REPRODUCTIVE BARRIERS AND EARLY DEVELOPMENT FROM HYBRIDIZATION EXPERIMENTS IN TWO SYMPATRIC SPECIES OF THE GENUS SARSIA (CNIDARIA, HYDROZOA, ANTHOATHECATAE, CORYNIDAE)

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REPRODUCTIVE BARRIERS AND EARLY DEVELOPMENT FROM HYBRIDIZATION EXPERIMENTS IN TWO SYMPATRIC SPECIES OF THE GENUS *SARSIA* (CNIDARIA, HYDROZOA, ANTHOATHECATAE, CORYNIDAE)

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SARSIA
CNIDARIA
HYBRIDIZATION
REPRODUCTIVE ISOLATION

ABSTRACT. – Mechanisms which isolate two sympatric species of the genus *Sarsia, S. apicula* (Murbach & Shearer, 1902) and *S. tubulosa* (M. Sars, 1835) in a sheltered bay off southern Vancouver Island, Canada, were investigated. Cross-breeding experiments using the two species revealed that there are two separate but linked factors which prevent hybridization. One barrier is an 8 to 9 h time difference in spawning time. The second is an inability of the two species to interbreed in interspecific crossings between ♂×♀, but not with ♀×♂ (asymmetrical breeding), even when spawning time is experimentally modified and made synchronous. The few embryos which resulted from *S. apicula* ♀ × *S. tubulosa* ♂ pairings under natural light were not viable beyond the cyst stage. Zygotes did not develop from *S. apicula* ♂ × *S. tubulosa* ♀ crosses under natural light because of decay of eggs after an 8-9h time lag. Viable hybrids were raised from pairings once spawning times had been experimentally synchronized, but only from *S. apicula* ♂ × *S. tubulosa* ♀ and not from the opposite combination. These hybrids, produced under experimentally altered light conditions, were comparable in numbers to the embryos and ensuing hydroids from intraspecific pairings of either species.

SARSIA
CNIDARIA
HYBRIDATION
ISOLEMENT REPRODUCTIF

RÉSUMÉ. – Les mécanismes isolant les deux espèces sympatriques du genre *Sarsia, S. apicula* (Marbach et Shearer, 1902) et *S. tubulosa* (M. Sars, 1835) d'une baie abritée au sud de l'île de Vancouver, au Canada, ont été étudiés. Des expériences de croisements des deux espèces en élevage révèlent que deux facteurs séparés mais liés empêchent l'hybridation. Une première barrière est un décalage de 8 à 9 h dans le moment de la ponte. La seconde barrière est l'impossibilité pour les 2 espèces de se croiser entre ♂ × ♀, alors que le croisement ♀ × ♂ est possible (croisement asymétrique), même si le moment de la ponte est modifié expérimentalement et rendu synchrone. Les quelques embryons résultant des croisements *S. apiculata* ♀ × *S. tubulosa* ♂ en lumière naturelle ne sont pas viables au delà du stade du cyste. Les zygotes provenant des croisements *S. apicula* ♂ et *S. tubulosa* ♀ sous lumière naturelle ne se développent pas à cause de la dégénérescence des œufs après 8 à 9 h de décalage. Des hybrides viables sont obtenus à partir de croisements opérés sur des pontes rendues synchrones, mais seulement avec *S. apicula* ♂ × *S. tubulosa* ♀ et non avec la combinaison inverse. Ces hybrides, produits expérimentalement en conditions lumineuses modifiées, sont comparables aux embryons et aux stades hydroïdes provenant de croisements intraspécifiques de chaque espèce.

INTRODUCTION

Several species of the genus *Sarsia* Lesson have been described from the southern British Columbia coast, Canada and the San Juan Islands, Washington, USA (Arai & Brinckmann-Voss 1980, Mills 1980, Miller 1982, Brinckmann-Voss 1985, 2000, Strathmann 1987). Some of these species morphologically resemble *S. tubulosa* (M. Sars, 1835), but they are difficult to distinguish, especially if the medusa stage only is considered. Miller (1980a, 1982), working in the nearby San Juan Islands (Washington state, USA) on the problem of sperm attractants in intraspecific and interspecific matings ("intraspecific" for crossings within one species and "interspecific for crossings between species is used throughout this paper; as used for instance by Szmant *et al.* 1997 and others), reported some biological separation of *Sarsia tubulosa* like

medusae. He therefore considered them "sibling" species, defined by Mayr (1982: 271, 281) as morphologically similar populations which are reproductively isolated. In later comparative studies of the genus *Sarsia* from the southern part of Vancouver Island and the San Juan Islands (Brinckmann-Voss 1985, 2000), "morphotypes of the *Sarsia tubulosa* complex" (see Miller 1980b,1982) were separated into species through morphological characters, such as egg size and structure of the hydroids. The *Sarsia tubulosa* morphotypes from Friday Harbor (San Juan Islands) should now be considered separate sympatric species, being morphologically different and having a certain degree of reproductive isolation as shown by Miller (1982) (note that Miller's *Sarsia* S is *S. apicula*, and *Sarsia* L is *Sarsia bella*; see Brinckmann-Voss 2000 for discussion of these species). Based on end-of-the-season collections of *Sarsia,* which were difficult to identify, Miller suspected hybridization among *Sarsia tubulosa* morphotypes from Friday Harbor, but this assumption needed confirmation. Experiments done as part of my studies, with material from Friday Harbor and Sooke Harbour (locations less than 65 km apart) did not produce viable zygotes in crosses between *S. tubulosa* morphotypes now identified as *S. bella* (*Sarsia L* in Miller 1982) and *S. apicula* (*Sarsia S* in Miller 1982).

