colour variety, and examined within 12 h for the presence of the following four symbionts: (1) the copepod *Cancerilla tubulata* Dalyell, 1851 (Fig. 2), which is an ectosymbiont that clings to the oral base of the



Figs 2–5. Scanning electron micrographs of the four symbionts from *Amphipholis squamata* (As). Fig. 2. *Cancerilla tubulata* (Ct) attached to the host. Fig. 3. *Parachordeumium amphiurae* (isolated specimen). Fig. 4. *Rhopalura ophiocomae* (Ro) in the host. Fig. 5. The new rhabdocoel turbellarian species (isolated specimen).

ophiuroid arm [17]; (2) the copepod *Parachordeumium amphiurae* Hérouard, 1906 (Fig. 3), which lives in the bursa of infested individuals that are then recognisable by an external swelling and a pore that the symbiont induces on the ophiuroid disc, and also by an orange tint of the symbiont visible through the ophiuroid skin [6]; (3) the orthonectid *Rhopalura ophiocomae* Giard, 1877 (Fig. 4), which infests the coelom and the coelom wall [5]; and (4) a new species of rhabdocoel turbellarian (in course of description) that occurs in the ophiuroid haemal system (Fig. 5). There are no external signs of the presence of the orthonectid and the turbellarian ([5]; Deheyn, personal observation; respectively). The disc of every ophiuroid was therefore dissected to check for their presence, which allowed simultaneous detection, under the compound microscope, of gonads and brooded juveniles.

2.2. Morphological observation

Infested ophiuroids were investigated by light microscopy to precisely localize the symbionts and to determine ophiuroid gonad development. A qualitative assessment was made of gonad size relative to "normal" in uninfested adult individuals. Some degree of reduction was noted as partial castration, while complete absence of testis or ovary constituted total castration. Isolated symbionts, as well as individuals in association with the ophiuroid, were fixed in 3% glutaraldehyde in cacodylate buffer (0.1 M, pH 7.8, adjusted to 1030 mOsM with NaCl) at 4°C for 12 h, dehydrated in graded ethanol, critical-point dried (using CO_2 as transition fluid), sputter coated with gold and observed with a JEOL JSM 6100 scanning electron microscope. Specimens for histology were fixed in 3% glutaraldehyde in cacodylate buffer, rinsed in buffer, post-fixed for 30 min in 1% osmium tetroxide in the same buffer, and rinsed again. They were then decalcified in the dark for 12–15 h at 4°C in 2% ascorbic acid in 0.3 M-NaCl [18], dehydrated in graded ethanol, and embedded in spurr resin. Semi-thin sections (0.3 μm) were stained with a 1:1 methylene blue–azur II mixture [19] and examined using a Leitz laborlux D light microscope.

2.3. Measurement of bioluminescence

Only arms produce light in the ophiuroid; therefore light production was measured from isolated arms of infested individuals. (When the symbiont *C. tubulata* occurred it was removed prior to light measurement.) Light production was also measured from 12 to 15 non-infested individuals of each variety, from each collection. When locations were sampled several times, results from the different collections were pooled. Light production was always measured within 12 h of collection, from adult individuals with intact arms (i.e. non-regenerating) with disc diameter 2–3 mm. Individuals were anaesthetised by immersion for 3 min in a 3.5% $MgCl_2$ solution and the length of arms measured before separation from the disc using a fine scalpel. The disc was dissected for symbionts, and the presence of brooded juveniles and developed gonads determined, while each isolated arm was measured for bioluminescence. The luminous reaction was triggered using KCl 200 mM so that light production was maximal and unimodal, and occurred within 180 s following KCl addition [12, 20]. Light was detected in a dark room at each research station, using a portable system consisting of a phototube IP21-S20 connected to a photomultiplier IL 760, the signal then passing through a radiometer IL 1700 before being graphically analysed using a Servogor S recorder [12]. The experimental device was calibrated once a week, using a tritium-phosphor light source of known intensity within the spectrum of the ophiuroid luminescence [12]. Two parameters were used to characterise the production, (1) the maximum light intensity LMax, expressed in megaquanta produced per s per mm of arm ($Mq\,s^{-1}\,mm^{-1}$), which reflects energy associated with the maximal flux of produced photons normalised per arm length, and (2) the time TLMax to reach the maximum light intensity, expressed in s, which characterises kinetic efficiency of the biological pathway leading to the maximal light production. When there were sufficient numbers of infested individuals to enable statistical analyses regarding their variety and location, differences in luminescence intensity and kinetics between infested vs non-infested individuals were tested for significance using nested analysis of variance

