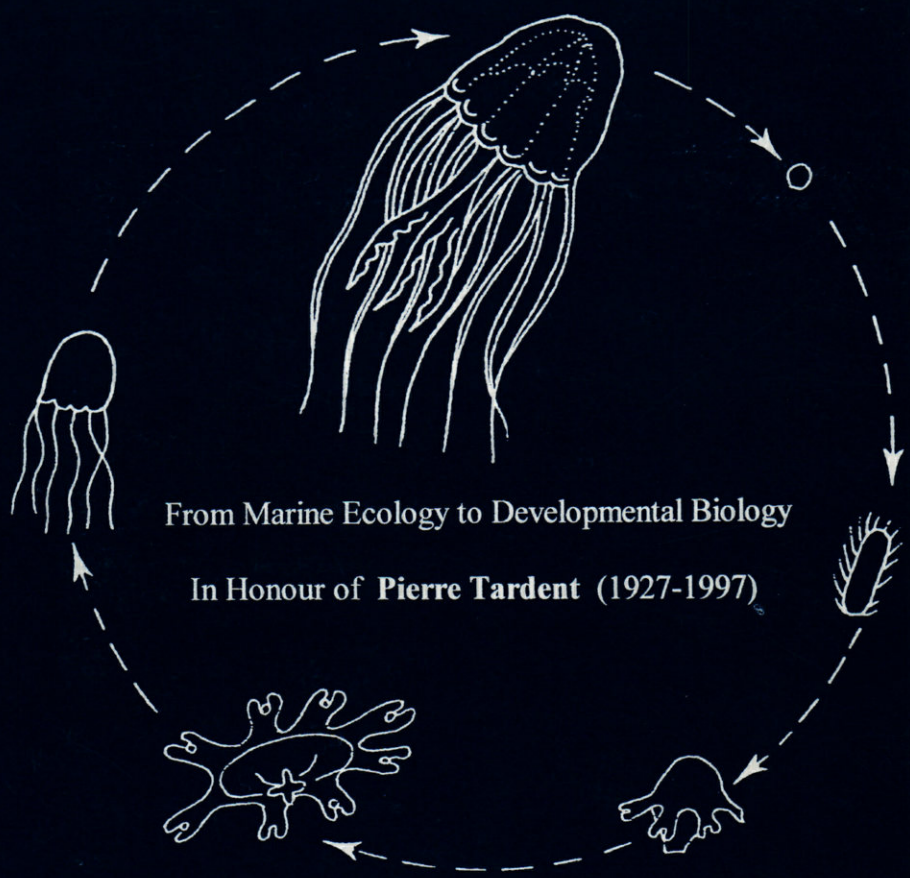
ISSN 0240-8759

Vie et Milieu

Life and Environment

Vol. 52 nº 4 – Décembre 2002



From Marine Ecology to Developmental Biology

In Honour of **Pierre Tardent** (1927-1997)

Périodique d'écologie - Publication trimestrielle

VIE ET MILIEU

Life and Environment

PÉRIODIQUE D'ÉCOLOGIE GÉNÉRALE
JOURNAL OF GENERAL ECOLOGY

LABORATOIRE ARAGO — UNIVERSITÉ P. et M. CURIE

Vie et Milieu – Life and Environment publie des contributions concernant les divers aspects de l'écologie, la biologie et la systématique dans les milieux marins, lagunaires et terrestres:

- biologie et écologie des organismes, des populations et des écosystèmes
- réseaux trophiques, flux énergétiques
- écologie benthique
- écophysiologie
- pollution
- écologie numérique, modélisation
- évolution et phylogénie

Un numéro à thème paraît chaque année.

Le périodique paraît tous les trimestres; chaque volume annuel comprend 4 fascicules groupant 400 pages et présente des articles originaux, des communications de synthèse et des notes brèves, en Français ou en Anglais.

Les manuscrits sont reçus par le Professeur G. BOEUF, Directeur de la Publication, ou par Mme N. COINEAU, Rédaction (Laboratoire Arago, 66650 Banyuls-sur-Mer, France). Ils ne seront acceptés qu'après avoir été soumis à l'approbation scientifique de deux lecteurs dont les commentaires restent anonymes.

Vie et Milieu – Life and Environment publishes contributions in the fields of Ecology, Biology and Systematics with emphasis on marine, lagoonar and terrestrial milieus:

- Organisms, communities and ecosystems biology and ecology
- Trophic webs, energetics
- Benthic ecology
- Ecophysiology
- Pollution
- Statistical analysis in ecology
- Evolutionary biology and phylogeny

Each year, a single or double issue is devoted to a special topic.

The journal is quaterly. Each volume (four fascicles) of ca 400 pages presents original articles, reviews and short papers, in french or english.

Manuscripts are received by Professor Dr. G. BOEUF, Editor in Chief, or by Dr. N. COINEAU, Managing Editor (Laboratoire Arago, F. 66650 Banyuls-sur-Mer). Papers are only accepted after review by two anonymous referees.