The regular abundance in sheltered Sooke Harbour of two morphotypes of *Sarsia tubulosa* now identified as *S. tubulosa* (M. Sars, 1835) and *S. apicula* (Murbach & Shearer 1902), were used in this study of reproductive isolation. This seemed interesting because Miller & Babcock (1997) and Wallace & Willis (1994) studying sympatric corals, and Pernet (1999) sympatric polychaetes, have reported that even morphologically distinct sympatric species seem to hybridize in the laboratory, with the barriers of reproductive isolation apparently not functioning. Reproductive isolation had generally been considered an effective barrier between sympatric species until now, defining species through their biological properties. Notably Mayr (1982: 273) considered the biological species concept superior to the older morphological species definition. The authors noted above sought explanations to seeming contradictions of biological versus morphological species in the "ecological niches" of each species.

The term "biological" species versus the "morphological "species (discussed and summarized in Mayr 1982), was based mainly on terrestrial organisms, where there are enough different barriers to allow for the evolution of allopatric and sympatric species (defined in Mayr, Linsley & Usinger 1953). Palumbi (1994) however, discussed the application of this concept to the marine environment, which superficially seems to lack the various natural barriers known from the terrestrial habitats. Based on a number of studies he was able to report the existence of various barriers in the marine environment which would explain the diversity of species in numerous marine groups. The two sympatric hydrozoan species *Sarsia tubulosa* and *S. apicula* appeared to be good material for a study of these reproductive barriers.

Any research adressing evolution and maintenance of species should include genetic testing of the species, and if present, their hybrids. As my main research is morphology and biology of hydrozoans, molecular biological methods were not used here; however, material from the present work was preserved for genetic testing.

MATERIAL AND METHODS

Collection: the medusae were collected by dipping from a floating dock at a small marina in Sooke Harbour, where medusae drift by with the tide. Medusae were most abundant near the surface about three hours after low tide especially during spring tides, when water currents were maximal. The medusae were put in a large insulated container, and returned immediately to the laboratory. There they were placed in a large glass bowl under natural light, not later than 30 min. after collecting, to prevent spawning (see below). The animals were then separated into the two species and each species into ♂ and ♀ specimens. From these "bulk" glasses single pairs in different combinations for intraspecific and interspecific crosses were placed in individual glass cups holding either 140-150 or 240-250 ml water. Cups with rounded walls are preferable to glasses with straight walls because counting of gametes, embryos and primary hydranths was easier in glasses with rounded walls. To facilitate counting, the outer bottoms of the glasses were divided in 8 or 16 numbered segments with a marker. Sea water used for maintaining collected medusae and for all experiments was collected from as unpolluted areas as possible, paper filtered, and left standing at least a week in to avoid any contamination with sperm or eggs. Moreover, additional ♀ only medusae were used in all sets of breeding experiments to find any contamination with sperm which presence could be detected from fertilized eggs. All sets of crossings between *Sarsia tubulosa* and *S. apicula* were done with four to eight separate breeding pairs, with half of them opposite sexes to detect individual variations in hybridization pattern. In addition each set of crosses included a separate pair of *S. tubulosa* ♂♀ and *S. apicula* ♂♀ as control. Each set of breeding experiments, consisting of a total of 6 to 10 separate breeding pairs, was repeated with medusae from new collections 17 times during April/May 2002 and 10 times during April/May 2001. Many more breeding experiments involving the two species were done in previous years, but due to different spawning times of the two species, and different life span of male and female gametes in the case of *S. tubulosa* (see below), results were often erratic and difficult to interpret. Results became repeatable and clearer to analyze only, when methods were found in 2001-2002

to synchronize spawning times of *Sarsia tubulosa* and *S. apicula.*

Sperm density was not measured, but in order to keep sperm density fairly constant each set of matings used same size glasses containing either 140-150 ml or 240-250 ml of sea-water. As the intraspecific pairs of *S. tubulosa* ♂×♀ and *S. apicula* ♂×♀ in each set of experiments had a fertilization rate of 94-100 % (n=27) it was assumed that the sperm density was sufficient for fertilization and independent of the two glass sizes or individual variation in the male medusae. Insufficient sperm density could therefore be excluded as cause for the low fertilization rate in part of the interspecific crossings (additional intraspecific crossings of the two species done in the years before 2001 had always the same high fertilization rate).

Once zygotes reached 2-8 cell division stages the parent medusae were removed from the culture bowls and preserved in 95 % ethyl alcohol.

Primary hydranths were fed with nauplii of *Thisbe* sp. (Brinckmann-Voss 1985) within seven days of appearance from the cyst. If left unfed longer the tentacles of the primary hydranths regressed and did not regenerate. Large hydranths and whole colonies were fed with adult *Thisbe* sp., which is preferable to brine shrimps, because the hydroid colonies needed to be handled once a week only (this feeding method applies for *Sarsia* hydroids only; other hydroid genera or medusa may have to be fed more often).

Medusae liberated from their hydroids were also first fed with *Thisbe* sp. nauplii and later with brine shrimps and adult *Thisbe* sp. on alternate days.

For the breeding experiments the medusae bowls were kept either in natural light/dark or, in case of the light/ dark experiments, were put under a 60 w lamp during the night, followed by placing the glasses in a box during dawn and daylight.

Photographs were taken with a Wild/Leitz MPS 52 camera used either on a stereomicroscope M8, M 420 or on a compound microscope Leica DMLB equipped with interference phase contrast (Nomarski). Most pictures are from living specimens.

RESULTS

Morphological differences between Sarsia tubulosa *and S.* apicula

The medusae (Fig. 1 a,d) redescribed by Edwards 1978, Arai & Brinckmann-Voss 1980, & Brinckmann-Voss 1985, Schuchert 2001, are very similar, nearly impossible to distinguish when preserved. Their most distinctive characters when living are the bluish tentacles in *S. tubulosa* compared to bright red tentacles in *S. apicula* which can already be seen in the sea. The red tentacles are not dependent on food, but are a specific character as discussed by Brinckmann-Voss 1985.

