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LIFE HISTORY OBSERVATIONS ON A FEW INTERSTITIAL OPISTHOBRANCH  
GASTROPODS FROM THE GULF OF MARSEILLES,  
BOUCHES DU RHÔNE, FRANCE

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RESUMO - Foi estudada a vida reprodutiva das 5 espécies dominantes de opistobrânquios mediterrâneos intersticiais do Golfo de Marselha, Bouches-du-Rhône, França. Duas espécies hermafroditas *Philine catena* e *Embletonia pulchra*, provaram ser anuais, sendo a duração de vida da última ligeiramente maior que um ano no aquário. Essas duas espécies mostram, em biótopos de alta e média exposições, um único período de recrutamento no verão ou/e no outono, implicando em acasalamento no outono precedente, ovipostura no outono-inverno, eclosão das larvas e estabelecimento dos estádios meiobênticos jovens na areia, durante a estação quente, na primavera-verão. O Acochlidae hermafrodito, *Hedylopsis spiculifera*, o qual pode sobreviver mais de um ano no aquário, é provavelmente também uma espécie anual, que passa por uma geração por ano. Contudo, uma fase flutuante de 4/5 meses, é registrada entre populações de biótopos de alta e média exposições: nos de média exposição há um recrutamento discreto na primavera enquanto que nos de alta exposição há um recrutamento mais importante no outono. Em biótopos de exposição intermediária há um prolongamento do período de recrutamento, do fim da primavera ao fim do outono e adultos podem ser observados no inverno e no verão. Os dois Acochlidae de sexos separados, *Pontohedyle mi-laschewitschii* e *Unela glandulifera*, mostraram ser espécies sub-anuais, cuja duração de vida é suposta ser não maior do que 6 a 7 meses; de fato, sua manutenção no aquário não ultrapassou algumas semanas. Essas duas espécies podem passar por 2 gerações por ano, desde que elas estejam no seu ambiente ótimo e se 2 estações de aquecimento (ou uma prolongada) forem registradas durante um ano, geralmente primavera e outono no Mediterrâneo. Uma primeira estação de ovipostura é presumida no inverno, eclosão e estabelecimento dos estádios jovens na areia, durante a primavera; esses jovens correspondem, onde e quando sobrevivem, à primeira geração descendente dos estádios adultos, alguns com espermatozoides, do outono precedente,

morrendo no inverno após a ovipostura. Uma segunda estação de ovipostura, no verão ou/e outono, eclosão e estabelecimento no outono, corresponde à segunda geração; a última, bem sucedida na maioria dos cascalhos e areias, descende dos estádios adultos da primavera precedente, mortos no outono após a ovipostura. Uma certa fase flutuante é registrada entre populações de biótopos de exposição alta e média e em exposição intermediária parece haver reprodução quase o ano todo, exceto no inverno ou primavera. Os espermatóforos observados em *P. milaschewitschii* (até 3 por indivíduo) e *U. glandulifera* (1 ou 2 por indivíduo) são relevantes nos períodos de acasalmento na primavera e no outono.

ABSTRACT - The reproductive life of the 5 dominant Mediterranean interstitial Opisthobranchs from the gulf of Marseilles, Bouches-du-Rhône, France, have been studied. Two hermaphroditic species, *Philine catena* and *Embletonia pulchra* proved to be annual ones, the life span of the latter slightly longer than one year in the aquarium. These two species exhibit both in high and mean exposure biotopes a single striking recruitment period in Summer or/and Autumn, implying mating in preceding Autumn, Spawning in Autumn-Winter, and hatching of larvae and settlement of the juvenile meiobenthic stages in the sand during the warming season, in Spring-Summer. The hermaphrodite Acochlidiacea *Hedylopsis spiculifera* which can survive more than one year in the aquarium is probably also an annual species which undergoes one generation per year. However a phase shifting of 4/5 months is recorded between populations of high and mean exposure biotopes: in those from mean exposure there is a discrete recruitment in Spring, while in the high exposure ones, there is a more important recruitment in Autumn. In intermediate exposure biotopes, there is a prolonged recruitment period from end of Spring to end of Autumn and adults can be observed in Winter and in Summer. The two monosexual Acochlidiacea *Pontohedyle milaschewitschii* and *Unela glandulifera* proved to be subannual species, the life span of which is supposed to last no more than 6 to 7 months; indeed, their maintaining in aquarium did not exceed a few weeks. These species may undergo 2 generations per year provided they are in their optimal medium and if 2 warming seasons (or a prolonged one) are recorded during a year, generally Spring and Fall in the Mediterranean. A first Spawning season is presumed in Winter, hatching and settlement of the juvenile stages in the sand during Spring; these juveniles correspond when and where they survive to the first generation descending from the adult stages, some with spermatophores, of the preceding Autumn, dying in Winter after spawning. A second spawning season in Summer or/and Autumn, hatching and settlement in Autumn, correspond to the second generation; the latter, successful in most gravels and sands, descends from the adult stages of the preceding Spring, died in Autumn after spawning. A certain phase shifting is recorded between populations of mean and high exposure biotopes, and in intermediate exposure there seem to be a year-round breeding except in Winter or Spring. The spermatophores observed in *Pontohe* -

*dyle milaschewitschii* (upto three per individual) and *Unela glandulifera* (1 or 2 per individual) are relevant to mating periods in Spring and in Autumn.

## INTRODUCTION

In the interstitial fauna, year round breeding is widespread among many of the dominant meiobenthic groups. For example, in their study of the Copepods of the North Inlet Estuary, Georgetown, South Carolina, USA, Coull *et al.* (1975) found out that the dominant meiobenthic species were in a reproductive state all year while less abundant ones had distinct seasonal reproductive periods. According to Swedmark (1964, 1969) the limited production of gametes as a result of small body size of the organisms, is compensated by a prolonged reproductive period. Only a few species, mostly interstitial Opisthobranchs, are supposed to reproduce during a period limited to 3 or 4 months in Spring and Summer (Swedmark, 1959). As a matter of fact, there seem to be very few evidences about the spawning seasons, the duration of development (larval period), the life span and in general the reproductive life of the interstitial Opisthobranchs, as a result of lack of reliable techniques for laboratory cultures, and also difficulties of observations of these tiny molluscs in the aquarium and in their natural medium. However, in this work, it has been possible to deal with a few aspects of reproductive life: mating period - when and where spermatophores occur - recruitment of juveniles, presence of adults, from which have been deducted the spawning season, the duration of the larval period from eggs to juvenile benthic stages, life span, etc. This work is restricted to five interstitial opisthobranch species which proved to be dominant in the gulf of Marseilles through the period 1970-1977. Therefore, a lot of observations and experiments in the aquarium and in natural mediums remain to be done to get a better knowledge of the reproductive life of the interstitial opisthobranch Gastropods.