Publié avec le concours financier/Published with the support
du Conseil Régional du Languedoc-Roussillon
de l'Académie Suisse des Sciences Naturelles (SANW)
de l'Association Naturalia et Biologia
du Centre National d'Etudes Spatiales (CNES)

ABONNEMENTS/SUBSCRIPTIONS 2002

	FRANCE	AUTRES PAYS
Un an (4 numéros) / *Annual subscription (4 issues)*.....	164,60 €	180 €

Adresser le paiement à l'ordre de / Address payment to the order of:

Vie et Milieu, Laboratoire Arago, BP 44, 66651 BANYULS-SUR-MER CEDEX, FRANCE

Les abonnements partent du premier numéro de l'année / *subscriptions begin with the first calendar year*

Numéros séparés de l'année, volumes antérieurs, suppléments et faunes des Pyrénées-Orientales, collections complètes, disponibles / *Back Issues and volumes, supplements and Faunas of Pyrénées-Orientales, complete collections, available:*

VIE ET MILIEU — Laboratoire Arago — BP 44 — F. 66651 BANYULS-SUR-MER CEDEX

PREFACE

The title "From Marine Ecology to Developmental Biology" could mean different things to different readers. It may allude to a progress or to a range – or to both. Here we should like to emphasize both the progress from one to the other, and the topical range in which one is linked with the other.

The contributions assembled in this issue as a homage to Pierre Tardent deal with marine organisms. Although fresh-water organisms like *Hydra* were praised models in his research (Galliot & Schmid 2002*), the marine realm as a whole – and the marine biological stations which provide the means to approach it – were extremely important for the scientific career of Pierre Tardent (see the closing article by Honegger & Honegger). His work thus appears paradigmatic for a very promising way to use marine organisms as models in experimental research, especially in developmental biology. Surely modern aquarium technology allows biologists to work on marine organisms even in laboratories established far from the sea, but such research facilities cannot replace the natural conditions under which organisms live in the sea. Truly natural conditions subsume more than the minimal requirements for survival and reproduction of organisms in captivity. To some extent the original conditions of autecology and synecology may be mimicked in an aquarium, but they can never be copied as a facsimile of the ocean. Thus the route from marine ecology to developmental biology is not a one-way street.

The link between ecology and development turns out crucial when the evolutionary problems of adaptation and speciation are addressed. In a recent issue of *Nature* (418: 578-579), a News Feature raised the question: "Can studies of environmental influences on developing organisms provide the key to understanding the evolution of species and populations ?" and observed: "A growing number of researchers think so". Let us assume that Pierre Tardent thought so in his time. Indeed, we owe much to his creative mind and to his persistent encouragement in doing research both in topical fields and off the beaten path. Many of his former students will also remember his advice to "read old literature when looking for new ideas".

As guest editors, we should like to thank all the authors for their contributions to this special issue, and we gratefully acknowledge the untiring efforts of Dr Nicole Coineau (Managing Editor) and Mrs Véronique Arnaud (Secretary) in processing the articles for publication.

Special thanks for subsidies are due to the Swiss Academy of Natural Sciences (SANW), to the French Space Agency CNES, to the Regional Council of Languedoc-Roussillon, and to Naturalia and Biologia

S.v. Boletzky, J.-P. Féral, D.K. Hofmann & H.-J. Marthy

*Galliot B, Schmid V 2002. Cnidarians as a Model System for Understanding Evolution and Regeneration. *Int J Dev Biol* 46: 39-48.
Cover picture: Life cycle of *Pelagia noctiluca* (From P. Tardent: "Meeresbiologie", 1993; with permission of Georg Thieme Verlag, Stuttgart)

VIE MILIEU, 2002, 52 (4) : 121-130

From Marine Ecology to Developmental Biology
In Honour of Pierre Tardent (1927-1997)

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

A. BRINCKMANN-VOSS

Centre for Biodiversity and Conservation Biology, Royal Ontario Museum, 100 Queens's Park, Toronto, Ontario, Canada, M5S 2C6