The mesogloea at the margin is thicker in *S. apicula* than in *S. tubulosa,* which results in more bulging of the mesogloea around the marginal bulbs in *S. apicula* than in *S. tubulosa* (compare Fig. 1b with 1e; see also Fig. 4c in Miller, 1982 as *"Sarsia s."= S. apicula*).

The hydroid stages (Fig. 1c, f) of both species are morphologically more distinctive (Edwards 1978, Brinckmann-Voss 1985) than the medusa stage.

Ecological remarks

Adult medusae of *S. tubulosa* appear on the surface waters of Sooke Harbour between end of March and end of April at a water temperature of 6-9°C, and diminish by the beginning of June. *S. apicula* is present from middle of April to beginning of July, later than *S. tubulosa* which it slowly replaces from end of May on. During May, when both species are abundant, their populations may become very dense and often more than 10 specimens may be seen in an area about 10 square cm.

In the laboratory *S. tubulosa* hydroids bud medusae between 4° and 10°C from December to May. Four different colonies aged one to four years showed no variation in this pattern of medusa budding and all four colonies started budding within the same week in December. However these temperatures apply to the *S. tubulosa* hydroid colonies raised from medusa from Sooke Harbour only. *S. tubulosa* hydroids obtained by the author from Helgoland, North Sea bud at higher temperatures (above 8°C). Werner (1963) reported budding temperatures of *S. tubulosa* of 2-8°C from two locations in the North Sea, whereas Edwards (1978) reported a temperature range for budding between 2-20°C from Oban, Scotland. It may, however, be possible that Edwards worked with different colonies, spatially separated, which may have resulted in the unusual wide temperature range of budding. Hydroids of *Sarsia apicula* bud medusae in the laboratory at an average temperature above 9°C.

Development of embryos from spawning medusae to primary hydranths

Intraspecific crossing

Sarsia apicula, ♀×♂ (Plate I a-d), Table I, II: *Sarsia apicula* medusae spawned 3-4 hrs after sunrise. Spawning occurred for three to four days in unfed animals, with diminishing egg numbers on consecutive days.

Diameter of eggs were 82 µm (n=50). The first divisions were total, equal and mostly radial leading to a coeloblastula and a swimming, ciliated planula as described for various other hydrozoans (Uchida & Yamada 1957, Van de Vyver 1967, Tardent 1978). The rate of fertilization was 94-

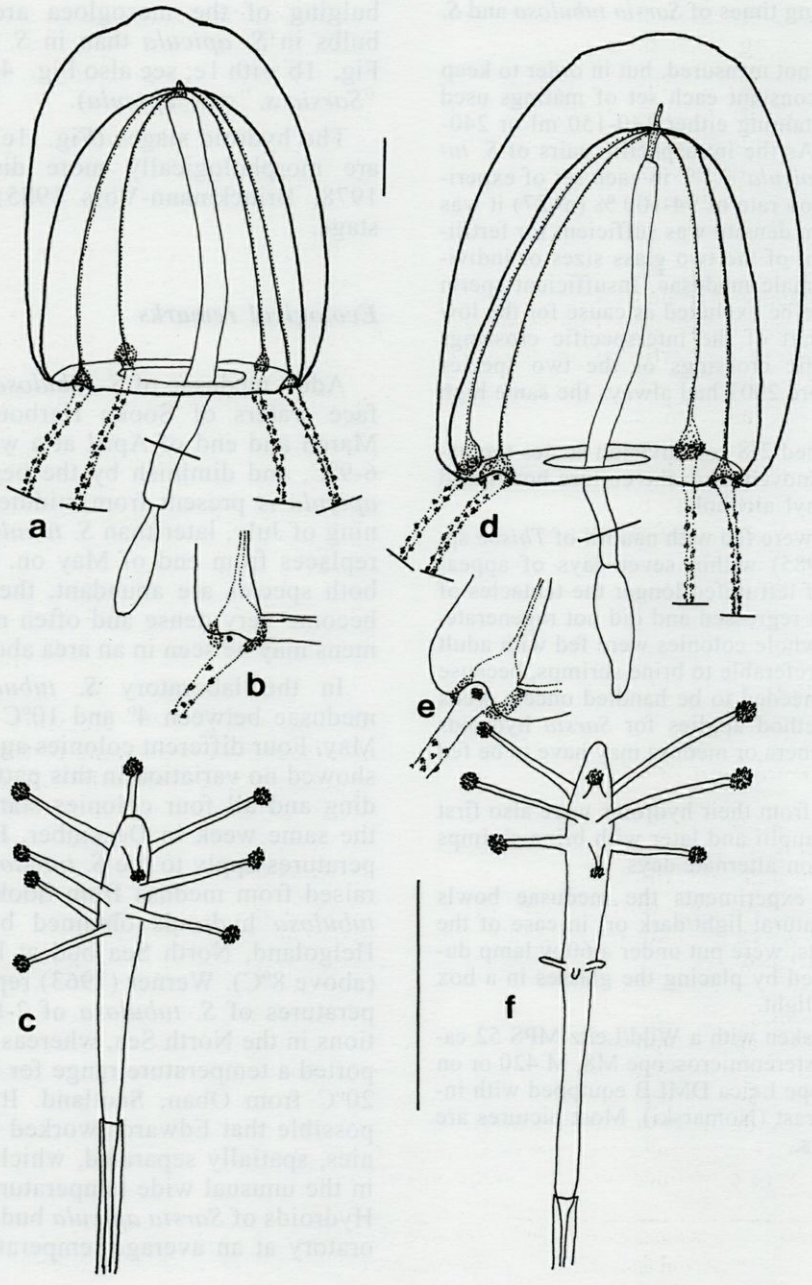

Fig. 1.– *Sarsia tubulosa* and *S. apicula* from sketches from living specimens. a-c *S. tubulosa*: a-adult medusa; b- marginal bulb; c- hydroid. d-f *S. apicula:* d- medusa; e- marginal bulb; f- hydroid. scale a, d: 1.4 mm; c, f: 1mm; length of manubrium and tentacles reduced in drawing as marked with line; natural length of tentacles and manubrium about four times length of umbrella.