## MATERIAL AND TECHNIQUES

### 1. Collecting stations, sampling and sorting of the interstitial Opisthobranchs.

The annual cycles of the interstitial Opisthobranchs have been reconstructed from informations obtained by means of regular seasonal samples. Among the 28 sampling stations prospected in the gulf of Marseilles (Fig. 1) only four have been presented in detail (Fig. 2): stations 6 and 8 of "high exposure" which proved to be "very favourable" to the interstitial Opisthobranchs (Poizat, 1978, 1981); while station 1 and 4 of "mean exposure" proved to be only "favourable" (Poizat, 1978, 1981). The "weak exposure" stations, "unfavoura

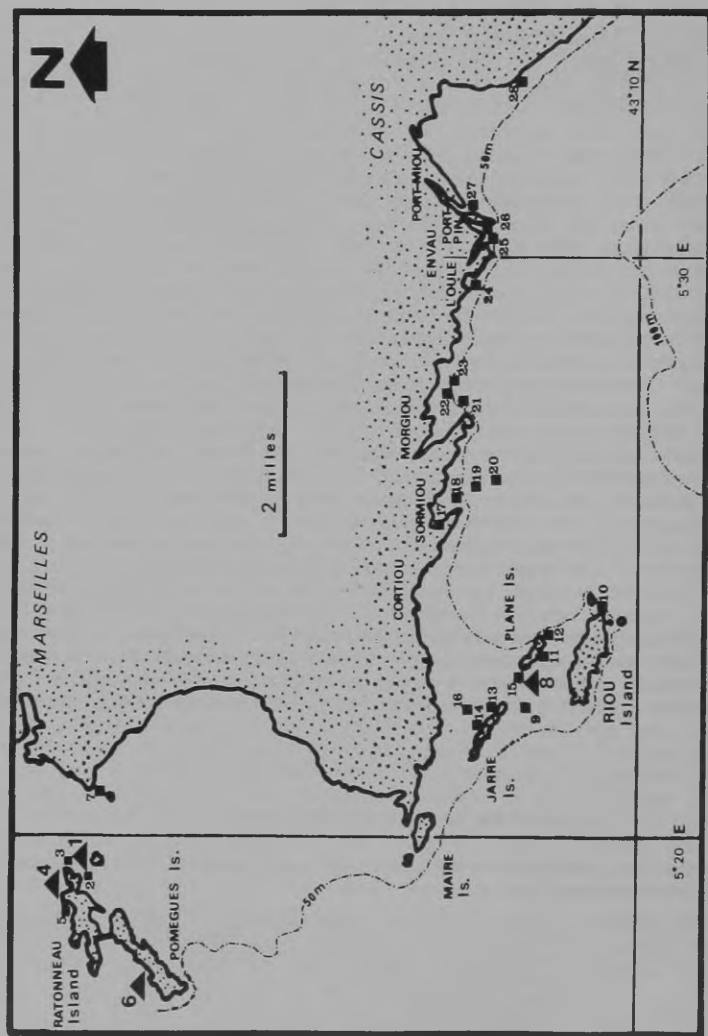


Figure 1 - Sampling stations of interstitial opisthobranch Gastropods in the gulf of Marseilles (Western Mediterranean, France): ▲ selected stations (see Fig. 2): 6 and 8 = high exposure (very favourable); 1 and 4 = mean exposure (favourable)

ble" ones, have not been considered here. In the discussion, the results of 13 (out of the 28 stations) "favourable" and "very favourable" stations are united and presented together (Figs. 6 and 7). The seasonal samples have been carried out by means of a "Charcot" dredge (Poizat, 1978) which allows the collect of huge volumes of sand (50 dm<sup>3</sup> at each station), while the Uhlig-Poizat fresh-water ice technique (Poizat, 1975; Uhlig, 1968; Uhlig et al., 1973) made sure an easy and quick separation and sorting of the interstitial Opisthobranchs which are to be observed and identified alive. Unfortunately this technique does not allow for the recolt of spawns attached to sand grains in gelatinous cocoons.

## 2. Laboratory rearing of the 5 dominant interstitial Opisthobranchs.

The studied species were maintained either in plastic boxes with shell sand or glass beads, changing the sea water every two days, or in a special sophisticated recirculated sea-water Aquarium (Poizat, 1972, 1978). The animals were kept completely dark inside small glass tubes, both extremities of which fitted with a nylon gauze of 100 µm mesh to prevent the organisms from creeping away. The glass tubes lay free on a gravel layer in 10 dark small vessels (1 dm<sup>3</sup>) fed by the circulating water of the aquarium. The water of each dark vessel was oxygenated by bubbling.