(Nested ANOVA, arms being nested within individual) and multiple mean comparison test (Tukey) ($P < 0.05$ for all analyses). Brooding influences the ophiuroid light-production performance [12], so results from brooding and non-brooding individuals were analysed separately.

3. Results

3.1. Geographical variation in infestation

Table 1 summarises all data regarding *A. squamata* infestations for the colour varieties in the various locations. No symbionts were found in/on individuals of *A. squamata* from three of the eight locations investigated, i.e. at New Haven (U.K.), Millport (U.K.) and Laing Island (Papua New Guinea). At the remaining five locations, infestation among locations varied qualitatively (symbiont species differed) and quantitatively (the same symbiont infested different numbers of individuals). Total infestation with all symbionts was highest at Wembury (U.K.) (15.1%), and lowest at Roscoff (France) (8.6%). Of the four symbionts found, three occurred at more than one location, while the orthonectid *R. ophiocomae* was found only at Langrune-sur-Mer (France). When the same symbiont occurred at several locations, its prevalence varied with location, e.g., *P. amphiurae* infestation ranged from 0.7% at Portobello to 15.1% at Wembury.

At two locations, only one symbiont species was present, namely *P. amphiurae*, at Wembury and Prawle Point. At the other three locations, two or three symbiont species were present in the population but, remarkably, the different species were never found together in a single host individual. The total intensity of infestation at a location was unrelated to the number of symbiont species occurring at that location. For example, the highest total infestation (15.1% at Wembury) was due to a single symbiont species, while a lower infestation (8.6% at Roscoff) was composed of two species.

3.2. Variation in infestation with colour variety

Specimens of *A. squamata* from the eight locations investigated were categorised into a total of 11 different colour varieties (orange, beige, dark brown, grey, black, spotted, black-brown, ochre, speckled, brown-grey and pale beige as listed in order of increasing light-production performance). The number of colour varieties present per location ranged from one to six (Table 1). It was observed from locations sharing varieties (e.g., Langrune-sur-Mer and Roscoff, or Prawle Point and Portobello) that the symbionts did not preferentially infest one or another variety. The most heavily infested varieties at the different locations were the grey one at Langrune-sur-Mer (14.9%), the black one at Roscoff (16.5%), the beige one at Prawle Point (14.5%), and the beige one at Portobello (11.3%). Some varieties were infested at one location but not at another (compare the black variety at Langrune-sur-Mer and Roscoff), and individuals from the orange variety were never found to be infested while those of the dark brown and grey varieties were infested at all locations. Considering each location separately, distribution of the symbionts was not homogenous among the colour varieties, as some were heavily infested and some lightly. This was especially conspicuous for the grey and the black varieties at Langrune-sur-Mer (14.9 and 0% of infestation, respectively) and for the black and the beige ones at Roscoff (22.8 and 5.1%, respectively).

3.3. Effect of infestation on light-production characteristics

Amphipholis squamata has the ability to produce light and the photocytes (i.e. the light-producing cells) occur in each arm segment at the periphery of the nerve ganglion that lies under each spine (there are six spines and thus six spinal ganglia per arm segment [21]). All nerve ganglia are interconnected through the main radial nerve cord [21, 22]. Since the latter lies between two perihaemal sinuses and the spinal ganglia are close to coelomic spaces, it is believed that both neuronal and endocrinal factors may affect the light production of photocytes, and thus there may be two avenues of influence resulting from the presence of a symbiont.