100 % (in this paper "fertilization" was used as the first two cleavage stages compared to undivided eggs). The planula became spherical before settling as a cyst, making it difficult to distinguish between anterior (=animal) and posterior (=vegetative) poles typical for numerous other hydrozoan larvae (Freeman 1980, Werner 1984, Van de Vyver 1995). The pattern of early development of *Sarsia apicula* is similar to that described by Bodo & Bouillon (1968) of *S. eximia.* Differences noted in their development are probably caused by the larger egg size in *S. eximia,* which was recently moved to the genus *Coryne* (Petersen 1990, Schuchert 2001). Three days after cyst formation about half of the encysted embryos became elongated, and two days later all embryos changed from round disks to an oblong shape with the thickening of one end as the future hypostome and a slight thickening of the aboral end, giving the embryo the shape of a dumbbell. The embryos were 250 µm long at this stage (average of 10). Tentacles, usually three, devel-

oped on the sixth day after spawning, with the primary hydranths emerging from their cysts.

Feeding of primary hydranths started at this stage. Growth of the hydrorhiza commenced about 6 days after the first feeding, although subsequent rate of growth depended on feeding and crowding. All well spaced primary hydranths developed into small colonies.

Sarsia tubulosa ♀ × ♂ (Plate II a-f), Table I, II: *S. tubulosa* medusae spawned one to three hours after sunset under natural light. Egg diameter was 84 µm (n=50). The rate of fertilization was 97-99 %. The pace and pattern of early development appeared to be the same as for *S. apicula,* although with a considerable time difference because *S. tubulosa* spawns 8-9 hours before *S. apicula*, whose spawning is induced by light. *Sarsia tubulosa* is the only species of the genus *Sarsia* from the research region covering south Vancouver Island and San Juan Islands whose release of gametes is triggered by darkness.

Interspecific crossings of *Sarsia apicula* × *S. tubulosa*

Breeding under natural light conditions

Sarsia apicula ♂ × *S. tubulosa* ♀: Placed under natural light, *S. apicula* ♂ crossed with *S. tubulosa* ♀ produced no zygotes. As there was an 8 to 9 hour difference in spawning time (Table I), *S. tubulosa* eggs were already disintegrating by the time *S. apicula* had spawned.

Sarsia apicula ♀ ×*S. tubulosa* ♂: This combination produced a small percentage of zygotes, some reaching the planula and cyst stage. The discrepancy between these two sets of matings may be caused either by the longer viability of the sperm than of the eggs or males may spawn over a period of several hours compared to the females which shed their eggs within half an hour. Therefore, to ascertain the longevity of the male gametes or the longer period of spawning compared to the shorter life span of the eggs, a separate intraspecific spawning experiment was done: *S. tubulosa* ♀ were treated with light/dark, (see also below) postponing the spawning time of the female by 8 hours. A male *S. tubulosa* which had spawned under natural light conditions 8 hours earlier, or continuously spawned for some hours, was then added together with its water to the treated female. A small percentage of eggs developed into zygotes, all of which developed into normal primary hydranths and colonies. The opposite with ♂ postponed spawning for 8 hours and ♀ spawning under natural lights, did not produce zygotes because the eggs disintegrated in less than 8 hours. This explains why in interspecific crossing of *S. apicula* ♀ ×*S. tubulosa* ♂ there is some fertilization, but not with opposite sexes (Table III). Ability of sperm to fertilize was discussed by Miller & Staub (1982) & Rosen-Runge (1962) for different species of hydromedusae.

Although the crossing of *S. apicula* ♀ with S. *tubulosa* ♂ under natural light produced some zygotes, fertilization (for practical purposes success-

Table I. – Spawning times of *Sarsia tubulosa* and *S. apicula* under natural and experimental light conditions. Spawning time defined in this paper as appearance of eggs. As spawning in the first species is induced by darkness, culture bowls were checked for eggs only each 15 or 30 min to expose medusae to as little light as possible. Therefore spawning may have occurred 15-30 min earlier than listed in the table. Sperm may be released 15 min earlier. See Miller (1982). Times are Pacific daylight saving time. The capital letters indicate the different, separately kept medusae of each spawning. Additional spawning observations were made with more than 100 specimens of both species, and results tabulated above were confirmed with this additional material.

Species	Spawning time under natural light conditions	Spawning time under experimental dark conditions	Spawning time under experimental light/dark conditions
S.tubulosa	A spawning:23 h (sunset 20.25h, (29.4.2002);. . B,C,D spawning:23.45h (sunset 20.28h, 1.5.2002)	A: dark from 18.35 h on; spawning: 20.30 h (23.1999). B: dark from 14h on; Spawning: 16 h (25.51999)	A:light from previous day to 6h following day, then dark; spawning: 8.30h (19.6.2001) B,C,D, and glass with more than 10 ♀: light from previous day to 5.45h following day, then dark ; spawning: 8.30h.(2.5.2002)
S.apicula	A,B,C spawning:8.30 h (sunrise 5.56h, 30.4. 2002); D spawning: 9.00 h (sunrise 5.52h, 2.5. 2002)	A: dark 16.10-18.50 h followed by natural daylight; no spawning in remaining daylight (26.5.1999) B: dark 14.30-16.45 h followed by natural daylight; spawning : 20 h (24.5.1999)	No light/dark trial with *S.apicula,* as this species spawns always after exposure to light

Table II. – Development of from intraspecific pairing of *Sarsia tubulosa* ♀×♂, *S. apicula*♀×♂ Times refer to age of embryos after spawning see table I. % is given as number of completed developmental stages compared to earlier stages or undeveloped eggs. Development times given as completion of each stage. * Irregular stages seem slightly more common than in *S.tubulosa,* but never as abundant from the interspecific crosses of *S.apicula*♀×*S.tubulosa* ♂; nd.nc. normal development observed, but embryos not counted. n: number of embryos counted. Capital letters designate the different single pairs of medusae.