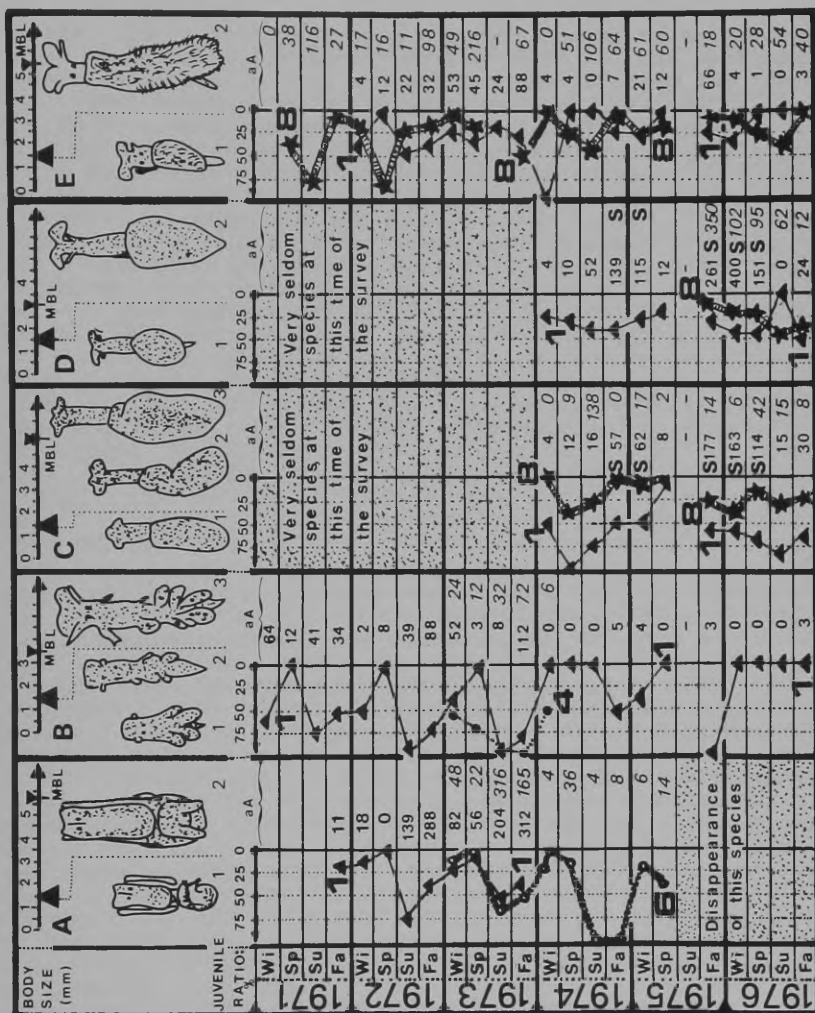
## 3. Biological material.

Ten species have been recorded in the gulf of Marseilles (Poizat, 1978, 1981, 1983a and b, 1984, 1985) during a seven-year survey (1970-1976) but only five of them, the dominant ones, have been considered in this paper. These are the following: *Philine catena* (Montagu, 1803) = Fig. 2A; *Embletonia pulchra* Alder & Hancock, 1844 = Fig. 2B; and three Acochliidae (revised taxonomy according to Salvini-Plawen in Arnaud et al., 1984): *Pontohedyle milaschewitschii* (Kowalevsky, 1901) = Fig. 2C; *Unela glandulifera* (Kowalevsky, 1901) = Fig. 2D; *Hedylopsis spiculifera* (Kowalevsky, 1901) = Fig. 2E. *Hedylopsis spiculifera* is known to be a hermaphrodite Acochliidae, with penial insemination (series Euacochliidae: Salvini-Plawen in Arnaud et al. 1984) while the monosexual *Unela glandulifera* and *Pontohedyle milaschewitschii* reproduce by aphyallid spermatophoral transfer (series Microhedyllidae: Salvini-Plawen in Arnaud et al., 1984). According to Swedmark (1968) spermatophores increase the likelihood of fertilization and can be interpreted as an adaptation which provides guarantees for fertilization for these small animals which produce very few gametes.

## 4. Determination of population size class.

The differentiation of juvenile and adult stages has been essentially founded on body size. Measurement of the size was carried out on carefully preserved specimens maintaining the animals in expanded conditions thanks to their anesthetization (nembutal 1g/l sea-water: Poizat, 1978).

Figure 2 - Seasonal variations of juvenile stage frequency (%) and average seasonal abundance (aA/50 dm<sup>3</sup> of sand) of the dominant interstitial Opisthobranchs between 1971 and 1976: A = *Philine catena*: A1 = juvenile stage; A2 = adult. B = *Embletonia pulchra*: B1 and B2 = juvenile stages; B3 = adult. C = *Pontohedyle milaschewitschii*: C1 = juvenile stage; C2 and C3 : adults. D = *Unela glandulifera*: D1 = juvenile stage; D2=adult E = *Hedylopsis spiculifera*: E1 = juvenile stage; E2 = adult .. in different exposure biotopes; stations 6 and 8 = high exposure; stations 1 and 4 = mean exposure. aA = average seasonal abundance of species: plain number = station 1; italic number = station 4, or 6, or 8. MBL = maximum body length (mm) S = spermatophores.



However, size is not always a good indicator of maturity (Hadfield et al., 1984) because temperature and food reveal their influence by controlling the rate of sexual development. But, in the species studied here, it is supposed that size reflects a set of environmental parameters common to all the organisms living in each biotope. As proposed by Miller (1962) the first evidence of a new generation has been based on the presence of small benthic stages in the samples. In addition to the size, other features such as the number of cerata, the more or less important covering of the shell by the mantle have been also helpful. **Philine catena**: the body size limit between juvenile and adult has been subjectively established around 1.5 mm (Poizat, 1978) while the maximum body length observed in the samples was MBL = 5.75 mm (Fig. 2A). In small juvenile specimens (< 1.5 mm), the mantle covering of the shell is incomplete (Fig. 2A1); then gradually, the mantle expands enveloping the shell and finally the shell is entirely covered by the mantle (Horikoshi, 1967). **Embletonia pulchra**: the body size limit between juveniles and adults lies around 1.5 mm (Poizat, 1978), while the maximum body length (MBL) observed in the samples was 3.5 mm (Fig. 2B). In the juvenile specimens (< 1.5 mm) the number of cerata ranges from 0 to 4 pairs on both sides of the dorsum (Fig. 2B1 and 2); while the adult specimens in most cases exhibit from 5 to 7 cerata on the left side of the dorsum, up to 6 on the right side (Fig. 2B3); rarely 8 cerata on the left side and 7 on the right side. In the aquarium one 2.5 mm long specimen had 9 cerata on the left and 8 on the right side (Poizat, 1972, 1978). This remarkable specimen was preserved after anesthesia, and is kept in the Author's collection. **Pontohedyle milaschewitschii** is one of the relatively long but nevertheless monosexual Acochlidiacea. The body size limit between juvenile and adult stages lies around 1.5 mm (Poizat, 1978) while the maximum body length observed was 5.75 mm (Fig. 2C). In the juvenile specimens (Fig. 2C1) the palps are small and the front part of the head is rounded instead of bilobed (Fig. 2C2 and 3). **Unela glandulifera**: the size limit between juvenile and adult stages lies around 1.5 mm while the maximum body length in the samples was 3 mm (Fig. 2D). In juvenile specimens (Fig. 2D1) the visceral hump is very short and therefore the rear part of the foot still remains visible in dorsal view, no matter whether the organism is in movement or not. In contrast, the visceral hump of adult specimens (Fig. 2D2) hides the rear part of the foot completely when the organism is motionless. **Hedylopsis spiculifera**: the size limit between juveniles and adults lies around 1.5 mm (Fig. 2E) while the maximum body length (MBL) of the samples was 5.5 mm. In juvenile specimens, the visceral hump is very short (Fig. 2E1) and does not cover the rear part of the foot. In contrast the adult specimens (Fig. 2E2) have a long spiculous hump with a typical short median depression at the rear.