Mailing address: P.O.B. 653, Sooke, B.C.,Canada, VOS 1NO. anitab-voss@shaw.ca

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-

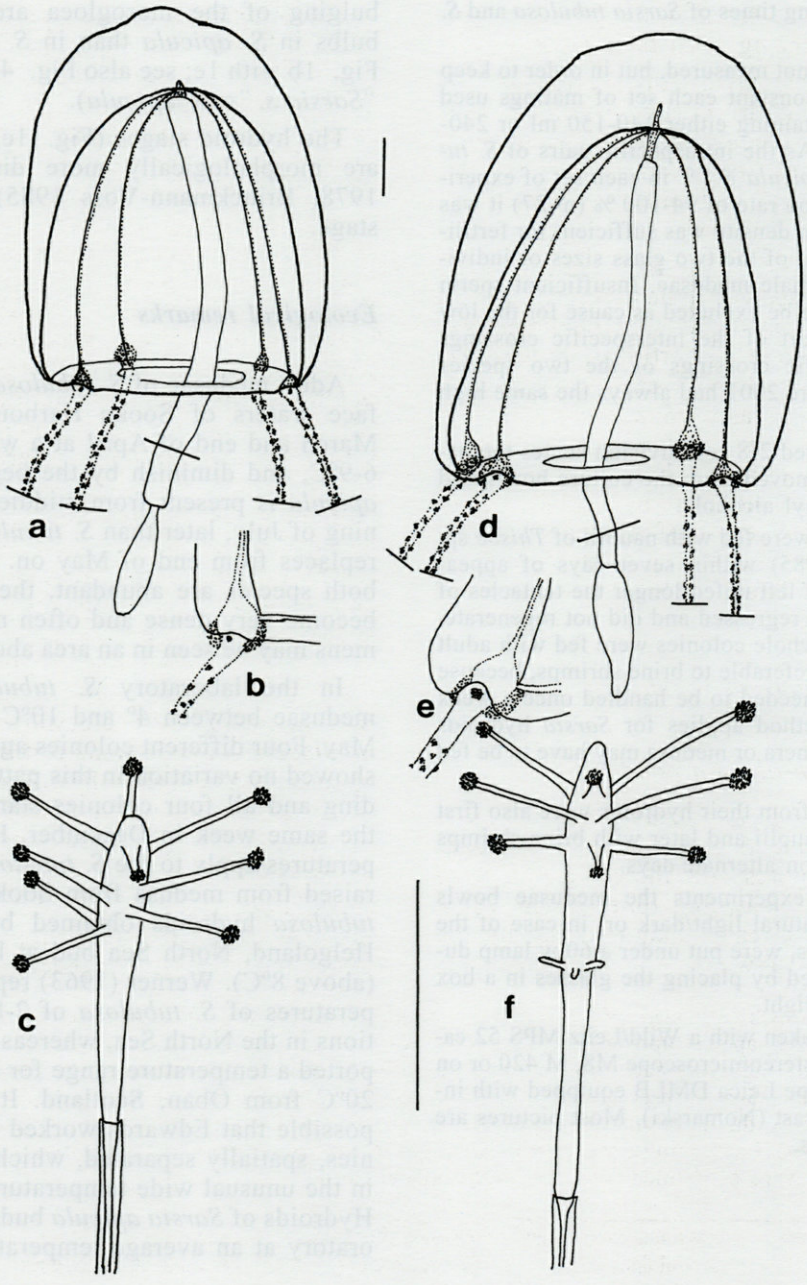


Fig. 1.– *Sarsia tubulosa* and *S. apicula* from sketches from living specimens. a-c *S. tubulosa*: a-adult medusa; b- marginal bulb; c- hydroid. d-f *S. apicula:* d- medusa; e- marginal bulb; f- hydroid. scale a, d: 1.4 mm; c, f: 1mm; length of manubrium and tentacles reduced in drawing as marked with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