Species	2-4 cell	Coelo blastula	Planula	Cyst	Dumbbell see text	Primary hydranth
S.tubulosa ♂×♀ average devel.. times	50min-2h	4-7h	15-24h	32-48h	53-72h	6 days
S.tubulosa development rates and percentages	Regular divisions typical; irregular divisions minimal	A:99% , n=100 B: 97%, n=121 C:nc	Planula swimming and crawling	Most planula settled as cysts	Elongation of embryos and thickening at both ends	total number A: nd.n.c. B: 523 C: 698
S.apicula♂× ♀; average devel. Times	1-2 h	4-6h	12-24h	24-60h	72-96h	6 days
S.apicula development rates and %	Regular divisions typical; irregular divisions minimal *	A:100%; n=127 B:94%; n=235 C:96%; n=225	Planula swimming and crawling	Most planula settled as cysts	Elongation of embryos and thickening at both ends	A: 895 B:nd. nc C:nd.nc. D:917
S.tubulosa ♀ Ctrl	No development					
S.apicula ♀ Ctrl	No development					

ful fertilization was registered as the appearance of the first or second division, although this should be called cleaving. See also Lesson & Cunningham 1990) was of a much lower percentage than the intraspecific crossing of either *S. tubulosa* or *S. apicula*. Besides the low fertilization rate of crosses of *S. apicula* ♀×*S. tubulosa* ♂, different pairs from the same collection varied much more in their fertilization rate than in intraspecific pairings. The few zygotes of *S. apicula* ♀×*Sarsia tubulosa* ♂ crosses would reach the planula stage and settle as cysts. But whereas encysted embryos from intraspecific matings reached the primary hydranth stage in 6 days (Table II, plate I,i), interspecific hybrids from the *S. apicula* ♀×*S. tubulosa* combination shrunk slowly in their cysts, rarely reaching the dumbbell stage and not producing viable primary hydranths (plate I, h).

Breeding under experimentally changed light conditions:

In order to synchronize spawning times of *S. apicula* and *S. tubulosa*, medusae of the latter were exposed to continuous light from daylight the previous day to 6 h the following day, followed by dark for two or three hours. Spawning then occurred between 8-9h in the morning at the same time *S. apicula* spawned under natural light conditions (Table I).

S. tubulosa ♀ × *S. apicula* ♂: Table III shows a high fertilization rate, nearly equal the intraspecific pairings of either *S. tubulosa* or *S. apicula;* in further development a large percentage of zygotes metamorphosed into primary hydranths (Table III). The time needed for their development from the first two cleavages to the primary hydranth was the same as for the intraspecific crossings. Variation between different pairs of *S. tubulosa* ♀×*S. apicula* ♂ was small. The primary hydranths of these hybrids (from year 2002) grew into small colonies within the same time span as those from intraspecific crossings, but had not budded medusae in the time this paper went into press. However, one hybrid colony raised in 2001 did bud medusae; but these medusae never became fertile, although medusae raised from non hybrid hydroid colonies (*Sarsia apicula* ♂×♀ and *S. tubulosa* ♂×♀) raised in the same time span did so. Results from one colony however, are not sufficient to ascertain the fecundity of a hybrid.

S. tubulosa ♂×*S. apicula* ♀: the fertilization rate varied between 50 % and 98 % in different pairs (Table III). After the first regular cleavages

