Cah. Biol. Mar. (2005) 46 : 399-402

Note on the association between *Plakobranchus ocellatus* (Mollusca: Gastropoda: Opisthobranchia) and *Holothuria atra* (Echinodermata: Holothuroidea)

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Abstract: This paper presents evidence of a commensal association between an opisthobranch (*Plakobranchus ocellatus*) and a sea cucumber (*Holothuria atra*). Field observations revealed that occurrences of *P. ocellatus* specimens and egg filaments were much more frequent on the body wall of *H. atra* than on that of two other holothurian species living in the same habitat. Roughly 95% of *H. atra* were colonized by *P. ocellatus* during the night, which corresponds to the active period of *H. atra* and the resting period of *P. ocellatus*. The proportion decreased to ca. 21% in the daytime when sea cucumbers are resting and most opisthobranchs are foraging. Laboratory trials confirmed the daily pattern of association and the fact that *P. ocellatus* lay their eggs on the body wall of *H. atra*. Furthermore, multiple-choice experiments showed that ca. 71% of *P. ocellatus* favoured *H. atra*, whereas only ca. 9% colonised other species of sea cucumber. Because *H. atra* is known to exude toxic chemicals that deter several species of invertebrates and fishes, the opisthobranch may use the sea cucumber as a refuge against predators and as a secure spawning ground.

Résumé : *Note sur l'association entre* Plakobranchus ocellatus *(Mollusca: Gastropoda: Opisthobranchia) et* Holothuria atra *(Echinodermata: Holothuroidea).* Cet article présente les évidences d'une association commensale existant entre un opisthobranche (*Plakobranchus ocellatus*) et une holothurie (*Holothuria atra*). Les données recueillies sur le terrain révèlent que les occurrences de *P. ocellatus* et de leurs oeufs sont considérablement plus fréquentes sur la paroi corporelle de *H. atra* que sur les autres espèces d'holothuries issues du même habitat. Approximativement 95% des *H. atra* examinés étaient colonisés par *P. ocellatus* pendant la nuit, période à laquelle *H. atra* s'active et *P. ocellatus* demeure au repos. Cette proportion n'atteignait plus que ca. 21% durant la journée lorsque les holothuries étaient immobiles et la plupart des opisthobranches fourrageaient. Des études en bassin ont confirmé le cycle quotidien de l'association et le fait que *P. ocellatus* pond ses oeufs sur la paroi corporelle de *H. atra*. En outre, les expériences de choix multiples ont montré que *P. ocellatus* favorise *H. atra* dans une proportion de ca. 71%, contre seulement ca. 9% pour les autres espèces d'holothuries. Puisque *H. atra* libère des substances toxiques qui repoussent plusieurs espèces d'invertébrés et de poissons, l'opisthobranche utilise vraisemblablement l'holothurie comme site refuge pour favoriser sa survie et celle de sa progéniture.

Keywords: Opisthobranchia, Holothuroidea, Sea cucumber, Echinoderms, Molluscs, Commensalism

Reçu le 15 mars 2005 ; accepté après révision le 19 septembre 2005.
Received 15 March 2005; accepted in revised form 19 September 2005.

Introduction

Sea cucumbers are known to harbour a multitude of internal and external symbionts, mainly commensals and parasites (Trott 1981; Jangoux 1990), among which various species of crustaceans, molluscs, worms and fishes (Jangoux 1990; Hamel et al. 1999; Eeckhaut et al. 2004). The species *Holothuria atra* Jaeger, 1833 is reported to host pearlfishes (Schultz 1960; Strasburg 1961), worms, crabs (Cannon 1982; Seeto 1994), copepods (Stock 1968; Ummerkutty 1970; Seeto 1994) and gastropods (Risbec 1953; Jones & James 1970; Seeto 1994). To our knowledge no opisthobranch has ever been described to associate with holothurians or with any echinoderm. The present study brings strong evidence that such an association exists between *H. atra* and *Plakobranchus ocellatus* van Hasselt, 1824, based on field surveys and laboratory trials.

Materials and Methods

The field work was performed in August and September 2001 in the lagoon of Majuro Atoll, Marshall Islands (7°09' 00" N: 171°11' 60" E). The reef flat of the intertidal zone and the subtidal area, down to ca. 5 m, was randomly walked or snorkelled on three separate occasions at one-week intervals, both in day time (between 10:00 and 12:00) and night time (between 19:00 and 20:30). On each survey, ca. 150 *H. atra* were examined for the presence of adults, juveniles and egg filaments of *P. ocellatus* on their body wall. Complementary observations were made on two other holothurian species that were present in the same habitat: *H. leucospilota* (Brandt, 1835) and *Thelenota anax* H.L. Clark, 1921. Between 30 and 50 individuals were scrutinized to establish the incidence of *P. ocellatus* on their body wall.