## RESULTS AND DISCUSSION

The typical opisthobranch life cycle includes embryo - nic, larval, juvenile stages (benthic but not reproductive) and adult stages (reproductive) (Hadfield et al. 1984) The present work is restricted to the mating period, especially when spermatophores are involved (Microhedylacea series) the recruitment of juveniles and the presence of adult stages at different times of the year supplemented by a few observations in aquarium to look for the life span. The recruitment periods have been detected by an increase of the juvenile ratio (Fig. 2) while increase of the corresponding seasonal average abundance (aA in Fig. 2) of species is considered as relevant to both recruitment and vertical upward migrations of the organisms in their substrate and their concentration in the boundary layer as a result of variation of the ecological conditions: hydrodynamism, temperature and oxygen content of the interstitial water light, etc. (Poizat, 1983a and b, 1984) Indeed the spasmodic variations in numbers of Nudibranchs can be linked with their migratory habits (Guberlet, 1928) but also to annual die-off (Bleakney et al. 1977) particularly during the warming season after spawning.

**Philine catena.** the life cycle of this species has been satisfactorily followed between 1971 and 1975 (Fig. 2A) in a high exposure station (st. 6) and in a mean exposure one (st.1). In both mediums, in addition to a moderate presence of juvenile stages during the greater part of the year, except Spring, throughout this three-year period of observations, there has been a striking recruitment in Summer 1972 (juvenile ratio = 75% while abundance aA = 139 individuals /50 dm<sup>3</sup> sand in station 1); in Summer 1973 (ratio = 50%, aA = 204 in station 1; ratio = 70%, aA = 316 in st. 6) This striking recruitment cannot be explained by allochthonously-produced larvae as supposed by Clark (1975) for Nudibranchs of the Northwest Atlantic, because, in interstitial Opisthobranchs, larvae lack photic reaction and have relatively weak development of larval organs of locomotion, a brief swimming period (Swedmark, 1968); all of which are adaptations for animals producing few gametes and a guarantee for the survival of the species, which counteract dispersion of the larvae in the open water and keep most of them within the territory of the population (Swedmark, 1968) Adult individuals with moderate size which did not exceed 4.5 mm, were seen at the end of Spring; later, at the end of Autumn other adult specimens but with a maximum body size up to 5.75 mm, were recorded. Striking recruitment in Summer, maximum body size in Autumn imply a spawning period during the cold water season, in Winter or/and in Spring, followed by a complete die-off of the Adults. In and after 1975, a complete disappearance of this species (Fig. 2A) was recorded in the gulf of Marseilles as a result of coastal pollution (Poizat, 1978, 1983a, 1984 and 1985) The life span of this species remains doubtful since it was impossible to maintain it more than 189 days in the

plastic boxes and more than a few weeks in the aquarium as a result of starvation and/or metallic contamination (Poizat 1972, 1978) However, *Philine* may live longer than a year: according to Horikoshi (1967) *Philine scabra* may live 1 year, while according to Blegvad (1928) and Brown (1934), the life span of *Philine aperta* is up to 4 years.

***Embletonia pulchra***: life history observations of this species have been possible from 1971 to 1975 (Fig. 2B) in the mean exposure stations 1 and 4 (Fig. 1). In addition to the moderate presence of juvenile stages during the greater part of the year, except Spring, a striking recruitment of juvenile stages was recorded in Summer 1971 (ratio = 75%, aA = 41 in station 1), in Summer-Fall 1972 (ratio = 95%, aA = 39, ratio = 75%, aA = 88 in station 1) and in Summer & Fall 1973 (ratio = 98%, aA = 8 in station 1, ratio = 100%, aA = 72 in station 4). The maximum body length and maximum number of cerata (adult stages) were much delayed and observed only in Winter 1971: 5.4 left, 4.5 right; 1972: 5.5 left, 5.5 right; 1973: 5.4 left, 4.9 right. These observations may imply a spawning season in Winter or/and Spring and therefore hatching in early Summer and settlement of juvenile stages in the course of Summer *Embletonia pulchra* seems to grow very quickly, between Summer and following Winter (5 months) According to Clark (1975), the possession of cerata appears to be an adaptation permitting increased rates of assimilation and metabolism. Two individuals of *Embletonia pulchra* were maintained in satisfactory conditions in the aquarium for 1.5 year (Poizat, 1972, 1978); this exceptional life span for a rather small species is due to its great resistance to anoxic conditions (Løyning, 1927) and presumably to the presence of the necessary food, bacteria and detritus, and possibly diatoms in absence of hydroids and also to the stability of the experimental conditions (Poizat, 1972). But it is difficult to presume for this hydroid-feeder (Thompson & Brown, 1976) a life span exceeding 1 year in its natural medium. Other experiments and observations in an aquarium are needed. ***Pontohedyle milaschewitschii***: as a result of its very rare occurrence till the end of 1973, observations of the life cycle of *P. milaschewitschii* were only possible in and after 1974 (Fig. 2C) in station 1 (mean exposure) and 8 (high exposure) In addition to a relatively high but unstable juvenile ratio (25%) in both high and mean exposure biotopes during the greater part of the year except Fall 1974 (st. 8), Winter (st. 8) and Spring 1975 (st. 1 and 8), there were only a few moderate peaks: successively in Spring 1974 (st. 1 and 8); in Winter (st. 8) and Summer 1976 (st. 1 and 8). The corresponding abundance peaks slightly delayed occurred the following seasons, successively in Summer 1974 (st. 8: aA = 138 individuals/50 dm<sup>3</sup> sand) in Spring 1976 (st. 8: 42 individuals), in Winter and Spring 1976 (st. 1: 163 and 114 individuals) The presence of long specimens (adults, some with spermatophores: Fig. 2C and 3a) was recorded in Autumn 1974, Winter 1975 (st. 1 and 8) and also in Autumn 1975, Winter and Spring 1976 (st. 1 and 8). Survival of this relatively large species in the aquarium did not exceed