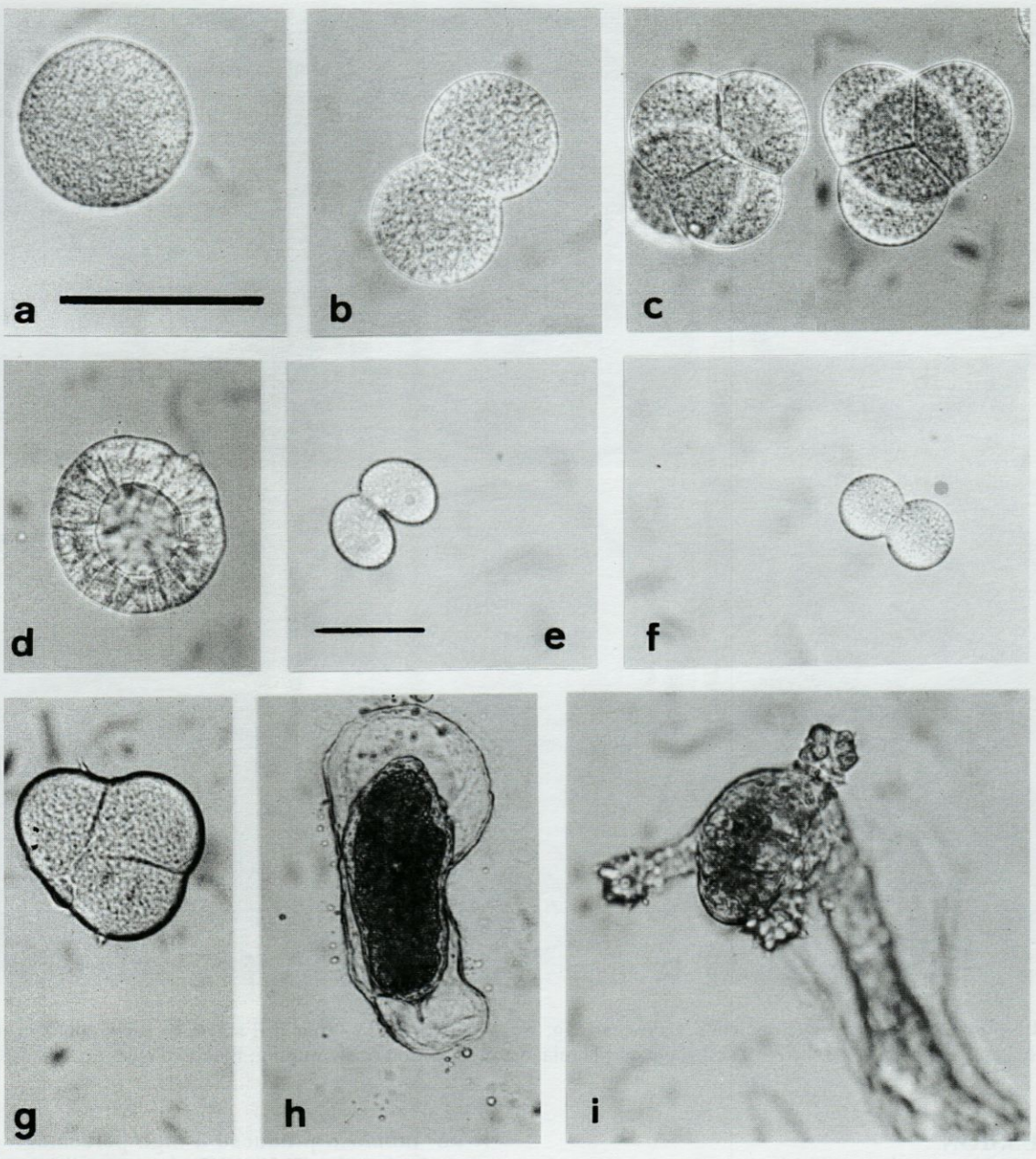

Plate I. – a-d: *Sarsia apicula* developmental stages; a- egg; b- two cell stage; c- several 4 cell zygotes; d- coeloblastula. e-h: *S. apicula* ♀×*S. tubulosa* ♂ developmental stages, often irregular after the first division. e-first cleavage; f- two cell stage; scale e-f:100µ; g- four cell stage with irregularly shaped blastomeres; h- shrinking embryo of *S.apicula*♀ ×*S.tubulosa* ♂ in its cyst; i- primary hydranth of *S. apicula*♂ ×*S. tubulosa*♀, same age as h. scale a-d, g-i:100 µm

(Plate I, e, f) development in these hybrids showed a large percentage of irregular embryos (Plate I g). These irregular early division stages however, often developed into seemingly normal embryos by the time they reached the late blastula (a similar development pattern was reported in Miller 1982 as personal information from G. Freeman). Although blastulae developed into swimming planula and cysts, none of these "irregular-regular" early embryos developed further than the cyst stage (Plate I, h). Those embryos of *S. tubulosa* ♂ ×*S. apicula* ♀ with regular division stages throughout early development developed to the cyst stage, but also these hybrids with a seemingly normal early development did not metamorphose into primary hydranths and disintegrated then slowly within their cysts as reported above for the few *S. tubulosa* ♂ ×*S. apicula* ♀ hybrid cysts from breeding under natural light conditions. Some cysts elongated slightly as if to form primary hydranths, but they died and no primary hydranths ever developed from the *S. tubulosa* ♂× *S. apicula* ♀ combination.