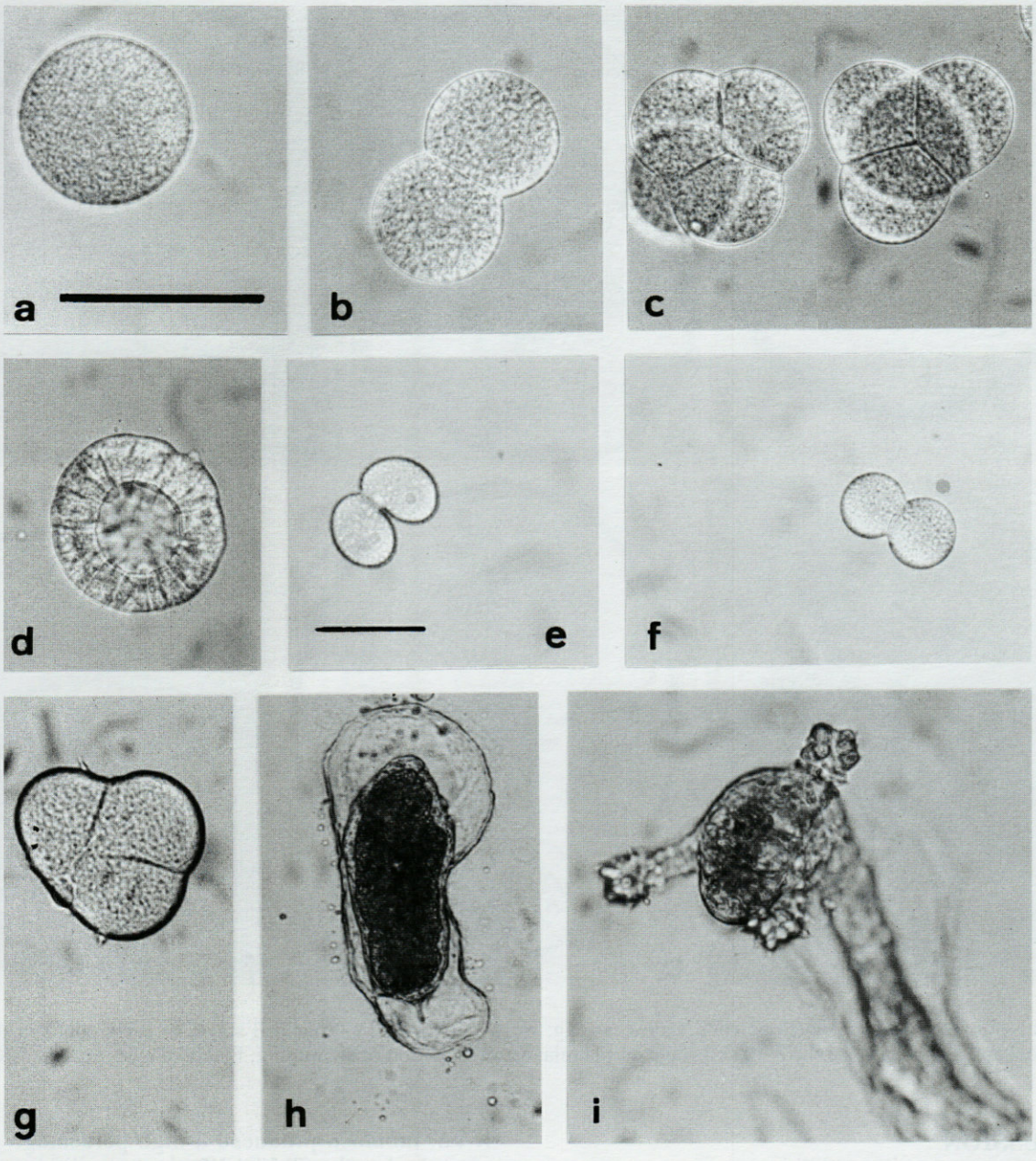


Plate I. – a-d: *Sarsia apicula* developmental stages; a- egg; b- two cell stage; c- several 4 cell zygotes; d- coeloblastula. e-h: *S. apicula* ♀×*S. tubulosa* ♂ developmental stages, often irregular after the first division. e-first cleavage; f- two cell stage; scale e-f:100µ; g- four cell stage with 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.

