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# Seasonal release of the egg capsules of *Anoplodium parasita* Schneider, 1858, intracoelomic turbellarian (Platyhelminthes, Rhabdocoela) symbiotic of the sea cucumber *Holothuria tubulosa* Gmelin, 1788 (Echinodermata, Holothuroida)

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ABSTRACT: The endosymbiotic umagillid *Anoplodium parasita* Schneider, 1858 (Platyhelminthes, Rhabdocoela) lives in the perivisceral coelom of the sea cucumber *Holothuria tubulosa* Gmelin, 1788 (Echinodermata, Holothuroida). The flatworms release their egg capsules in the coelomic cavity of their host, where they are embedded in brown bodies concentrated in the posterior part of the body, near the cloaca. The infestation of the holothurians by turbellarian egg capsules was very high: 128 out of the 202 holothurians inspected from January 1999 to July 2000 at Banyuls-sur-mer (France) contained at least 6 and up to 10,000 egg capsules (mean 1433). Quite uniform throughout the year, the number of holothuroids infested as well as the number of egg capsules found per individual drastically fall in July, the period when the gonads of holothurians are highly developed. We suggest that the release of egg capsules might be an indirect result of the spawning of the hosts. To discharge their gametes, holothurians raise their body and adhere on the substratum by their posterior end. This behaviour would cause a surpressure in the peri-cloacal coelomic area of the holothurians and consequently the expulsion of the coelomic fluids through their coelo-cloacal ducts, carrying brown bodies and egg capsules to the outside.

KEYWORDS: Evisceration, gonad, infestation, symbiosis, umagillidae

## 1 INTRODUCTION

Turbellarians (Platyhelminthes) are, with gastropods, among the most common symbionts of echinoderms (Jangoux 1990). About 100 such flatworm species have been described since 1858, 25 of which in the last decade. Most species belong to the family Umagillidae (Rhabdocoela) and infest mainly echinoids and holothuroids. The symbiotic relationships between turbellarians and echinoderms are highly specific: all representatives of the genus *Anoplodium* Schneider, 1858, for example, are exclusively associated with aspidochirote holothurians of the families Holothuriidae and Stichopodidae. There are 12 species of umagillids reported to inhabit the coelomic cavity of holothuroids (Cannon 1990, Jangoux 1990) and, among these, only four have been observed to release their egg capsules in the coelomic cavity of their hosts: *Anoplodium hymanae* Shinn, 1983 (Shinn 1984, 1985a, b), *A. leighi* Cannon, 1990 (Cannon 1990), *A. parasita* (Schneider 1858, Arvy 1957, Changeux 1961, Kroll & Jangoux 1989) and *A. stichopi* Bock, 1925 (Jespersen & Lützen 1971). For these species, the egg capsules have been found embedded in the brown bodies, pigmented coelomic aggregates of host defensive cells (coelomocytes) which retain unwanted material (Jans et al. 1996). However, all reports were occasional observations from time-limited collection periods and there is no information from a long term study focused on turbellarian egg capsules.

The present work aimed to describe the infestation of the common European sea cucumber *Holothuria*

*tubulosa* by the egg capsules of *Anoplodium parasita*, a species whose life cycle remains unknown. *A. parasita* is widely distributed in the Mediterranean Sea and has been reported from Naples (Schneider 1858, von Graff 1882, Wahl 1906, Kroll & Jangoux 1989), Nice (Schmidt 1861), Corfou (Schmidt 1861), Trieste (von Graff 1882, Wahl 1906), Marseille (Briot 1906a, b), Villefranche-sur-mer (Arvy 1957) and Banyuls-sur-mer (Changeux 1961, present study). *A. parasita* apparently infests three species of holothurians: *H. tubulosa*, *H. polii* and *H. stellati*, and is said to live in the coelomic cavity of its host, rarely in the digestive tract or respiratory trees.

## 2 MATERIAL AND METHODS

202 individuals of *Holothuria tubulosa* were collected by SCUBA diving in the *Posidonia oceanica* meadow of Banyuls-sur-mer (Pyrénées-Orientales, France) from January 1999 to July 2000 and 20–21 specimens were inspected every two months. Samplings were done on a defined perimeter, 5 meters deep, about 50 meters West from the "Ile Grosse", close to the marine station. Specimens were brought into the Arago laboratory and maintained in a large acclimatization tank (open circuit marine aquarium; water temperature: 20°C in July and 12°C in January; salinity: 38‰) for two days at maximum. They were then "anaesthetized" in refrigerator at 4°C for 45 min, measured and dissected.