The light production was affected by three of the four symbioses investigated. The effect of each particular symbiont on host bioluminescence was

Table 1
For the eight locations investigated, number and percentage of *Amphipholis squamata* individuals that were non-infested and infested by the four symbionts considered[a]

Station	*A. squamata* variety	Non-infested individuals		Infested individuals: *C. tubulata*[b] (crustacean)		*P. amphiurae* (crustacean)		*R. ophiocomae* (orthonectid)		n.sp (turbellarian)		Total
Laing Island (Papua New Guinea)	Pale beige	387	(100%)	—		—		—		—		387
New Haven (U.K.)	Beige	620	(100%)	—		—		—		—		620
	Black-brown	431	(100%)	—		—		—		—		431
	Total	**1051**	(100%)	—		—		—		—		**1051**
Millport (U.K.)	Beige	627	(100%)	—		—		—		—		627
	Black-brown	435	(100%)	—		—		—		—		435
	Ochre	192	(100%)	—		—		—		—		192
	Speckled	26	(100%)	—		—		—		—		26
	Total	**1280**	(100%)	—		—		—		—		**1280**
Prawle Point (U.K.)	Beige	497	(85.5%)	—		84	(14.5%)	—		—		581
	Black-brown	227	(91.5%)	—		21	(8.5%)	—		—		248
	Ochre	38	(92.7%)	—		3	(7.3%)	—		—		41
	Speckled	87	(100%)	—		—		—		—		87
	Total	**849**	(88.7%)	—		**108**	(11.3%)	—		—		**957**
Wembury (U.K.)	Orange	6	(100%)	—		—		—		—		6
	Beige	52	(81.5%)	12	(18.8%)	—		—		—		64
	Dark brown	76	(89.4%)	9	(10.6%)	—		—		—		85
	Grey	87	(86.1%)	14	(1.9%)	—		—		—		101
	Black	77	(77.8%)	22	(22.2%)	—		—		—		99
	Spotted	44	(91.7%)	4	(8.3%)	—		—		—		48
	Total	**342**	(84.9%)	**61**	(15.1%)	—		—		—		**403**
Roscoff (France)	Orange	6	(100%)	—		—		—		—		6
	Beige	447	(94.9%)	2	(0.4%)	22	(4.7%)	—		—		471
	Dark brown	488	(92.1%)	8	(1.5%)	34	(6.4%)	—		—		530
	Grey	545	(90.4%)	18	(3.0%)	40	(6.6%)	—		—		603
	Black	44	(77.2%)	4	(7.0%)	9	(15.8%)	—		—		57
	Spotted	169	(86.5%)	6	(3.1%)	18	(9.3%)	—		—		193
	Total	**1699**	(91.3%)	**38**	(2.0%)	123	(6.6%)	—		—		**1860**
	Orange	11	(100%)	—		—		—		—		11
	Beige	767	(89.8%)	—		—		69	(8.1%)	18	(2.1%)	854
	Dark brown	397	(87.9%)	—		—		53	(11.7%)	2	(0.4%)	452
	Grey	384	(85.1%)	—		—		63	(14%)	4	(0.9%)	451
	Black	349	(100%)	—		—		—		—		349
	Spotted	177	(87.6%)	—		—		25	(12.4%)	—		202
Langrune/Mer (France)	**Total**	**2085**	(89.9%)	—		—		**210**	(9.1%)	**24**	(1.0%)	**2319**
	Beige	125	(88.7%)	11	(7.8%)	—		—		5	(3.5%)	141
Langrune/Mer (France)	Black-brown	100	(89.3%)	—		2	(1.8%)	—		10	(8.9%)	112
Langrune/Mer (France)	Brown-grey	18	(90.0%)	2	(10.0%)	—		—		—		20
Portobello (New Zealand)	**Total**	**243**	(89.0%)	**13**	(4.8%)	2	(0.7%)	—		**15**	(5.5%)	**273**

[a] Individuals in each location were distinguished by colour variety. Numbers in bold are the total numbers (all varieties together) of investigated individuals.