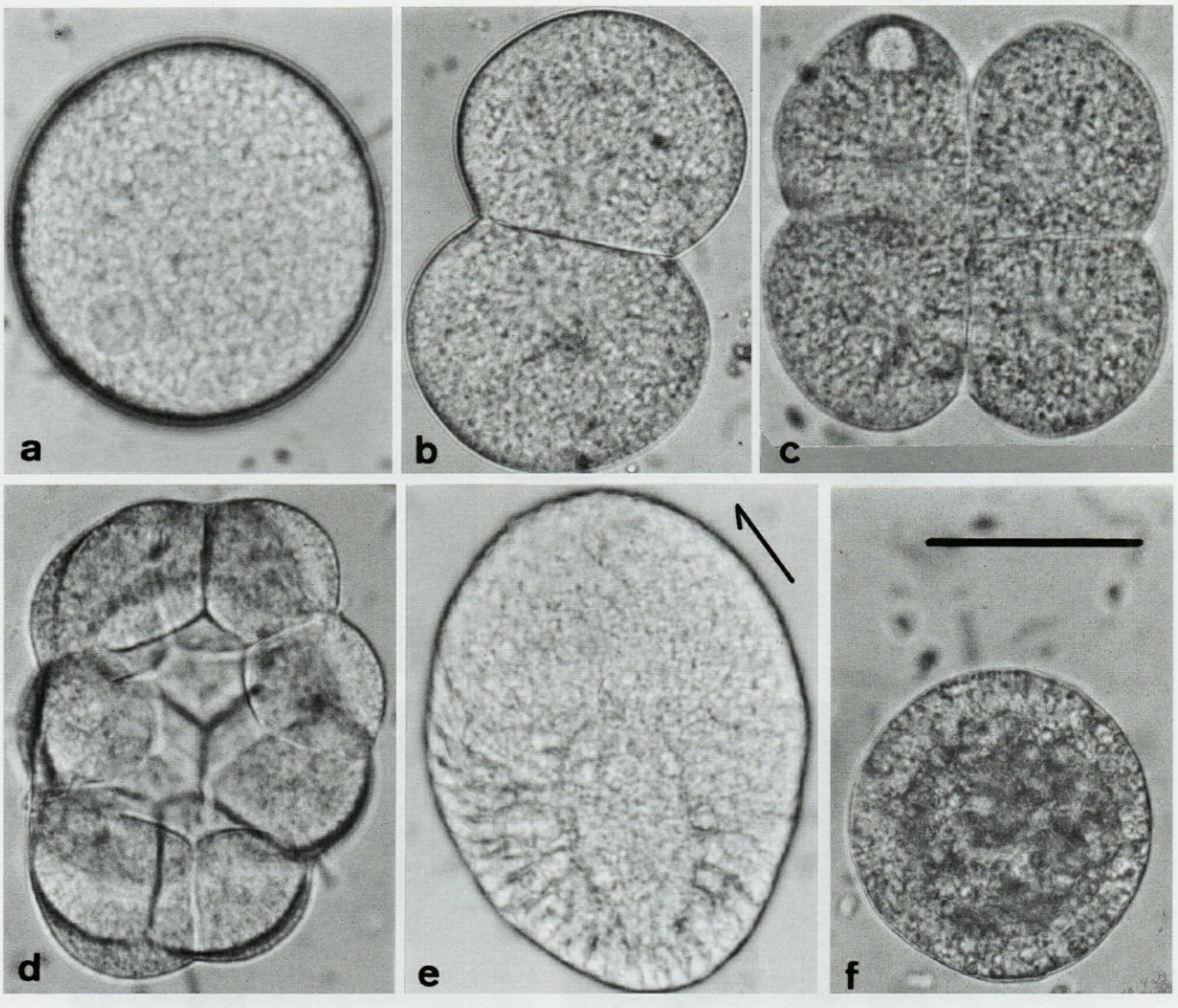


Plate II. – *Sarsia tubulosa:* developmental stages; a- egg; b- two cell stage; c- four cell stage; d- early coeloblastula; the blastocoel appears already on the 8-16 cell stage; e- planula; arrow direction of swimming; f- settled cyst. Scale a-f:50 μm.

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.

ACKNOWLEDGEMENTS. – I am very grateful to D Calder for his detailed editing and revising of this paper. I thank also my second reviewer, B Pernet for reviewing this manuscript. C Mills helped a great deal introducing me to people in Friday Harbor who work in a similar field as the topic of this paper. I thank Claudia very much for her time and assistance at that laboratory. Both she and M Needler Arai retrieved and copied numerous references from their institutions for which I am very thankful.

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.

Reçu le 18 juillet 2002; received July 18, 2002
Accepté le 9 septembre 2002; accepted September 9, 2002

VIE MILIEU, 2002, 52 (4) : 131-140

From Marine Ecology to Developmental Biology
In Honour of Pierre Tardent (1927-1997)

STAGING AND INDUCTION OF MEDUSA METAMORPHOSIS IN *CARYBDEA MARSUPIALIS* (CNIDARIA, CUBOZOA)

K. STANGL,**, L.v. SALVINI-PLAWEN***, T.W. HOLSTEIN**

* *Institut für Zoologie, Technische Universität Darmstadt, Molekulare Zellbiologie, Schnittspahnstrasse 10, 64287 Darmstadt, Germany*
** *Institut für Medizinische Biochemie, Vienna Biocenter, 1030 Wien, Austria*
*** *Institut für Zoologie, Universität Wien, 1090 Wien, Austria*

Address for correspondence: T.W. Holstein
holstein@bio.tu-darmstadt.de

CNIDARIA
CUBOZOA
CARYBDEA
METAMORPHOSIS
DEVELOPMENT
EVOLUTION
ECOLOGY

ABSTRACT. – The cubozoan life cycle is characterized by the transformation of a solitary benthic polyp into a pelagic medusa. We investigated the morphodynamics and kinetics of this metamorphosis in *Carybdea marsupialis*. Nine stages in metamorphosis are defined. Metamorphosis is induced by a stop of feeding and culturing polyps at temperatures > 28°C. Metamorphosis is inhibited by feeding, but stimulated in the presence of a metamorphosing polyp. The transformation of the polyp into the medusa is localized to the apical half of the polyp, which is reminiscent to metamorphosis in Stauromedusae. The relationship of cubozoan metamorphosis to other scyphozoan and hydrozoan medusa formation modes is discussed.

CNIDARIA
CUBOZOA
CARYBDEA
MÉTAMORPHOSE
DÉVELOPPEMENT
ÉVOLUTION
ÉCOLOGIE

RÉSUMÉ. – Le cycle de vie d'un Cubozoaire est caractérisé par la transformation d'un polype benthique solitaire en une méduse pélagique. Nous avons étudié la dynamique morphogénétique de cette métamorphose chez *Carybdea marsupialis*. La métamorphose, que nous avons divisée en 9 étapes, est induite par une privation de nourriture ainsi que par une température > 28°C. Le phénomène est inhibé par l'ajout de nourriture dans le milieu, et est stimulé en présence de polypes en cours de métamorphose. La transformation en méduse se produit dans la partie apicale du polype. Ce processus est très similaire à la métamorphose des Stauroméduses. La métamorphose chez les Cubozoaires, ainsi que le mode de formation des méduses chez les Scyphozoaires et les Hydrozoaires sont discutés.

INTRODUCTION

An important question about the cnidarian life cycle is whether the polyp or the medusa represents the ancestral typ (Nielsen 2001). Some hydrozoans and scyphozoans lack a polyp stage and their life cycle has been interpreted as ancestral, and regarding the polyp stage, as a larval specialisation (Boero *et al.* 1998, Bouillon & Boero 2000, Brusca & Brusca 1990, Hyman 1940, Piraino *et al.* 1996). The contrary hypothesis holds that the ancestral cnidarian was a polyp and the medusa is considered to be a specialized sexual state (Collins & Valentine 2001, Nielsen 2001, Salvini-Plawen 1978, Werner 1984).

Phylogenetic analyses of Cnidaria have determined the relationship among the four main taxa that compose it, Anthozoa, Cubozoa, Hydrozoa, and Scyphozoa (Bridge *et al.* 1992, 1995, Brusca & Brusca 1990, Meglitsch & Schram 1991, Odoric & Miller 1997, Petersen & Eernisse 2001, Salvini-Plawen 1978, Schuchert 1993, Werner 1973). From these investigations, a framework has emerged that Anthozoa are the sister group of the remaining Cnidarians which are collectively referred to as Tesserazoa (Salvini-Plawen 1978) or Medusozoa (Petersen 1979). The interpretation of Anthozoa as a sister group of Medusozoa is also supported by a number of morphological characters (Nielsen 2001, Salvini-Plawen 1978) and by mitochondrial chromosome structure (Bridge *et al.* 1992).