The body wall was sliced open with a razor blade, the coelomic fluids were collected in a Petri dish and, as the coelomic cavity itself, inspected under the stereomicroscope. Worms found in the coelomic fluids or on the epithelium of the coelomic cavity were transferred to individual Petri dishes, filled with 0.2 µm filtered sea water. The same was done for the turbellarian egg capsules that were then counted under microscope between slide and cover slide. The digestive tract and respiratory trees of the sea cucumber were then removed, dissected open in a Petri dish filled with filtered sea water and examined for additional specimens. Host gonads were observed under microscope to determine host sex then dried (24 h at 70°C) and weighted (d.w.), as the rest of the body (body wall and viscera).

## 3 RESULTS

All umagillid turbellarians found in the perivisceral coelom of *Holothuria tubulosa* from Banyuls-sur-mer were specimens of *Anoplodium parasita* and fit the descriptions of Schneider (1858), Wahl (1906) and Westblad (1953). They were grey to translucent and up to 2 mm long. In total, 37 adult specimens were found, either swimming in the host's coelomic fluids or crawling on its coelomic epithelium. Data on the infestation variables will be presented in a forthcoming paper.

Numerous golden brown turbellarian egg capsules (183,455 in total) were observed in the body cavity of the holothurians. Sometimes solitary, more often embedded in brown bodies, these capsules were mainly found in the posterior part of the coelom, near the cloaca. These egg capsules are easily recognizable by their characteristic needle shape, with the head of the capsule, containing the developing embryo, about 100 µm in diameter, and the filament, coiled and narrow, up to 1 mm long.

While only 13% of the holothurians were infested by adult worms, 128 out of the 202 holothurians inspected (63%) contained at least 6 and up to 10,000 egg capsules (mean 1433). The number of egg capsules of *A. parasita* observed in 20–21 holothurians was high at each period, except in July (Table 1): we found 4617

Table 1. Characteristics of the infestation of *Holothuria tubulosa* by egg capsules of *Anoplodium parasita*.

| | Jan 1999 | Mar 1999 | May 1999 | July 1999 | Sept 1999 | Nov 1999 | Jan 2000 | Mar 2000 | May 2000 | July 2000 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of inspected holothurians | 20 | 20 | 20 | 21 | 20 | 20 | 20 | 21 | 20 | 20 | 202 |
| Number of infested holothurians | 16 | 16 | 18 | 3 | 13 | 13 | 14 | 15 | 18 | 2 | 128 |
| Frequency of infestation (%) | 80 | 80 | 90 | 14 | 65 | 65 | 70 | 71 | 90 | 10 | 63 |
| Rate of infestation | 709 | 781 | 2199 | 1539 | 1871 | 2512 | 1447 | 764 | 1317 | 1520 | 1433 |
| Min | 7 | 15 | 24 | 530 | 54 | 146 | 48 | 6 | 7 | 814 | 6 |
| Max | 4332 | 3297 | 10,000 | 3345 | 5122 | 9231 | 5316 | 2447 | 5712 | 2226 | 10,000 |
| Number of egg capsules | 11,337 | 12,495 | 39,584 | 4617 | 24,317 | 32,652 | 20,252 | 11,460 | 23,701 | 3040 | 183,455 |

and 3040 egg capsules in July 1999 and July 2000, respectively, whereas egg capsules were two times more numerous for the other periods, with a maximum of 39,584 egg capsules in May 1999. On the other hand, at least 65% of the hosts were infested at each period (up to 90% in May 1999 and May 2000) but only 10–14% of the holothurians presented egg capsules in July (Table 1, Fig. 1). Concerning these frequencies of infestation, the period of July is significantly different from the other periods (G square, $p < 0.0001$).

Interestingly, the periods during which the egg capsules were the least frequent are the ones during which the gonads of the holothurians were highly developed. As shown in Figure 1, the gonads were virtually absent from September to March, after what they started growing and becoming larger until the critical period of July, during which the gametes were quickly released in the field: the spawning occurs in Summer. There seems to be no relation between the presence of egg capsules and the sex, size or body dry weight of holothurians.

## 4 DISCUSSION

This study is the first one that focuses on the infestation of holothuroids by turbellarian egg capsules, described over a long period and compared with the annual biology of the hosts. Working on *A. parasita* infesting *H. tubulosa* at Naples (Italy), Kroll & Jangoux (1989) found 50 to 1000 egg capsules (mean 274 for a total of 6568 egg capsules counted) in 24 out of 50 holothurians. Even though they did not mention the time of sampling, it can be said that the infestation of *H. tubulosa* by *A. parasita* is much higher in Banyuls-sur-mer, since the lowest rate of infestation, with 709 egg capsules per infested host, is three times higher than the record from Naples. Shinn (1985b) counted 0–3327 (mean 474) egg capsules of *Anoplodium hymanae* in 54 individuals of *Parastichopus californicus* from June 1981 to April 1983 (five different periods). This rate is again much lower than what we observed in Banyuls-sur-mer.