[b] Identified as *Cancerilla neozelanica* Stephensen in New Zealand (see [48]).

similar across all locations and host colour varieties, but the different symbionts had different effects on the ophiuroid ability to produce light (Fig. 6). Symbiont species also differed in their effects on host reproduction, through affecting the development and/or occurrence of gonads and brooded juveniles in the bursae.

Cancerilla tubulata did not alter gonad development or brooding ability of the ophiuroid: all infested individuals were found to be brooding, sometimes with well-developed gonads. Most often only one copepod individual occurred per ophiuroid and the host brooding ability and light production were unaffected; light-production intensity and kinetic parameters (LMax and TLMax, respectively) were not significantly different between infested and non-infested individuals (Fig. 6A, B).

Parachordeumium amphiurae induced partial castration of the host (testes and ovaries were always reduced) and appeared to reduce its ability to brood juveniles (brooded juveniles were very few and consistently smaller). Multiple infestation with this symbiont was common. As many as four of the 10 bursae were found to be infested, always with one pair of symbionts (the large female and the dwarf male). Light-production intensity was enhanced by the infestation ($P<0.0001$; Fig. 6C), while light-

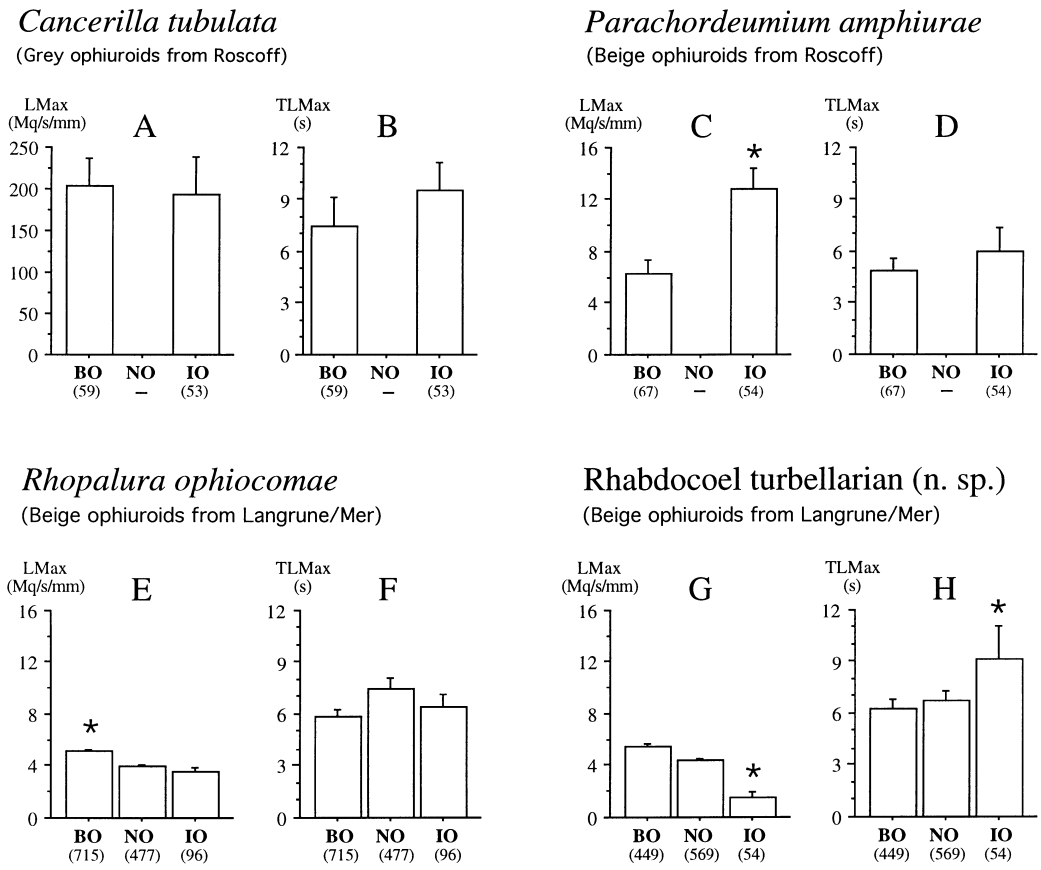


Fig. 6. Intensity (LMax) and kinetic (TLMax) parameters of *Amphipholis squamata* luminescence: non-infested and brooding ophiuroids (BO), non-infested and non-brooding ophiuroids (NO), and ophiuroids infested (IO) by (A, B) *Cancerilla tubulata*, (C, D) *Parachordeumium amphiurae*, (E, F) *Rhopalura ophiocomae*, or (G, H) the new rhabdocoel turbellarian species (the number of stimulated arms are indicated in parentheses) (mean values ± 95% confidence limit; *statistically different). All ophiuroids were of the beige colour, except those infested by *C. tubulata*. At Roscoff, all non-infested individuals were brooding, hence there was no measurement available for NO.

production kinetics (TLMax) did not vary significantly (Fig. 6D).

Rhopalura ophiocomae was found by the hundreds infesting the host. The symbiont clearly caused total castration of the ophiuroid, as neither ovaries nor brooded juveniles were observed in the host (small testes were sometimes observed). Light production was decreased but only when compared with non-infested individuals that were brooding ($P<0.0001$; Fig. 6E). Non-brooding individuals routinely exhibit lower light-production performance than brooding ones, and the decreased light-production intensity of infested individuals was similar to that of non-infested ones when non-brooding (Fig. 6E). Kinetics of the light production were always similar between individuals, whether or not they were infested or brooding (Fig. 6F).