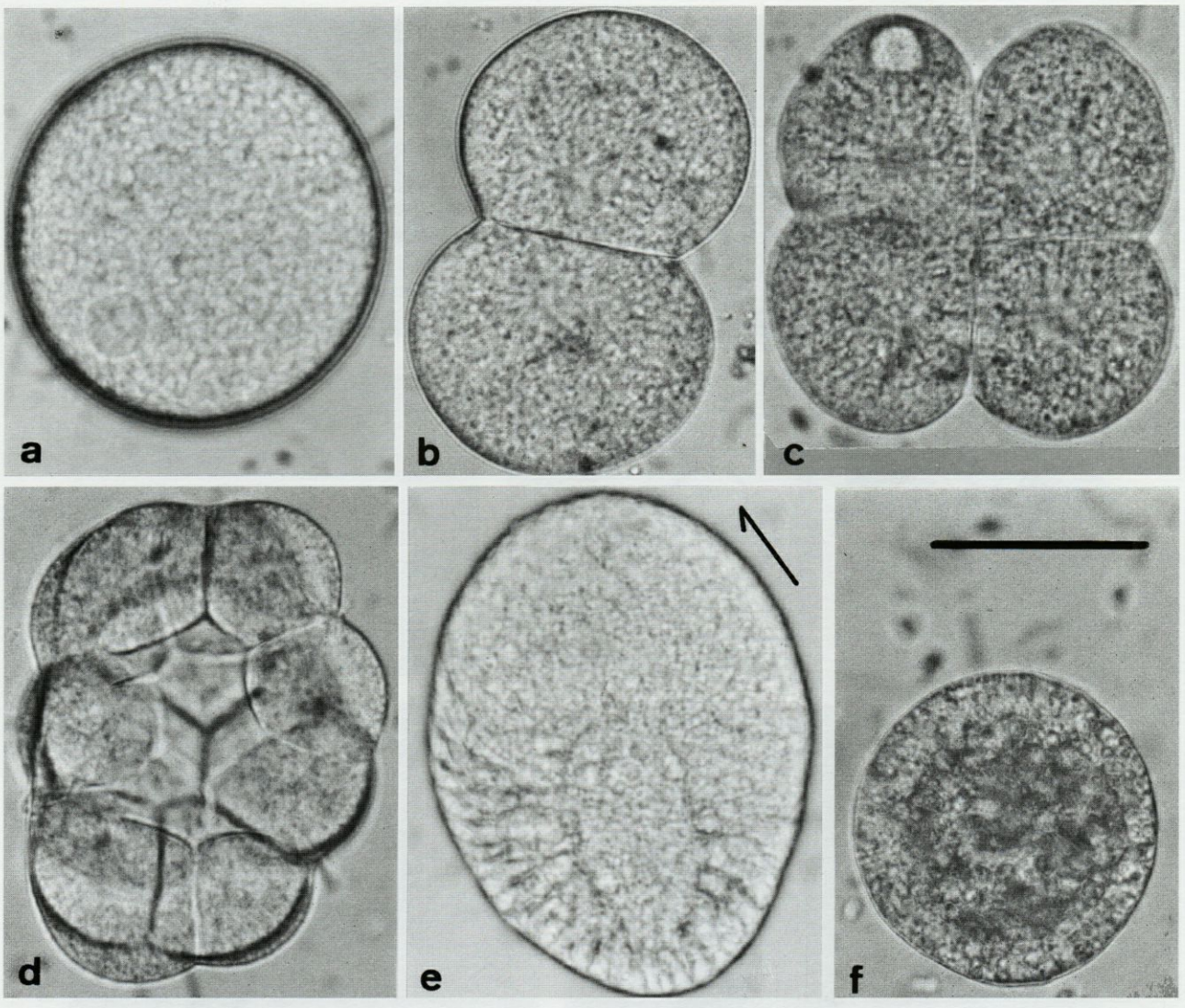

Plate II. – *Sarsia tubulosa:* developmental stages; a- egg; b- two cell stage; c- four cell stage; d- early coeloblastula; the blastocoel appears already on the 8-16 cell stage; e- planula; arrow direction of swimming; f- settled cyst. Scale a-f:50 µm.

DISCUSSION

Results from hybridization experiments of *Sarsia tubulosa* and *S. apicula* revealed two barriers which keep the two species reproductively isolated and prevent their hybridization under natural conditions. First, a difference of 8-9 hours in spawning time between the two species results in female gametes of *S. tubulosa* already disintegrating at a time when male medusae of *S. apicula* are spawning. Although the opposite *S. apicula* ♀ with *S. tubulosa* ♂ cross produced a low percentage of hybrid zygotes under natural light conditions, these were not viable and died off before metamorphosing into primary hydranths. Even when spawning times were synchronized experimentally in the laboratory the two species did not interbreed freely as in intraspecific pairings. Although ♂ *S. apicula* × ♀ *S. tubulosa* produced a high percentage of primary hydranths (Table III) the opposite cross ♀ *S. apicula* × ♂ *S. tubulosa* resulted in a much lower fertlization rate, often irregularly shaped embryos and cysts which did not metamorphose into primary hydranths. A second barrier thus exists to hybridization, which mostly affects pairing with one set of sexes and not when done with opposite sexes. This "asymmetrical" barrier seems more to affect the viability of the embryos in the cyst stage than the fertilization and early division stages, and thus acts more post than prezygotically.

"Asymmetrical" hybridization with synchronized spawning times may also explain why in pairings under natural light, and therefore also in nature, those few embryos which may get fertilized in crossings of *S. apicula* ♀ × *S. tubulosa* ♂ are affected by the barrier of asymmetrical development and do not develop beyond the cyst stage. In the

Table III. – Development from interspecific breeding of *Sarsia tubulosa*×*S. apicula*
From spawning under natural light (8-9 hours time difference between spawning of the two species) and experimentally changed light to achieve synchronized spawning for both species. n=number of embryos counted; Capital letters designate the different breeding pairs. All numbers and percentages are from a one day single spawning. The percentage of coeloblastulae was calculated against non developed eggs or irregular earlier stages. Controls consisted of several ♀ from either species. Nc: development was observed but single stages not counted.

	2-4 cell	% coeloblastula	cysts, total	Primary hydranths, total
Natural spawn. *S.ap.*♂×*S.tub.*♀	Disintegrating eggs only	nil	nil	nil
Natural spawn *S.ap.*♀×*S.tub.*♂	Regular and irregular	A: 12% n=250 B: 50% n=168 C: 9% n=274 D: 8% n=214	A: 75 B: 172 C: nc D: nc	No primary hydranths developed from cysts
Synchronized spawn. *S.ap.*♀×*S.tub.*♂	Regular and irregular	E: 51%n=98 F: 71%n=105 G: 98%n=693 H: 59%n=343	E: 371 F: 333 G: nc H: nc	No primary hydranths developed from cysts
Synchronized spawn. *S.ap.*♂×*S.tub.*♀	Mostly regular	I: 97%n=204 J: 98%n=105 K: 98%n=109 L: nc	I: nc J: 790 K: nc L: nc	I:nc J: 744 K: 602 L: 734 Five additional spawnings produced more than 600 primary hydranths each
Ctrl: *S.tub.*♀	Disintegrating eggs only			
Ctrl: *S.ap.*♀	Disintegrating eggs only			

opposite cross (S. *tubulosa* ♀×*S. apicula* ♂) under natural light hybridization can not occur at all because of the decay of eggs over the eight hours time difference in spawning of the two species. "Asymmetrical" hybridization has been reported from different phyla as reported by Strathmann (1981), Miller (1982), Lesson & Cummingham (1990), Miller & Babcock (1997) and Pernet (1999).

Clearly, *S. apicula* and *S. tubulosa* from Sooke Harbour are efficiently isolated reproductively both by the difference in spawning time and by a second isolating mechanism affecting mostly the viability of embryos in the cyst stage of *S. apicula* ♀×*S. tubulosa* ♂ but not the opposite cross.

These results differ from those of Miller (1982), because Miller's species from Friday Harbor were: *Sarsia bella* and *S. apicula* (*Sarsia L* and *Sarsia S* in Miller 1982). According to his work the two species had a spawning difference of only about one hour, which did not prove to be an effective isolating mechanism between his species (not as interpreted in Schuchert 2001). Miller suspected the presence of additional isolating mechanisms acting as reproductive barriers in the Friday Harbor natural populations.

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REFERENCES

Arai M, Brinckmann-Voss A 1980. Hydromedusae of British Columbia and Puget Sound. *Can Bull Fish Aquat Sci* 204: 1-192.

