International Research: **Biological Studies**

Symbiont – host interraction in the association of the scaleworm *Branchipolynoe* aff. *seepensis* (Polychaeta: Polynoidae) with the hydrothermal mussel, *Bathymodiolus* spp. (Bivalvia: Mytilidae)

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Introduction

Symbiotic polynoids of the genus *Branchipolynoe* associated with mussels are a common component of hydrothermal vent and cold-water seep communities. Recent detailed studies carried out on the Atlantic scale-worm *B.* aff. *seepensis* Pettibone, 1986 (Jollivet *et al.*, 2000) revealed some interesting features of its reproductive biology: external sexual dimorphism, internal fertilisation and dominance of females in the population. Nevertheless, the role of symbiotic polychaetes in the communities and their relationships with their hosts remain virtually unknown. In fact, the association of the different species of *Branchipolynoe* with their host mytilids *Bathymodiolus* spp. has been usually regarded as commensalism (Pettibone,1986; Chevaldonne *et al.*, 1998). However the finding of bits of mussel gills in the gut of *B. symmyitilida* from the Galapagos Rift (Desbruyères *et al.*, 1985) pointed to a parasitic behaviour.

The present paper reports preliminary results based on material collected mainly during a recent cruise of the *R/V Akademik Mstislav Keldysh* (2002) at the hydrothermal vent fields along the MAR and complimentary data of previous French and Russian expeditions which allowed to better understand the true nature of the relationships between the Atlantic scale worm *B.* aff. *seepensis* with their mytilid hosts *Bathymodiolus puteoserpentis* and *B. azoricus*.

Material and methods

The bivalves were collected from hydrothermal vent fields along the MAR. At the Logatchev, Snake Pit, Lucky Strike, and Rainbow vent fields mussels were sampled durig expeditions of the Russian *R/V Akademik Mstislav Keldysh* (1998, 1999, 2002). In addition, material obtained in expeditions of the French *R/V Nadir* (1993, 1995, and 1997) at Snake Pit, Lucky Strike and Menez Gwen was also used. At present two species of mytilids are known from the MAR: *Bathymodiolus puteoserpentis* occurs at the Logatchev, Snake Pit and Broken Spur and *B. azoricus* at Lucky Strike, Rainbow and Menez Gwen. In total 300 specimens of *Bathymodiolus puteoserpentis* and 680 specimens of *Bathymodiolus azoricus* were examined. In the former 41 and in the latter 400 specimens of *Branchipolynoe* aff. *seepensis* were found.

Firstly, the length, width and height were measured for each mussel. Then the shell was opened, the number and location of symbiotic polychaetes were recorded, the polychaetes were measured and modifications in the morphology of soft tissues of the host were quantified and analysed.

Gut content was analysed in 13 symbionts associated with *B. azoricus* and 9 symbionts associated with *B. puteoserpentis*. To analyse the gut content, polychaets were dissected along the dorsal side.

All statistical analyses were carried out using the SYSTAT 5 (vers. 5.2.1) statistical package.

Table 1. Characteristics of the infestation by *Branchipolynoe seepensis* of the two hydrothermal mytilid hosts *Bathymodiolus puteoserpentis* and *Bathymodiolus azoricus*.

Host	Locality	Prevalence (n)	Abundance (Intensity)
Bathymodiolus puteoserpentis	Logatchev, 1998	7.2% (180)	0.07 (0-1)
	Snake Pit, 2002	23.3% (120)	0.275 (0-6)
Bathymodiolus azoricus	Rainbow, 1999	65.0 % (20)	0.70 (0-2)
	Rainbow, 2002	60.8% (51)	0.80 (0-2)
	Lucky Strike, Statue de la Liberte, 1993	76.5 % (34)	0.73 (0-1)
	Lucky Strike, Elisabeth, 1995	54.8% (445)	0.47 (0-3)
	Lucky Strike, 2002	71.5% (130)	0.80 (0-6)

Results

Characteristics of infestation

Infestation varied from 7.2% to 90.5% and was significantly lower ($p < 0.02$) for the host *B. puteoserpentis* (Table 1). Abundance was also highly variable, ranging from 0.07 to 0.8 symbionts per host, and significantly lower ($p < 0.007$) for the host *B. puteoserpentis* (Table 1). Infestation increased with increasing mussel size (Fig. 1). In the case of *B. azoricus*, this trend was recorded