a few weeks (Poizat, 1978) Therefore, its life span is not known with certainty but may not exceed 7 or 8 months. The spermatophores of *P. milaschewitschii* proved to be very small (Fig. 3a) conform with Swedmark (1968) During the reproductive period, the spermatophores are attached to the animal waiting for fertilization. One, sometimes two, exceptionally three spermatophores are attached to each animal (Fig. 3a) most commonly on the dorsal part of the visceral sac (Fig. 4a): frequency of spermatophore placement = 10 in zone A3; 16 in zone A4. Sometimes, spermatophores were present in front of the head-foot complex (frequency = 1 in A1 and B1) They were always absent in the radula zone (A2 and B2) **Unela glandulifera** (Fig. 2D): observations of the life history of this species were carried out in station 1 (mean exposure biotope, Fig. 1) and 8 (high exposure) throughout the years 1974, 1975 and 1976. In contrast to the three preceding species, relatively regular high values of the juvenile ratio (> 25%) were recorded during all the year, except in Summer 1976, while very moderate peaks occurred in Summer-Fall 1974 and Winter-Spring 1976. The corresponding abundance peaks were recorded in Autumn 1974 (st. 1: aA = 139 individuals/50 dm<sup>3</sup> sand) Winter 1975 (aA = 115), in Autumn 1975 (st. 1: aA = 261; st. 8: aA = 350); in Winter 1976 (st. 1: aA = 400; st. 8: aA = 102) and in Spring 1976 (st. 1: aA = 151; st. 8: aA = 95). Large specimens (adults, some with spermatophores) were observed in Autumn 1974, Winter 1975, Autumn 1975, Winter and Spring 1976 (st. 1 and 8: Fig. 2D). In the mean exposure biotope (st. 1) the more favourable biotope for this species (Poizat, 1978, 1981), the recruitment seems to occur during a more prolonged period of the year (peaks in Spring and Fall) except during the warm season (Summer) while copulation may occur during colder months. In contrast, in high exposure biotopes (st.8), less favourable ones for this species (Poizat, 1978, 1981), the recruitment peak is restricted to the warm season (Summer) The life span of **Unela glandulifera** remains doubtful since it was very difficult to maintain it in the aquarium more than a few weeks as a result of starvation (Poizat, 1978) In **Unela glandulifera**, the spermatophores are very long (Fig. 3b) almost as long as the visceral sac (Swedmark, 1968). In most cases, only one spermatophore (rarely 2) is attached (Fig. 4b) generally on the dorsal part of the visceral sac (frequency of spermatophoral placement = 13 on zone D3 and 9 on zone C4). Contrary to the preceding species (**Pontohedyle milaschewitschii**) spermatophores can be observed in front of the head-foot complex (frequency = 5 in zone D1) while no spermatophores at all were recorded in the radula zone (region C2 and D2: Fig. 4b). **Hedylopsis spiculifera** (Fig. 2E): the reproductive cycle of **Hedylopsis spiculifera** was studied (Fig. 1) in station 1 (mean exposure) and 8 (high exposure) from 1971 to 1976. In both stations, juvenile specimens were collected the year round. In the mean exposure station 1, less favourable medium for this species (Poizat, 1978, 1981) moderate peaks occurred in Summer 1972 (juvenile ratio = 50%, aA = 12). Spring 1973 (juvenile ratio = 40%, aA = 45), Winter 1974 (ratio = 100%, aA = 4) In contrast, in the high exposure station 8, very fa

Figure 3 - Spermatophoral placement in: **Pontohedyle mi-laschewitschii** (a): 45 specimens observed, 33 with only one spermatophore, 8 with 2 and 4 with 3 (very small spermatophores in this species), - **Unela glandulifera** (b): 40 specimens observed, 34 with only one spermatophore and 6 with 2 (very long spermatophores in this species)