Bodo F, Bouillon J 1968. Étude histologique du développement embryonnaire de quelques hydroméduses de Roscoff: *Phialidium hemsphaericum* (L.), *Obelia sp.* Peron et Lesueur, *Sarsia eximia* (Allman), *Podocoryne carnea* (Sars), *Gonionemus vertens* Agassiz. *Cah Biol Mar* 9: 69-104.

Brinckmann-Voss A 1985. Hydroids and medusae of *Sarsia apicula* (Murbach and Shaerer, 1902) and *Sarsia princeps* (Haeckel 1897) from British Columbia and Puget Sound with an evaluation of their systematic characters. *Can J Zool* 63: 673-681.

Brinckmann-Voss A 2000. The hydroid and medusa of *Sarsia bella* sp. nov. (Hydrozoa, Anthoathecatae, Corynidae), with a correction of the "life cycle" of *Polyorchis penicillatus* (Eschscholtz). *Scientia Marina* 64 (Suppl 1): 180-195.

Edwards C 1978. The hydroids and medusae of *Sarsia occulta* sp.nov., *Sarsia tubulosa* and *Sarsia loveni. J mar biol Ass U.K* 58: 291-311.

Freeman G 1980. The role of cleavage in the establishment of the anteroposterior axis of the hydrozoan embryo. *In* P Tardent & R Tardent eds, Developmental and Cellular biology of Coelenterates. Proceed IV Intern Coelenterate Conference, Interlaken Elsevier/ North Holland Biomedical Press, Amsterdam: 97-108.

Lesson HA, Cunningham CW 1990. Gametic incompatibility between species of the sea urchin *Echinometra* on the two sides of the Isthmus of Panama. *Evolution* 44(4): 933-941.

Mayr E 1982. The growth of biological thought. The Belknap Press of Harvard University Press, Cambridge, Mass. USA, London, UK. 974 p.

Mayr E, Linsley EG, Usinger RL 1953. Methods and principles of systematic zoology McGraw-Hill Book Company inc. 336 p.

Miller K, Babcock R 1997. Conflicting morphological and reproductive species boundaries in the coral genus *Platygyra. Biol Bull* 192: 98-110.

Miller R L 1980a. Species-specificity of sperm chemotaxis in the Hydromedusae. *In* Developmental and cellular biology of Coelenterates, Tardent P & R Tardent eds, Elsevier/North-Holland Biomedical Press, Amsterdam, NewYork, Oxford: 89-94.

Miller R L 1980b. *Sarsia tubulosa* at Friday Harbor Labs. consisting of two sympatric Species. *Am Zool* 20(4): 848.

Miller R L 1982. Identification of sibling species within the "*Sarsia tubulosa* complex" at Friday Harbor, Washington (Hydrozoa, Anthomedusae). *J exp Mar Biol Ecol* 62: 153-172.

Miller R L, Staub G 1982. Evidence for age-related in Hydromedusae sperm Chemotaxis. *Int J Invert Reprod* 4: 267-271.

Palumbi S R 1994. Genetic divergence, reproductive isolation, and marine speciation. *Annu Rev Ecol Syst* 25: 547-572.

Pernet B 1999. Gamete interactions and genetic differention among three sympatric polychaetes. *Evolution* 53(2): 435-446.

Petersen KW 1990. Evolution and Taxonomy in Capitate Hydroids and Medusae. *Zool J Linnean Soc* 100: 101-231.

Rosen-Runge EC 1962. On the biology of sexual reproduction of hydromedusae, genus *Phialidium* Leuckart (Leptomedusae). *Pac Sci* 16: 15-24.

Schuchert P 2001. Survey of the family Corynidae (Cnidaria, Hydrozoa). *Rev Suisse Zool* 108 (4): 739-878.

Strathmann M 1987. Reproduction and development of marine invertebrates of the Northern Pacific Coast. University Washington Press, Seattle, London. 670 p.

Strathmann R R 1981. On barriers to hybridization between *Strongylocentrotus droebachiensis* (O.F. Müller) and *S. pallidus* (G.O. Sars). *J exp mar Biol Ecol* 55: 39-47.

Szmant AM, Weil E, Miller MW, Colón DE. Hybridization within the species complex of the scleractinan coral *Montastraea annularis. Mar Biol* 129: 561-572

Tardent P 1978. Coelenterata, Cnidaria. *In* Morphogenese der Tiere by Friedrich Seidel, Lieferung 1, A-I: 69-415.

Uchida T, Yamada M 1957 (in Japanese), English translation 1968. Invertebrate Embryology Cnidaria. *In* Invertebrate Embryology, ed M Kume & K Dan: 86-117. English edition published for the National Library of Medicine, Public Health Service, U.S. Dpt Health, Education and Welfare and the National Science Foundation, Washington, D.C. by Nolit Publishing House, Belgrade, Yugoslavia, 168.

Van de Vyver G 1967. Étude de développement embryonaire des hydraires athécates (Gymnoblastiques) à gonophores.1. Formes à planula. *Archs Biol Paris* 78: 451-518.

Van de Vyver G 1995. Reproduction Sexuée – Embryologie. *In* Traité de Zoologie Masson, Paris 3 (2): 417-471.

Wallace CC,Willis BL 1994. Systematics of the Coral Genus *Acropora:* Implications of new biological findings for species concepts. *Annu Rev Ecol Syst* 25: 237-62.

Werner B 1963. Experimentelle Beobachtungen über die Wirksamkeit von Aussenfaktoren in der Entwicklung der Hydrozoen und Erörterung ihrer Bedeutung für die Evolution. Veröffentlich Inst Meeres Forsch Bremerhaven, Sonderb 3 meeresbiologisches Sympos: 153-177.

Werner B 1984. Cnidaria. *In* Lehrbuch der speziellen Zoologie, begründet A Kaestner: Gustav Fischer Verlag, Stuttgart Band 1(2): 1-305.

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