Individuals of the new species of rhabdocoel turbellarian occurred in numbers usually ranging from four to 10 symbionts per ophiuroid. The symbiont clearly reduced the host's ability to brood (no brooded juveniles were ever observed in infested individuals) with no obvious effects on the gonads as they were always well developed. The symbiont strongly reduced the host's intensity of light production ($P<0.0001$; Fig. 6G), and significantly increased the time to maximum output ($P<0.006$; Fig. 6H).

4. Discussion

4.1. Distribution and prevalence of symbionts among locations

The four symbiont species were not present at all locations, nor were all the species present at any one location. The ophiuroid was free of symbionts at three locations, and at the other five locations one, two or three symbiont species were found. The four symbionts considered here do not, therefore, have the same widespread geographical distribution as does the host. Two of the locations sampled have been the subject of earlier surveys investigating the same symbionts (except for the newly discovered rhabdocoel turbellarian) (Roscoff: [17, 23, 24] [F. Goudey-Perrière, *Amphiurophilus amphiurae* (Hérouard), crustacé copépode endoparasite de l'ophiure *Amphipholis squamata* Della Chiaje (Echinoderme): étude du développement et données biologiques. PhD thesis, Paris VI University, 1972] and Prawle Point: [8, 10]). Comparing those results with ours reveals wide fluctuations in prevalence of symbionts, suggesting that particular local and temporal conditions may influence infestation.

The copepod *C. tubulata* is an external symbiont, and at Roscoff it infested 2% of the ophiuroids. From the same location, Bocquet [23] reported 1% infestation and Carton [17] mentioned 8%. Such variability may be explained in relation to the ecology of this symbiont, as *C. tubulata* may also exist free living [1, 25]. A stable "odour" of the ophiuroid is necessary for the *C. tubulata* to detect and find the host for infestation, but also to ensure durable linkage with the host [3, 26, 27]. Thus, the low infestation reported in the present study may be indicative of particular environmental conditions.