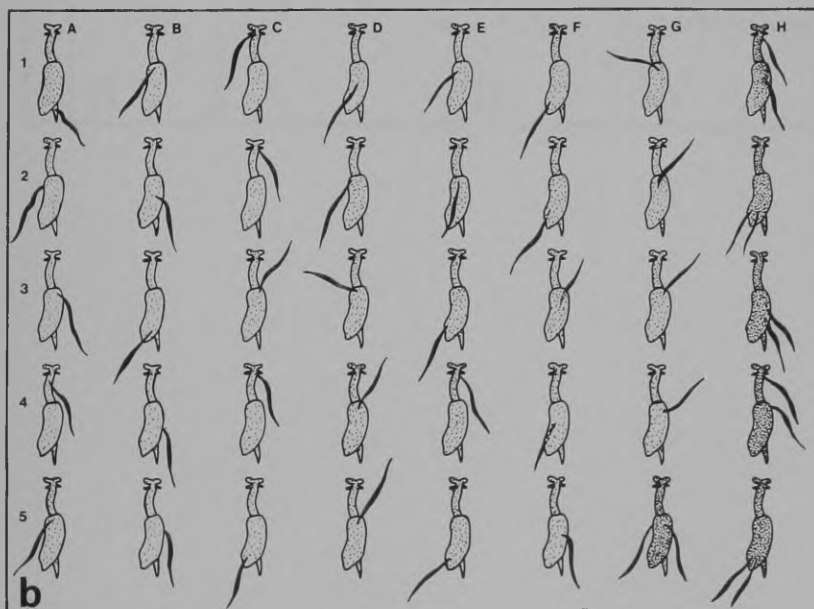
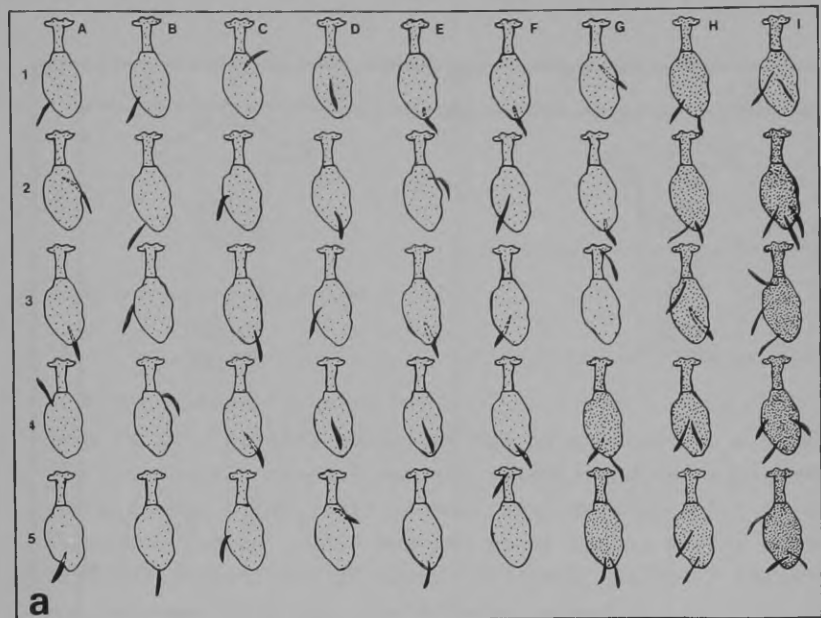
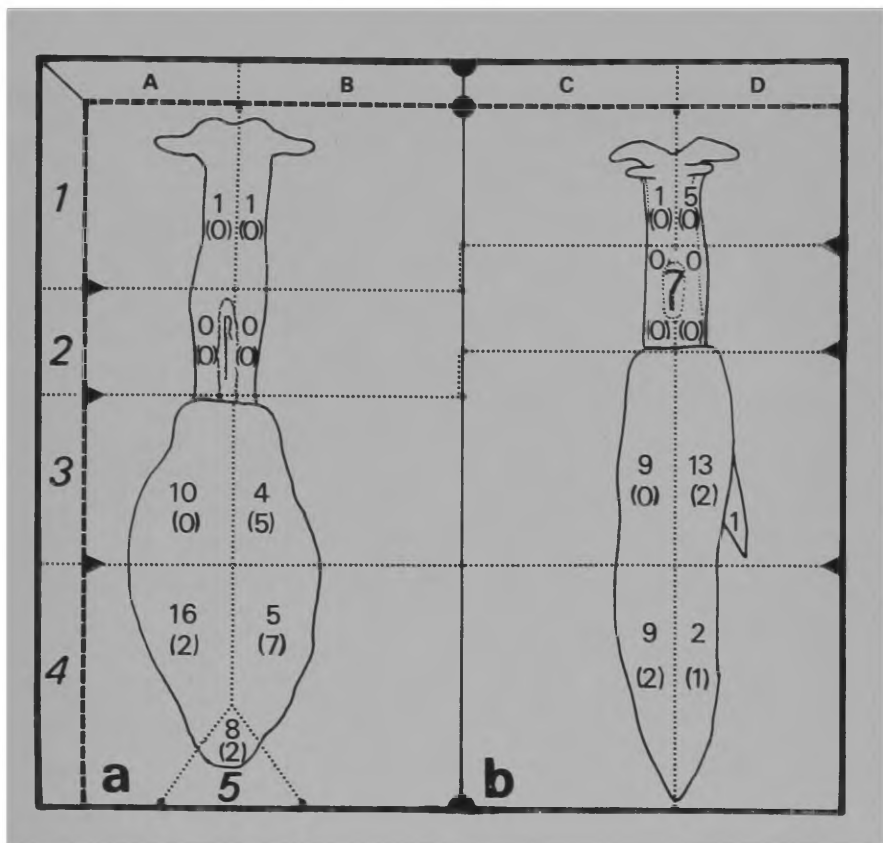


Figure 4 - Frequency of spermatophore position in: a) **Pon-**  
**tohedyle milaschewitschii**: maximal frequency in dorsal zo-  
nes A4 (16 spermatophores) and A3 (10) while none are re-  
corded in the radula zones (A2 and B2) Values between  
brackets = ventral position. Total of specimens taken into  
account = 61. b) **Unela glandulifera**: maximal frequency on  
right side of the dorsum D3 (13 spermatophores) while none  
are recorded in the radula zones (C2 and D2) Total of spe-  
cimens taken into account = 43.



avourable medium for this species, striking recruitment peaks (ratio varying from 25 to 90%) occurred in Summer 1971 (aA = 116), Spring 1972 (aA = 16), Spring & Fall 1973 (aA = 67), Summer 1974 (aA = 106, Summer 1976 (aA = 54). 19 individuals of this species survived from 8 months to 1.5 year in the aquarium (Poizat, 1972, 1978) but with a significant loss of size presumably as a result of starvation. The sizes of the animals in the experiments decreased from 2.20-2.40 mm to 0.86-0.90 mm, and from 2.74-3.25 mm to 1.64-1.68 mm (Poizat, 1978). The necessary food, mainly diatoms, was lacking in the experimental conditions, but it is admitted that the life span of this species can exceed 1 year in natural medium.

In his study of the Manx Nudibranchs, Miller (1962) arranged the species in two groups on the basis of the number of generations produced in each year: group 1 with usually one, exceptionally 2 generations (for example: *Elysia viridis*); group 2 with two or more, often several generations (for example: *Limapontia capitata*). Thompson (1964) also distinguished two groups in the British Nudibranch fauna: the first with an annual life cycle, dependant of abundant and stable prey such as Tunicates and Bryozoans; a second with a shorter life, year round breeding and dependance on unstable prey such as hydroids. Todd (1981) recognized 3 ecological groups of Nudibranchs: "subannual" species with a life span of a few weeks or months undergo several overlapping generations per year and generally feed on seasonally transitory prey. Reproductive individuals may be found sporadically at several times in the year or if there is considerable asynchrony among generations, reproductive individuals may be found the year round. Many aeolids and smaller dorids fit into this category; "annual" species usually have a single generation per year, a discrete, synchronized spawning period, and feed on temporally stable prey. They correspond to larger species (mostly dorids) and feed upon animals that live all the year such as sponges, barnacles, bryozoans, etc. ; "biannual" species have only a single spawning period after a post larval life of almost 2 years. Large animals (Dendronotaceans, Dorids) fit into this category. They have large, long-lived prey such as sponges and alcyonarians.

According to the classifications proposed by Miller (1962), Thompson (1964) and Todd (1981) on the one hand, the literature and the few experimental results and observations in the aquarium on the other hand, the dominant interstitial Opisthobranchs studied here can be interpreted either as "subannual": *Pontohedyle milaschewitschii*, *Unela glandulifera*; or "annual" *Philine catena*, *Embletonia pulchra* and *Hedylopsis spiculifera*.