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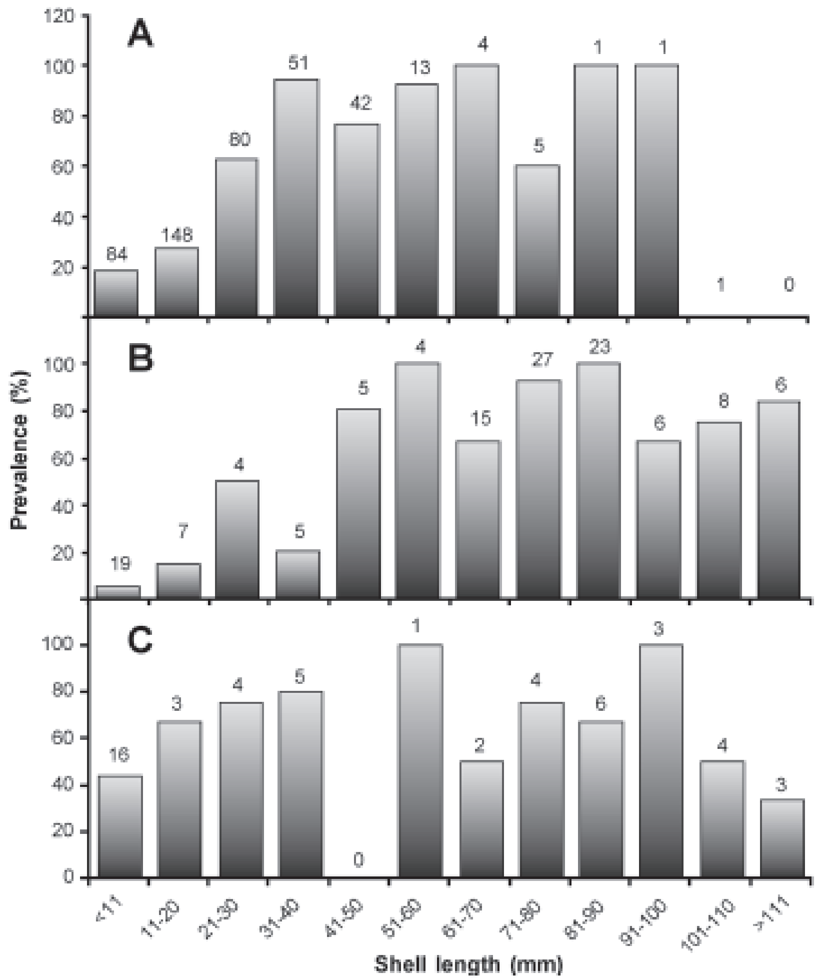

Figure 1. Relationships between prevalence and host size. *Bathymodiolus azoricus* from Lucky Strike: (A) 1995; (B) 2002; *B. puteoserpentis* from Snake Pit, 2002; (C) Figures on the top of bars indicate the number of mussels.

only for mussels with the shell length less than 40 mm (Fig. 1A, B). Infested host can harbour from 1 to 6 worms (Table 1), in most cases one mollusc harboured only 1 polychaete.

Location of symbionts

The polychaetes were most frequently located in the mantle cavity of the molluscs between the mantle wall and the external demibranchs, while the positions between the internal and external demibranchs and between the internal demibranch and the foot were less frequent (Fig. 2A). The prostomia (Fig. 2B) were mostly directed towards the siphon opening, but the orientation towards the host palps was also frequent. Orientations towards the body centre or the outer edge of the valves were remarkably less frequent.

Symbiont's gut content

Six from the 13 studied guts of the symbionts of *B. azoricus* contained food remains. The guts were filled with detritus and a suspension of inorganic particles, including the peculiar ochroid particles, which are found on the host shell surface. In addition, four specimens contained polychaete chaetae belonging to the same species. Single findings of crustacean chaetae, diatom theca and fragments of a pelagic predator copepod of the family Oncaeidae were also observed.

Among nine guts of symbionts associated with *B. puteoserpentis*, four contained identifiable food remains. Detritus and suspended inorganic particles were found in 3 worms, while ochroid particles, diatom theca and fragments of a non-identified crustacean occurred in 1 worm.

Analysis of traumatism in the host Bathymodiolus

The traumas observed in mussels included shortenings of some ctenidial filaments and deformation of labial palps and feet (Fig. 3). Among the different samples, the number of mussels with traumas varied from 59.1% to 72.2%. The number of traumas was significantly higher in infested (n=204) than in non-infested molluscs (n=248, ANOVA, $p < 0.001$). Among the infested hosts, traumas of palps were most frequent, followed by those of ctenidia, which were usually located in the anterior parts of the demibranches (Fig. 3). The hosts harbouring female symbionts showed a higher number of traumas, particularly on palps.

The symbionts were frequently found inside tunnel-like structures formed by gill filaments between demibranches. Sometimes, an epithelial ridge (or callosity) outlining the polychaete body is developed on the mantle wall. These structures were present sometimes in non-infested mussels.