The copepod *P. amphiurae* is an intrabursal symbiont that infested 6.6% of the individuals at Roscoff and 11.3% at Prawle Point, while earlier surveys reported less than 1% [23] and 13% ([24]; F. Goudey-Perrière, PhD thesis, 1972) at Roscoff, and 10%, 14.1% and 30% at Prawle Point ([8–10], respectively). Such variation in infestation between years and locations is most likely related to environmental and biological factors [28]. Success of infestation can be affected by seawater currents ([24]; F. Goudey-Perrière, PhD thesis, 1972), by temperature and by photoperiod; these factors determine the ophiuroid physiological condition [V. Alva, Reproduction, développement, incubation et dynamique de population de l'ophiure *Amphipholis squamata* (Echinodermata) en baie de seine. PhD thesis, Free University of Brussels (ULB), 1996], which in turn influences the probability of infestation, which also depends on the ophiuroid size and on whether or not it is brooding or already infested ([6, 9]; F. Goudey-Perrière, PhD thesis, 1972). Variation in infestation could also be related to populational organisation of the host (whether forming dense aggregates or not), since frequency of contact between infesting larvae and host individuals could determine the level of infestation ([24, 29]; F. Goudey-Perrière, PhD thesis, 1972).

The orthonectid *R. ophiocomae* is an internal symbiont found in the coelom and the coelom wall

of the ophiuroid disc. It was found only at Langrune-sur-Mer in 9.1% of the individuals. This is less than the 18% minimal infestation reported at San Juan Island, U.S.A. [7], and more than the 6.5% maximal infestation mentioned from Roscoff [30]. The absence of the orthonectid from the other locations investigated is contrary to the reported worldwide distribution of the symbiont ([30], for review). This discrepancy may reflect the biology of *R. ophiocomae* which is well adapted for rapid and heavy infestation of hosts [30, 31] but apparently sensitive to ecological parameters, making its abundance variable over time. Little is known about the ecology of this symbiont and the environmental parameters that influence its infestation, except that density and biological conditions of the host are determinant [7, 30]. Also, the orthonectid causes noticeable effects on the host, whose biological activity, growth rate and regeneration are reduced [7], and survival of infested individuals may then be more threatened.

The four investigated symbionts of *A. squamata* appear to be mutually exclusive, since no host individual ever harboured more than one species of symbiont. This was suggested earlier by J. Johnson [The biology of *Amphipholis squamata* Delle Chiajei (Echinodermata: Ophiuroidea). PhD thesis, University of Newcastle, 1972], who found only two cases of double-infestation out of 66 infested individuals at Northumberland (U.K.) where the two copepods and the orthonectid are present.

4.2. Distribution of symbionts among colour varieties

The varieties of *A. squamata* differ in their innate ability to produce light [12]. Gotto [32] suggested that the distribution of symbionts could be correlated with light-production intensity of the host, with apparently the more luminous the individual, the higher the frequency of infestation. Such a relationship was not substantiated in the present study. Symbionts occurred regardless of host variety, without any correlation with their bioluminescence or their degree of pigmentation. Yet, considering each location separately, it is obvious that the symbionts were not distributed homogeneously amongst the varieties, some being heavily infested and some others not, but varying from one location to another (e.g., compare the grey and black varieties at Langrune-sur-Mer and Roscoff; Table 1). It is a common occurrence, however, that frequency of infestation of a widely distributed species of host varies from one location to another, the causes being related to environmental and/or populational differences between locations, and sometimes also to genetic variance in the host and/or the symbiont between locations [27, 29]. *Amphipholis squamata* from distinct geographical regions have been demonstrated to be genetically different (D.R. Murray, Master thesis, 1989). Genetic differences probably also exist between individuals of the different colour varieties [11, 12], which could result in differences in susceptibility/tolerance to infestation between varieties even at a given location. *Amphipholis squamata* may thus resemble another polychromatic ophiuroid (*Ophiocoma echinata*) in which "colour morphs—from different habitat—will eventually be recognised as distinct substrate-specific or host-specific taxa" [33].