Laboratory experiments were carried in 3 round 20-ton tanks (ca. 2.7 m wide and 1.5 m high) under a flow through of 1200 L h^{-1} and natural lighting. Fifteen *H. atra* (between 20 and 25 cm long) and 30 *P. ocellatus* (between 3 and 4 cm long) were placed in each tank and left to acclimate for 4 days. Afterwards, the proportion of *P. ocellatus* found on *H. atra* was noted twice daily, between 10:00 and 12:00 and between 19:00 and 20:00, on 3 successive days. The presence of egg filaments was also recorded.

The extent of host specificity was determined in the same tanks and under the same conditions. Thirty *P. ocellatus* were released in each tank together with 5 *H. atra*, 5 *H. leucospilota* (between 25 and 30 cm long) and 5 *T. anax* (between 40 and 50 cm long). All sea cucumbers were visually inspected between 19:00 and 20:30 every day for 5 days in search of *P. ocellatus* and their egg filaments. *H. leucospilota* was chosen because they were present in the same habitat as *H. atra*, and *T. anax* because they coexist with *H. atra* in deeper waters in the Majuro lagoon.

Results and Discussion

Field observations brought the first evidence of an association between *H. atra* and *P. ocellatus* over the day/night cycle. Night surveys revealed that 94.7 ± 0.6% (mean ± S.D.) of all *H. atra* had opisthobranchs on their body wall. This proportion decreased drastically to 20.7 ± 4.5% during the day (Table 1). At night, 73.3 ± 2.1% of *H. atra* hosted a single *P. ocellatus*, 13.0 ± 6.6% hosted two ore more *P. ocellatus* (with a maximum of 7 *P. ocellatus* per host) and 8.3 ± 4.9% harboured *P. ocellatus* <1 cm long, presumably juveniles (Table 1). Laboratory trials also showed a higher incidence of association during the night: 95.2 ± 2.9% of the 30 specimens of *P. ocellatus* present in a tank were recovered on *H. atra* at night, whereas only 17.4 ± 8.3% dwelled on *H. atra* during the day (Table 2). Hence, the occurrence of *P. ocellatus* on *H. atra* is apparently cyclic and cannot be explained by fortuitous encounters. The fact that nearly all *P. ocellatus* converge on *H. atra* at night in the holding tanks suggests that the opisthobranchs can detect and seek their holothurian hosts.

The proportion of other sea cucumbers that harboured *P. ocel-*

Table 1. Incidence of the association between *Plakobranchus ocellatus* and *Holothuria atra* in the lagoon of Majuro Atoll. Data are expressed as the proportion (%) of *H. atra* (n = 150) that were found to harbour *P. ocellatus* specimens or egg filaments on each weekly survey.

Tableau 1. Fréquence de l'association entre *Plakobranchus ocellatus* et *Holothuria atra* dans le lagon de l'atoll de Majuro. Les résultats expriment la proportion (%) de *H. atra* (n = 150) hébergeant des spécimens ou des masses d'œufs de *P. ocellatus* à chacun des relevés hebdomadaires.

	Proportion of *Holothuria atra* (%)				
Survey	**Without *P. ocellatus***	**With a single *P. ocellatus***	**With > 2 *P. ocellatus***	**With *P. ocellatus* < 1 cm**	**With *P. ocellatus* egg filaments**
1st day	84	16	0	0	8
1st night	5	75	14	6	4
2nd day	79	19	1	1	5
2nd night	6	74	6	14	9
3rd day	75	12	10	3	5
3rd night	5	71	19	5	11

Table 2. Incidence of the association between *Plakobranchus ocellatus* and *Holothuria atra* in the laboratory. Data were noted in 3 separate tanks containing 30 *P. ocellatus* and 15 *H. atra*, and results are presented as mean ± standard deviation.

Tableau 2. Fréquence de l'association entre *Plakobranchus ocellatus* et *Holothuria atra* en laboratoire. L'étude fut menée dans 3 réservoirs contenant 30 *P. ocellatus* et 15 *H. atra*, et les résultats présentent la moyenne ± déviation standard.