Shell length-width ratio in infested and non-infested hosts

To determine the possible influence of symbionts on the host shell morphology we compared the shell length-width ratio of infested and non-infested mussels. The cross correlation analysis demonstrated the existence of a significant association between the two patterns in the case of *B. azoricus*. In this species, the maximal Pearson's correlation index had a position lag of 0.012 in width-length ratio (correlation = 0.974, $p<0.001$), indicating that the infested shells were relatively wider than the non-infested ones (Fig. 4).

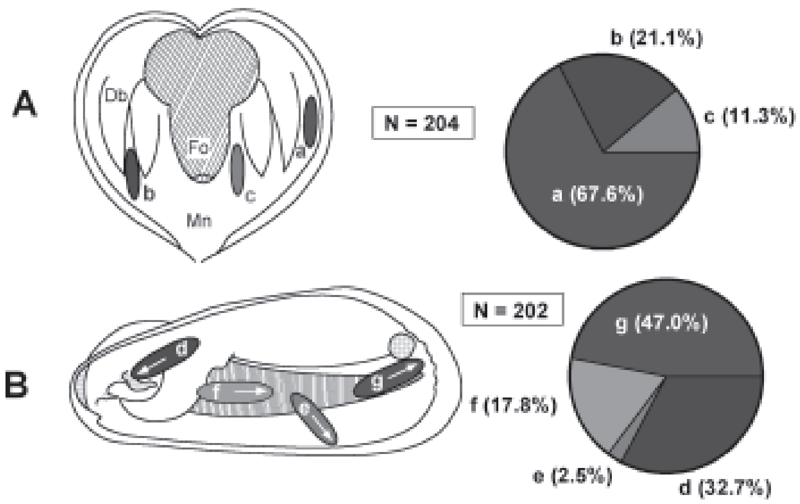

Figure 2. Location of the symbionts inside the host Bathymodiolus azoricus. (A) Relative to the ctenidia and foot: between the mantle wall and the external demibranch (a), between the internal and external demibranch (b) and between the internal demibranch and the foot (c). (B) Orientation of the prostomium: towards the host palps (d), towards the outer edge of the valves (e), towards the body centre (f), towards siphon opening (g). The figures of percentages on the circled diagrams indicate the frequency of associations with given location of worms. Do - demibranch, Fo – foot of mussel.

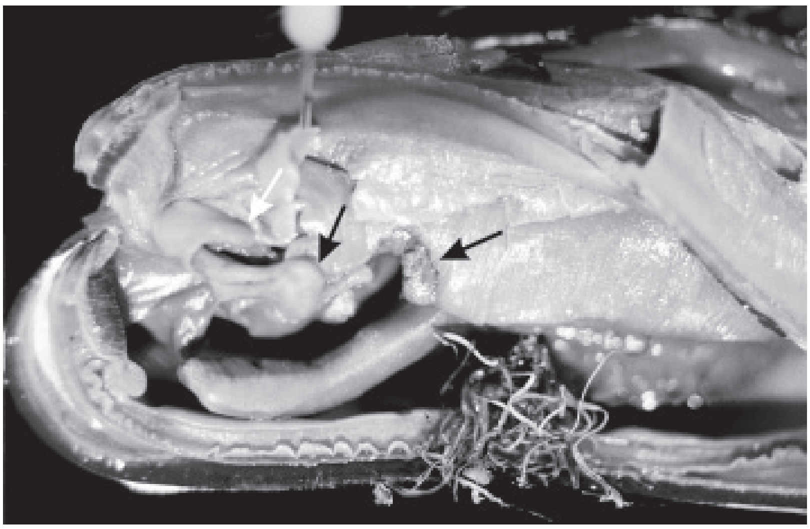

Figure 3. Light micrograph of traumas observed in *Bathymodiolus azoricus*. Anterior pair of palps is intact (white arrow); posterior palps are shortened and have bulbous thickenings, and also ctenidial filaments are shortened (black arrows).

Discussion

Infestation of mussels by *B.* aff *seepensis* varies substantially between different hydrothermal fields and sites, depending on the host species and size. Although two parts of a mussel population separated only by a few meters from each other may show different infestation levels, some general trends may be inferred. The infestation by *B.* aff *seepensis* is higher in larger than in smaller hosts, as is common among other symbiotic polychaetes . Conversely, it already occurs among the smallest mussel size classes. This is a very rare trend for a symbiotic association and may be related to the suggested continuous reproduction and settlement of the symbionts (Jollivet *et al.*, 2000). On the other hand, infestation of *Bathymodiolus azoricus* is significantly higher than in *B. puteoserpentis*. According to our data, this trend is more likely related to environmental differences of localities of both species rather than to the peculiarities of host's biology.