4.3. Effect of symbionts on the host light-production characteristics

The presence of the external symbiont *C. tubulata* had negligible impact on the host. Most often only one copepod was present, and gonad development, brooding ability and light-production parameters were all unaffected. The three internal symbionts, however, were present in greater numbers, and each caused some degree of reproductive impairment and induced changes in light-production parameters. Maximum light output was decreased when *R. ophiocomae* or the rhabdocoel turbellarian was present, but increased in the presence of *P. amphiurae*. Kinetics of the light production (TLMax) were affected only by the presence of the rhabdocoel turbellarian.

Bioluminescence is a biological property associated with considerable energetic cost [34], and each of the symbionts also induces some energetic loss for the host (see [7, 8, 35]; F. Goudey-Perrière, PhD thesis, 1972). The observed reductions in light-production intensity in the presence of the orthonectid and the rhabdocoel turbellarian might therefore be interpreted as due simply to the energy cost

of infestation. The real explanation, however, is likely to be more complex. Although nothing is known about the chemistry of bioluminescence in *A. squamata*, light production is known to be under the neurophysiological influence of brooding and increases when at least one juvenile is brooded in the bursae [12]. The orthonectid caused total castration and total absence of brooded juveniles in the bursae, and the reduced light output was equivalent to that seen in non-infested, non-brooding individuals. The effect may therefore have been entirely due to the absence of juveniles in the bursae. On the other hand, the copepod *P. amphiurae* lives specifically in the bursae, usually in reproductive pairs, inhabiting up to four of the 10 bursae. The significant increase in light output observed from individuals infested with this symbiont may therefore also be due to a "brooding" effect, the presence of the copepod stimulating the neurophysiological responses normally due to the presence in the bursae of brooded juveniles. The stimulatory effect then exceeded any diminution due to energy cost of the infestation.

Individuals of the new species of rhabdocoel turbellarian caused the greatest alterations in the ophiuroid light-production performance. Intensity of the bioluminescence (LMax) was strongly reduced and the kinetics (time to reach maximal output; TLMax) significantly increased by infestation. The turbellarian develops long processes that underlie the ophiuroid digestive epithelium (Deheyn, personal observation), suggesting nutrient transfer (energy) from host to symbiont. It also caused total absence of brooded juveniles in the bursae. The decrease in light intensity in the presence of this symbiont substantially exceeded that due to the orthonectid, and this implies an effect of the energetic loss as well as an effect due to the absence of brooding. Reduced intensity and increased kinetics could also be the result of interference with the pathway of light production. Symbiotic turbellarians are known to induce alterations of cellular and sub-cellular functions of their hosts by abstracting molecular oxygen from host tissues, utilising haemoglobin-like proteins [36–38]. The turbellarian from *A. squamata* is brown-red to orange in colour, which suggests it may contain a haemoglobin-like protein [36, 37]. The reaction of bioluminescence requires molecular oxygen [34], so an oxygen imbalance could also contribute to alteration of the light-production performance in individuals infested by the rhabdocoel turbellarian.

It is also possible, for any of the symbionts considered, that the variations of light-production performance from infested *A. squamata* are related to the oxidative immune reaction of the ophiuroid. Bioluminescence ability is affected by oxygen reactives, such as superoxide ions, free radicals, or polar steroids [39, 40]. These reactives are toxic and produced in response to infestation, their production being part of the organism's immune reaction [41, 42]. The molecules that are used in bioluminescence have a high affinity for such oxygen reactives [43, 44] and can be utilised by the host to neutralise the toxic, oxidative effects of these reactives. A luminous molecule that is used in this way for protection against the immune oxidative stress would then be unavailable for the production of light, and the light-production performance thus would depend on the individual's immune condition (see [44]). The superoxide immune reaction has been demonstrated in echinoderms [45–47], although not specifically in *A. squamata*.

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