Medusae are formed through different processes in the three medusa bearing classes. In cubozoans, the polyp goes through a metamorphosis and becomes a medusa, the tentacles of the polyp are reduced and become sense organs, and new medusa tentacles differentiate (Werner *et al.* 1971, Werner 1973, 1975, 1984). Cubozoans were orginally classified within the Scyphozoa (Cubomedusae) based on distinct morphological characters as rhopalia, gastric filaments, eyes and the tetraradial shape of the medusa (Berger 1898, 1900, Claus 1878, Conant 1897, 1898, Hyman 1940, Okada 1927,

Uchida 1929), these still represent synapomorphies between both classes (Salvini-Plawen 1987, Schuchert, 1993). In scyphozoans, medusae are formed through a process of transverse fission (strobilation) of the polyp below the tentacle disc or calyx. By comparison, in hydrozoans, the polyp typically forms lateral medusae buds, which detach as free-living medusae or remain attached as variously reduced medusoid reproductive units (for review see Bouillon 1981, 1985, Bouillon & Boero 2000, Brusca & Brusca 1990, Hyman 1940, Meglitsch & Schram 1991, Nielsen 2001, Salvini-Plawen 1978, Tardent 1978, Werner 1984). Thus, the evolutionary differentiation of medusae (Medusozoa, Medusogona) has been considered to be at least diphyletic (Salvini-Plawen 1987).

The cubozoan metamorphosis was detected by Werner *et al.* (1971) in *Tripedalia cystophora* and characterized as a complete transformation of the solitary radial symmetrical polyp into a tetraradial medusa. In a number of further studies histology and life cycle of *Tripedalia cystophora* and other cubopolyps has been described (Arneson & Cutress 1976, Chapman 1978, Cutress & Studebaker 1978, Werner 1975, 1983, 1984, Werner *et al.* 1976, Yamaguchi & Hartwick 1980, Yamasu & Yoshida 1976). Although these studies are nicely in accord with the classical concept that the medusa corresponds the (transformed) pelagic form of a solitary benthic polyp, it was less clear whether the entire polyp undergoes metamorphosis or only a part of it. In order to gain a better understanding of the kinetics and mechanisms of metamorphosis, we investigated the kinetics of metamorphosis in another cubozoan species, *Carybdea marsupialis*.

MATERIAL AND METHODS

Animals: Polyps of *Carybdea marsupialis* (Linné, 1758) were a kind gift of Dr J Jarms (Zoological Institute, University of Hamburg). The animals were the offspring of an original culture established by late Dr Bernhard Werner (Biologische Anstalt Helgoland, Hamburg) in 1978. Animals were kept in artificial seawater (Tropic Marin), pH 7.5-8.0 at 24 ± 0.3°C in the dark. They were grown on watch glass dishes as substrate and fed 4-5 times a week with freshly hatched brine shrimps (*Artemia salina*); the seawater was replaced about 8 hr after feeding. Mass cultures of the cubopolyps were kept in plastic dishes (500 ml, 5 cm depth, ~ 1000 polyps per dish), which were stored in an incubator allowing various temperatures between 10-30°C. For induction of metamorphosis adult polyps were carefully removed from the glass substrate and kept separately in 24 well microculture plates.

Microscopy: Polyps and medusae were analyzed by using a Wild MP5 stereomicroscope. For micrographs and fluorescence microscopy we used an Axiovert 100 (Zeiss) with neofluar optics and a MC 80 analog camera.

For DAPI-staining, animals were relaxed in Ca^{2+}-free sea water containing 0.2% $MgCl_2$ for 15 min and fixed with 4 % paraformaldehyde in seawater for 12 hr. DAPI staining of PBS (pH 7.2) washed specimens was performed using DAPI at a concentration of 15 µg/ml in PBS buffer (pH 7.2). Under these conditions not only the nuclei are stained, but also the gamma-polyglutamate matrix of differentiated nematocysts (Szczepanek *et al.* 2002). All experiments were carried out in the former laboratory of TW Holstein at the Zoological Institute of the JW Goethe-University at Frankfurt a. Main.

RESULTS AND DISCUSSION

Stages of Metamorphosis

In cubozoans the entire polyp goes through a metamorphosis and becomes a medusa (Werner 1975). To analyze this process quantitatively, we reanalyzed metamorphosis and subdivided it into nine characteristic developmental stages which can be easily distinguished by light microscopy (Fig. 1, 2). These stages define a continuous morphogenetic process which takes about two weeks; it can be reliably induced by a temperature shift from 20°C to 28° (see below).