	Proportion of *P. ocellatus* found on *H. atra* (%)		
Survey	**Occurring singly**	**Occurring in groups**	**Total**
1st day	10.0 ± 3.3	-	10.0 ± 3.3
1st night	57.8 ± 5.1	37.8 ± 5.1	95.6 ± 1.9
2nd day	12.2 ± 6.9	6.7 ± 0.0	18.9 ± 3.5
2nd night	50.0 ± 6.7	44.4 ± 5.1	94.4 ± 1.9
3rd day	15.6 ± 5.1	7.8 ± 6.9	23.3 ± 8.8
3rd night	26.7 ± 6.7	68.9 ± 1.9	95.6 ± 5.1

latus in the field was 5.1 ± 0.9% (mean ± S.D.) for *H. leucospilota* and 1.6 ± 0.4% for *T. anax*. Similarly, host preference was clearly expressed in the multiple-choice trials: based on the combined results for the 5 night surveys, 71.1 ± 9.1% of *P. ocellatus* were found on *H. atra*, compared to 6.9 ± 3.7% on *H. leucospilota* and 1.9 ± 1.5% on *T. anax* (Table 3). The rest (ca. 20%) was found on the bottom and sides of the tank. These results infer that *P. ocellatus* is able to discriminate between several holothurian species, possibly through chemical detection.

Table 3. Host selectivity of *Plakobranchus ocellatus*. Data are expressed as the proportion (%) of sea cucumbers from each species that harboured at least one *P. ocellatus*. Results were noted in 3 separate tanks holding 30 *P. ocellatus* and 5 specimens of each sea cucumber species. They are presented as mean ± standard deviation.

Tableau 3. Sélection de l'hôte par *Plakobranchus ocellatus*. Les données expriment la proportion d'individus hébergeant au moins un *P. ocellatus*, pour chaque espèce de concombre de mer. L'étude fut menée dans 3 réservoirs contenant 30 *P. ocellatus* et 5 individus de chaque espèce de concombre de mer. Les résultats présentent la moyenne ± déviation standard.

	Proportion of *P. ocellatus* found on the various sea cucumber species (%)		
Night	***Holothuria atra***	***Holothuria leucospilota***	***Thelenota anax***
1	64.4 ± 6.9	8.9 ± 5.1	-
2	73.3 ± 3.3	4.4 ± 1.9	-
3	65.6 ± 11.7	5.6 ± 5.1	2.2 ± 1.9
4	73.3 ± 12.0	6.7 ± 3.3	-
5	78.9 ± 5.1	8.9 ± 1.9	2.2 ± 1.9

Although the preference expressed by *P. ocellatus* for *H. atra* is clear, the reasons for it can only be hypothesized at this point. The fact that *T. anax* is uncommon in shallow water < 5 m deep suggests that the distribution of this species barely overlaps that of *P. ocellatus*. Hence, *T. anax* moving to much deeper waters could carry *P. ocellatus* to inadequate habitats or feeding grounds. The cryptic behaviour of *H. leucospilota* (Kerr et al. 1993) could justify the poor interest of *P. ocellatus* for this species in the field, although concealment cannot justify the results of the tank trials, since the sea cucumbers were not provided with any shelter. Either other factors are involved or the attraction of *P. ocellatus* to *H. atra* has evolved toward true species specificity.

Incidentally, egg filaments of *P. ocellatus* were exclusively found on the body wall of *H. atra*, never on other sea cucumbers or elsewhere in the tanks. Specifically, 4 egg masses were observed during the study. These results, combined with the incidence of egg filaments found on the body wall of 7.0 ± 2.8% of *H. atra* in the field, indicate that *P. ocellatus* voluntarily seek *H. atra* as a spawning ground. Hadfield & Switzer-Dunlap (1984) mentioned that numerous species of opisthobranchs spawn on or close to their food source. Here however, the exact relationship between the sea cucumber and the opisthobranch, and whether or not it could involve feeding habits, remains to be clarified.

The fact that *P. ocellatus* dwell on *H. atra* mostly at night when they are not actively foraging suggests that the sea cucumber is not a source of food. However, the body wall of *H. atra* exudes a toxin (holothurin) that is known to efficiently deter a variety of invertebrates and fishes (Bakus 1968; Rao et al. 1985; Sarma et al. 1987). The deterrent protects the sea cucumber and may also benefit *P. ocellatus*, which does not seem affected by it. The association can consequently be related to predator avoidance, the sea cucumber offering a safe refuge to the opisthobranch. This may also explain why *P. ocellatus* spawns on the body wall of *H. atra*, which grants protection together with a means of dispersion.

The sea cucumbers do not seem to derive any advantage from the association, or be harmed in any way by the presence of the opisthobranchs, indicating that the relationship fits the definition of commensalism.

Acknowledgements

We would like to thank the staff of the Marshall Islands Science Station (MISS) of the College of the Marshall Islands (CMI) for their help during the field and laboratory monitoring.

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