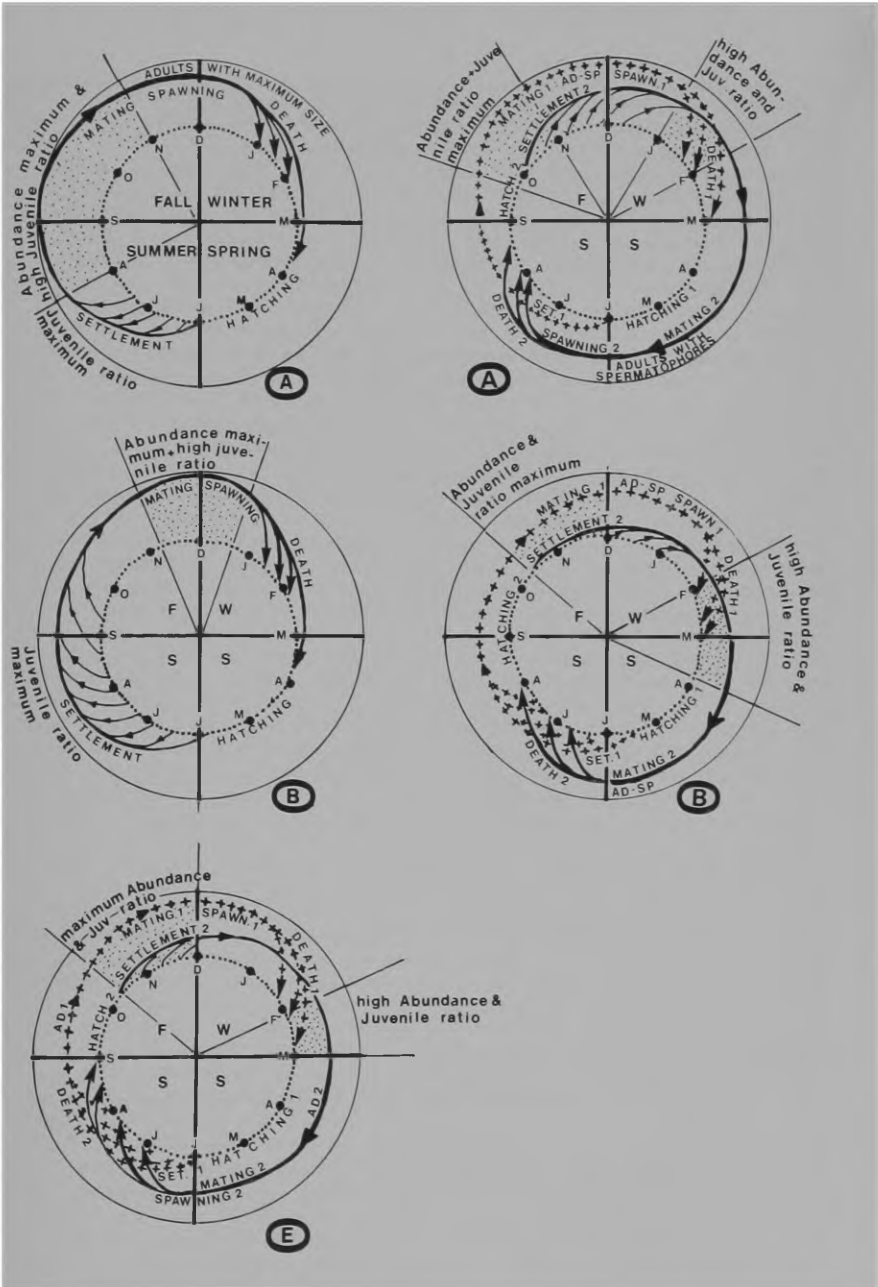
The "annual" interstitial Opisthobranchs: *Philine catena* (Fig. 2A) *Embletonia pulchra* (Fig. 2B) and *Hedylopsis spiculifera* (Fig. 2E). If we consider as a whole the united favourable and very favourable biotopes the two species *Philine catena* (Fig. 5A) and *Embletonia pulchra* (Fig. 5B) are characterized in both high and mean exposure biotopes by a striking unique recruitment in Summer (juvenile ratio peak and abundance peak more or less coinciding) juvenile stages during the greater part of the year except in Spring. Very few adults of



**Philine catena** were observed in Spring and they are characterized by a moderate size. They may represent the last survivors of the preceding year's generation, in which maturation of gonads and subsequently spawning has resulted in a loss of body size. Indeed, Seager (1982) found out that, in the Antarctic Opisthobranch **Philine giba** Strebel, a significant loss of weight occurred in half the fertile adults at spawning, subsequently leading to the die-off of these animals. In Autumn, the adult specimens of **Philine catena** exhibit a maximum body length. Mating occurs at this time of the year, spawning later on in Winter. **Embletonia pulchra**, hydroid-feeder (Thompson & Brown, 1976) is also certainly able to ingest detritus and bacteria during its interstitial incursions (Poizat, 1983b) while the microcarnivorous **Philine catena** (Ros, 1978) is suspected to feed upon other prey than Foraminifera, for example upon the Acochlidiacea **Unela glandulifera** (Poizat, 1983b). Both species therefore can take advantage of a stable year-round diversified food-supply. This set of characteristic enhances for these species the probability of only one generation per year (group 1 of Miller, 1982) with a life span slightly exceeding one year in their natural medium in contrast to the two preceding annual species, **Hedylopsis spiculifera** which proved to be an "annual" species in the aquarium, showed discrete recruitment peaks in mean and high exposure biotopes (Fig. 2E). If we consider as a whole the favourable and very favourable stations united (Fig. 6) for this species, in addition to abundance and juvenile ratio peaks at the end of Autumn, there is also a discrete abundance peak at the end of Winter, corresponding to a high juvenile ratio but not to a juvenile ratio peak. As a matter of fact, this late Winter abundance peak does not correspond to a second recruitment period; it merely represents a phase shifting between populations from high hydrodynamism (very favourable for this species: Poizat, 1981) and populations from mean hydrodynamism (favourable for this species: Poizat, 1981). Indeed, the life cycles of each population proved to be asynchronized from one yearly station to the other (up to 4 months), probably as a result of noticeable differences in the ecological parameters. The various degrees of exposure due to the topography of the coast and the bottom lead to differences in the annual curve of temperature. Mean exposure = sheltered biotopes generally have the warming season earlier than the high exposure ones and conversally the cooling season later. Therefore, the organisms grow quicker and reproduce earlier in the former since temperature plays an important part in the developmental rates of individuals (Dehnel & Kong, 1979; Edmunds, 1977).