The specimens of *Branchipolynoe*, like most symbiotic scale-worms, are able to move inside the mantle cavity of their host, as it may be inferred from the different adopted positions. However, they usually remain immobile for a long time, as indicated by the presence of epithelial ridges on the mollusc mantle walls along the polychaete body and tube-like structures formed by the host gill filaments. The location of the symbiont inside the mantle cavity can be particularly related to the peculiarities of their feeding behaviour. The two predominant head orientations, head-to-siphon and head-to-mouth, suggest that symbionts may consume filtered and agglutinated suspension particles, transported with the water flow to the host mouth, or suspended organic particles transported to the siphon opening. This is supported by the presence of detritus and suspended inorganic particles, including the ochroid ones that are often found on the shell surface, inside the polychaete intestine. The single finding of a planktonic copepod of family Oncaeidae also supports this suggestion. Similar behaviour (*i.e.* kleptoparasitism) is typical for some other symbiotic animals, such as the polychaete *Branchiosyllis exilis*, associated with the brittle star *Ophiocoma echinata* (Hendler & Meyer, 1982), or the nemertine *Malacobdella grossa*, associated with bivalves (Gibson, 1967).

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The only previous report on trophic preferences of *Branchipolynoe* showed the presence of pseudofaeces and bits of mussel demibranches in the gut of *B. symmitilidae* (Desbruyères *et al.*, 1985). We did not find traces of host tissues or pseudofaeces in the gut of *B.* aff. *seepensis*, which may probably result from the different food preferences in this species. However, it seems clear that the symbionts are the responsible for the traumatism on host palps and ctenidia, with the relationship between the level of damage and the presence of symbionts being indirect evidence. According to our data, the host traumas seem to be an accidental result of "normal" symbiont feeding activities, so that their behaviour may be more reliably considered as kleptoparasitic (stealing food from a host) than as true parasitic (feeding on the host) or commensal (sharing food with a host).

Additional information on the nature of a symbiotic association may be inferred from the influence of the symbiont on the host metabolism or growth. In the case of bivalves, decreasing growth rates may lead to changes in shell shape, which tend to become relatively wider. The studied populations of *B. azoricus* show a relative increase of shell width in infested mussels when compared to non-infested. Assuming that these shell modifications are caused by the inhibition of shell growth in infested hosts, this strongly supports that the behaviour of the symbionts is closer to parasitism than to commensalism.

In summary, our data point out that the symbiosis between *Branchipolynoe* aff. *seepensis* and its host mitylids is exceptional in that the association starts from the smallest mussel size classes and in that it can be demonstrated that the presence of the symbiont has a negative influence on the host growth (and, thus, on its productivity). Taking into account that the species of *Bathymodiolus*, *B. azoricus* and *B. puteoserpentis*, are among the most abundant inhabitants of Mid Atlantic hydrothermal vents and the high prevalence of the infestation by *Branchipolynoe*, the role of the symbiotic polychaetes in the functioning of the hydrothermal vent community appears to be substantially more significant than previously thought.

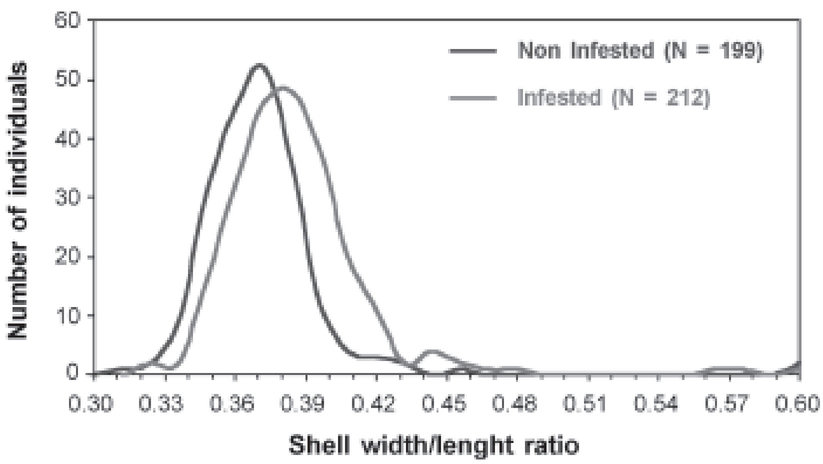

Figure 4. Patterns of length-width ratio in shells of infested and non-infested *Bathymodiolus azoricus*. The maximal Pearson's correlation index had a position lag of 0.012 in width-length ratio, indicating that the infested shells were relatively wider than the non-infested ones.

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