Several authors were intrigued by the way turbellarian egg capsules are released from the holothurian coelom. Changeux (1961) suggested that egg capsules of *A. parasita* are cast out when the host eviscerates. This phenomenon was observed by Jespersen & Lützen (1971) for the egg capsules of *A. stichopi* in *Stichopus tremulus* or by Byrne (1985) for the gastropods *Thyonicola americana* in *Eupentacta quinquesemita*. Although evisceration occurred for few *H. tubulosa* maintained in aquarium, none of the 202 holothurians dissected showed any signs of evisceration, which strongly suggests that evisceration in *H. tubulosa* does not occur in the field. One should particularly notice that all holothurians investigated in July, which were without egg capsules, presented an intact digestive tract. Shinn (1984, 1985a) elegantly suggested that turbellarian egg capsules might be released through coelo-cloacal ducts, which have been described ultrastructurally in *Parastichopus californicus* (Shinn et al., 1990). For this species, the diameter of the coelo-cloacal ducts, about 50 μm at rest, is not too small to allow the release of egg capsules because

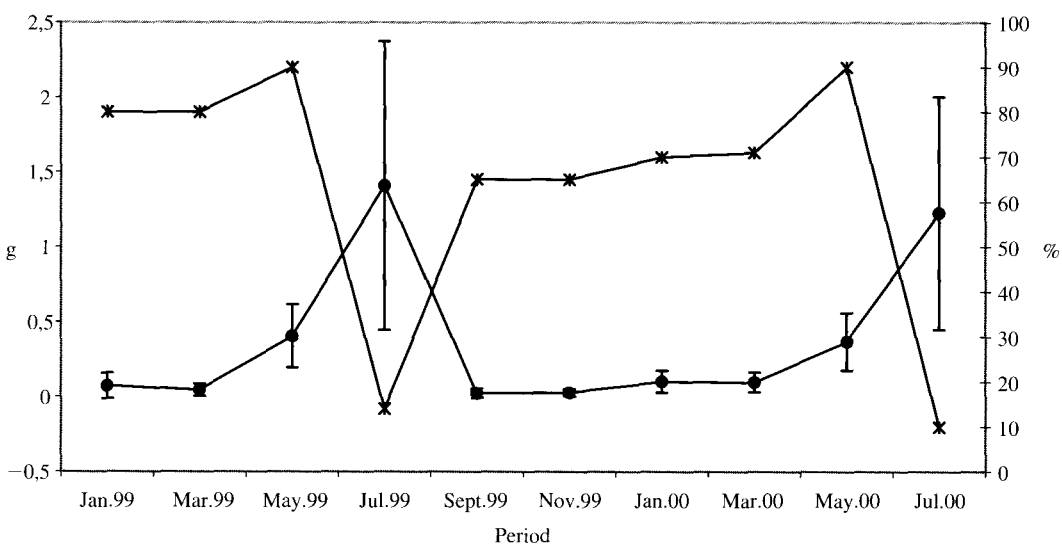


Figure 1. Frequencies of infestation (with asterisks) of *Holothuria tubulosa* by egg capsules of *Anoplodium parasita* and mean gonad dry weight (g = gram) of *Holothuria tubulosa* (with circles, bars indicate standard deviation).

these ducts can be enlarged under pressure of the coelomic fluids (Shinn 1985a). The same could logically happen in *H. tubulosa*, for which coelo-cloacal ducts have been mentioned by Jans et al. (1996).

The number of egg capsules found in an holothurian is certainly highly variable and must depend on different factors influencing their production (the number of adult worms and their reproductive capacities) and their maintenance in the coelomic cavity (depending on any of the biological changes in the behaviour of the host (contractions of the body) or its physiology (immunological response, for example)). According to Shinn (1985a, b), egg capsules are released to the environment at all times of the year. However, our results strongly suggest that most of the egg capsules accumulate in the coelomic cavity until a sudden discharge around July or just before (i.e. the spawning period of *H. tubulosa*). Our hypothesis is therefore that the release of the egg capsules is actually correlated with the spawning of holothurians. To optimize the swarming of their gametes, holothurians raise their body in the water column, adhering on the substratum only by the peri-cloacal end, and then contract the gonad and body wall muscles. During this process, a surpressure should occur in the coelom of the most posterior part of the animal. As a result, the coelomic fluids would be expelled through the coelo-cloacal ducts, carrying away their contents to the cloaca. Brown bodies with egg capsules are indeed mostly found in the posterior part of the coelomic cavity, often trapped in the suspensor strands of the cloaca, which gives our hypothesis further support.

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