Steady state polyps

The steady-state polyp, which is ready to undergo metamorphosis corresponds to Stage 0. This solitary polyp is of radial symmetry, has a size of about 1-3 mm and a uniform tube-shaped gastric cavity (Fig. 1A, 2). Three regions can be distinguished along the polyp's apico-basal axis: head region, gastric region, and foot region (Fig. 1A). The head region is comprised by a large, conus-shaped hypostom and a circle of capitate solid tentacles (n = 6-24) at the hypostomal base. On the ultrastructural level, the side of tentacle insertion is additionally characterized by a nerve-ring (Werner *et al.* 1976, Chapman 1978) located immediately apical to the tentacles ("supratentacular" region). Budding of new polyps occurs at the lower end of the gastric region at temperatures below 25°. At higher temperatures there was a significant shift of the budding region towards the apical end. Polyps undergoing metamorphosis can have buds initially, but they never begin to propagate asexually when metamorphosis was initiated. Throughout metamorphosis, the polyp is embedded with its foot region in a muccous-like peridermal cup, which attaches the polyp to the substratum.

Formation of the medusa anlage

During the first phase of metamorphosis (stage 1-4), several changes occur which are restricted to the

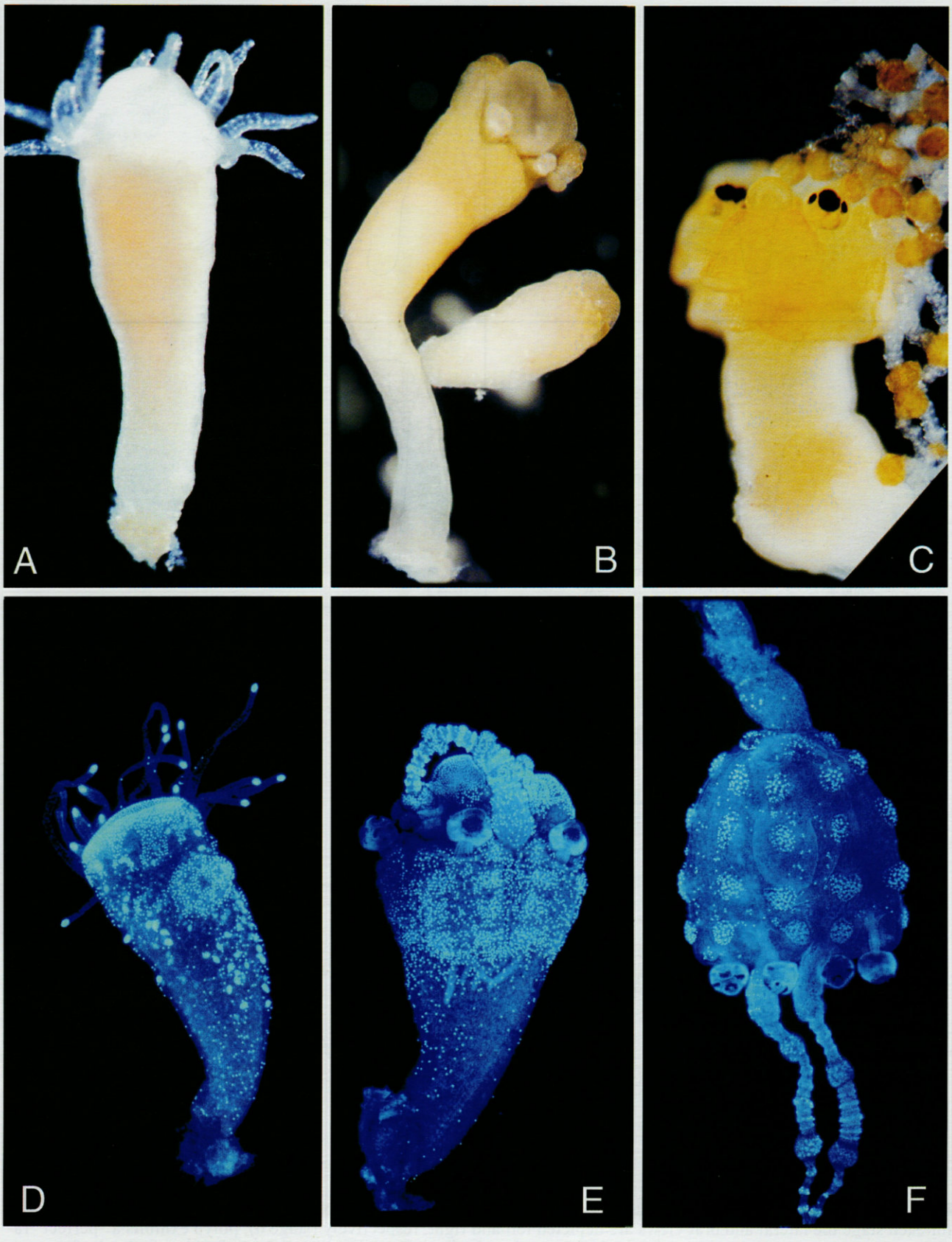


Fig. 1. – Metamorphosis of *Carybdea marsupialis*. A-C, macrographs show the transformation of a steady state cubopolyp (stage 0; A) into a medusa which occurs mainly at the oral end of the polyp; B, stage 5 and (C) stage 7 correspond to Fig. 2E, G, respectively. Note the polyp bud in (B) which also undergoes metamorphosis. D-F, Pattern of nematocysts as visualized by DAPI-staining in a stage 0 polyp (D), a polyp in the midth (stage 5; E) and a polyp at the end of metamorphosis (late stage 8; F). For clarity the stage 8 polyp (F) is inverted by 180°C which corresponds to the orientation of a detached medusa. Note the pseudostenoteles at the tip of the tentacles in (D) and the rudiment of the polyp's stalk at the top of the exumbrella in (F). Maginifications (A-C) X 47; (D-F) X 50.