The "subannual" interstitial Opisthobranchs: **Pontohedyle milaschewitschii** (Fig. 2C) and **Unela glandulifera** (Fig. 2D). In contrast to the preceding annual category, these two presumed sub-annual Acochlidiacea are generally characterized by a recruitment depending strikingly on the degree of exposure of the stations: **Pontohedyle milaschewitschii** (Fig. 2C) which prefers high exposure biotopes (Poizat, 1981) has 2 generations per year in high exposure station 8 where it exhibited two juvenile-ratio peaks in Winter and in Summer 1976,

Figures 5, 6 and 7 - Interpretative seasonal reproductive cycles (with favourable and very favourable stations united: see Figure 1) of: A = **Philine catena**; B = **Embletonia pulchra**; C = **Pontohedyle milaschewitschii**; D = **Unela glan**ulifera; E = **Hedylopsis spiculifera**.



a year of abnormal warming (Poizat, 1978, 1983a), while the same species only had one generation in the less favourable mean exposure station 1. *Unela glandulifera* (Fig. 2D) which prefers mean exposure biotopes (Poizat, 1981) had 2 generations in Spring and Autumn 1976 in the mean exposure station 1 while there was only one recruitment peak in high exposure station 8. According to Miller (1962), temperature and food supply have an effect on the number of generations produced in a year by members of group 2. An increase in either or both of these factors must accelerate the growth and attainment of maturity and thus increase the number of generations in a year. *Unela glandulifera* and *Pontohedyle milaschewitschii* sometimes undergo 2 generations per year in their optimal medium, while only one generation is recorded in the less favourable ones. The consideration as a whole of the favourable and very favourable biotopes united (Poizat, 1978) emphasizes this occurrence of two generations per year with particularly 2 abundance peaks coinciding with high and sometimes maximum juvenile-ratio: middle of Winter and middle of Autumn (*P. milaschewitschii*: Fig. 7A); end of Winter and end of Autumn (*Unela glandulifera*: Fig. 7B). In spite of the presence of juvenile stages the year round (except in Spring) there is not a great asynchrony among the generations since reproductive individuals, recognized by their spermatophores, are not observed the year round but only twice per year in Spring and in Autumn. For both species, our data may imply a first spawning season in Winter, hatching and settling of the juvenile stages in the sand in Spring corresponding to the first generation represented by the adult stages observed during the preceding Autumn and which die in Winter after spawning, resulting in a very low abundance of both species. This first generation is never successful in unfavourable biotopes; according to Seager (1982) in his study of the life cycle of the Antarctic *Philine gibba*, intraspecific competition for food may inhibit growth of juveniles to such an extent that they can be eliminated by starvation. In contrast, this first generation of *Unela glandulifera* and *Pontohedyle milaschewitschii* is more successful in favourable or very favourable biotopes where it is recognized by high abundance. A second spawning season in Summer, hatching and settlement in Autumn, correspond to the second generation represented by the adult stages observed during the preceding Spring and which die in Autumn after spawning (juvenile ratio at a maximum). The two Acochlidiacea (*U. glandulifera* and *P. milaschewitschii*) presumably belong to Miller's group 2 (1962) in which each Nudibranch has a life history adapted to make the most of fluctuating food supply. *P. milaschewitschii* is known to feed upon epipsammic diatoms and also upon detritus and bacteria (Hadl et al., 1970; Poizat, 1978, 1983b). Its food could therefore consist of diatoms in Spring (first generation, successful in very favourable biotopes) and sea-weeds and sea-grass detritus and bacteria in Autumn (2nd generation). These diatoms give the gut of these animals a light green colouration in Spring and this colouration varies seasonally becoming dark brown in certain individuals collected in Autumn. Similarly, *Unela*

**glandulifera** feeds on epipsammic diatoms and detritus at the surface of the bottom during the night (Poizat, 1983b), the abundance of which in the boundary layer depends on seasonal cycles of the ecological parameters (light, temperature, oxygen, hydrodynamism, etc. .) Differences in these ecological parameters, from one station to the other prove to have a considerable impact on subannual interstitial species, the latter in high temperature conditions being given a chance to have two generations in the year if they are in their optimal biotope.

#### CONCLUSIONS

Two recruitment periods corresponding to 2 generations in a year are deduced for the two interstitial Acochliidae: the monosexual species **Pontohedyle milaschewitschii** and **Unela glandulifera**, both appearing as "subannual" species, the life span of which does not exceed 7 to 8 months. The first generation (in Spring) is successful only if the organisms are in their optimal biotopes: high exposure for **Pontohedyle** and mean exposure for **Unela**. The second generation in Autumn is always more important (juvenile ratio and abundance peaks more or less synchronized), and occur in all the favourable or very favourable mediums. The respective importance of the two slightly asynchronized and overlapping generations is supposed to be linked to the variation of food supply at the surface of the bottom: mainly epipsammic diatoms in Spring and detritus + bacteria in Autumn. In contrast, **Philine catena** (microcarnivorous) and **Embletonia pulchra** (hydroid-feeder) both hermaphroditic interstitial Opisthobranchs, which exhibit only one striking recruitment period in Summer or/and Autumn proved to be "annual" species which are supposed to live more than a year and to feed upon a more permanent and diversified food supply consisting of diatoms + bacteria + hydroids and other interstitial organisms. **Hedylopsis spiculifera**, hermaphroditic Acochliidae is difficult to interpret. This species which proved to live more than one year in the aquarium (annual species), exhibits one discrete recruitment period depending on the degree of exposure: in Spring in the mean (sheltered) exposure biotopes = high abundance and juvenile ratio; in Autumn in the high exposure biotopes = maximal abundance and juvenile ratio. Therefore, in intermediate exposure biotopes, there is a prolonged recruitment period from the end of Spring to the beginning of Winter

No more than 2 long spermatophores are received by one and the same individual in **Unela glandulifera**, while **Pontohedyle milaschewitschii** can receive up to three very short ones. In both species, spermatophores were recorded on any part of the body, except in the radula zone (rear of head-foot complex). In most cases, spermatophores were observed on the dorsal part of the visceral hump, in the closest vicinity of the genital gland attached to the digestive one in these tiny species.

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