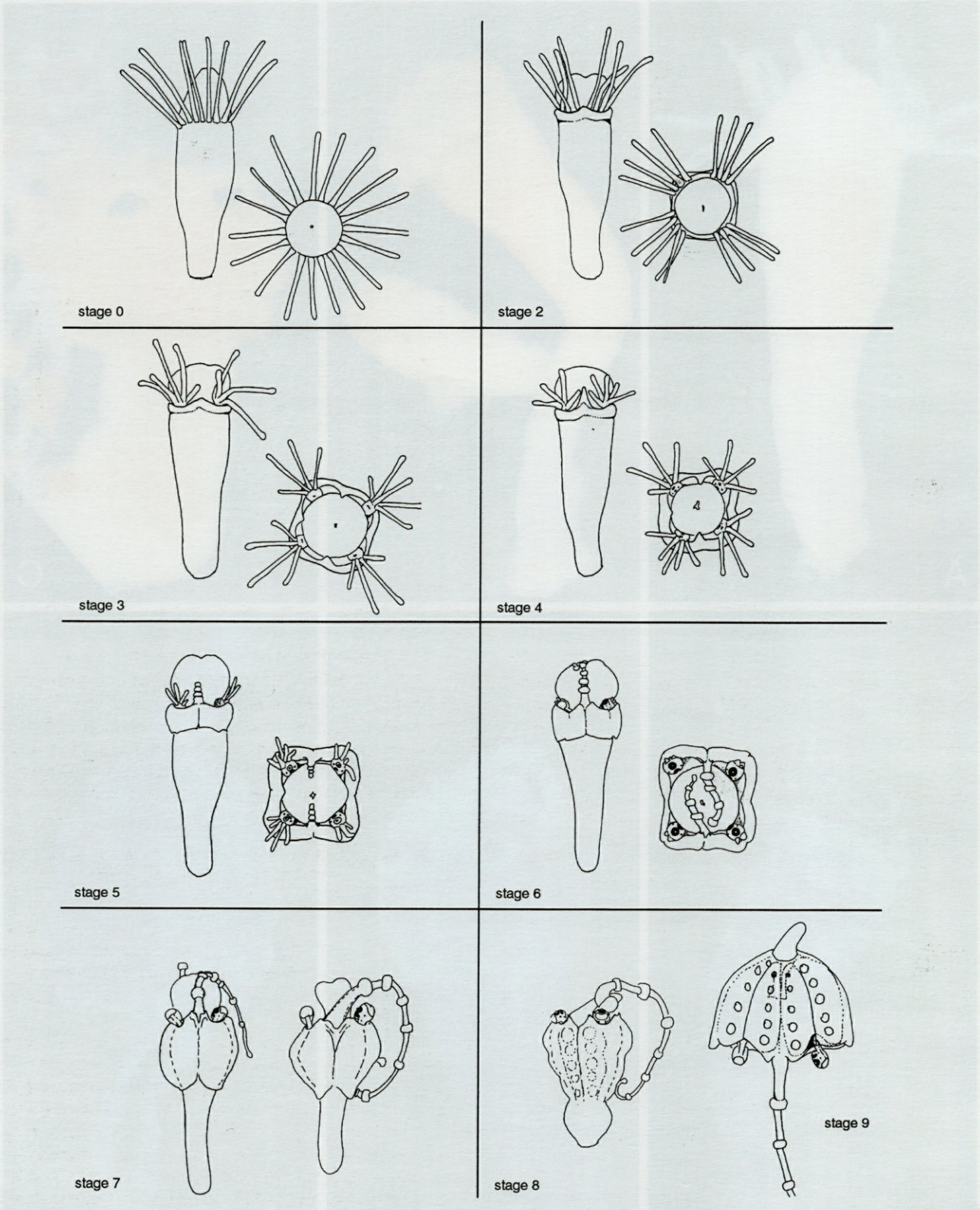


Fig. 2. – Schematic drawings of characteristic stages of cubozoan metamorphosis (*Carybdea marsupialis*). (Stage 0-6) for each stage the lateral and oral views are depicted left and right, respectively (stage 0), polyp exhibits a "perfect" radial symmetry; (stage 2), extension of the hypostomal region and concentration of the polyp's tentacles in four quadrants; (stage 3), fusion of the polyp's tentacle bases and appearance of pigmented eye spots; (stage 4), appearance of the medusa tentacles; (stage 5), fully differentiated eyes and rudiments of polyp' tentacles; (stage 6), disappearence of the polyp's tentacles, elongated medusa tentacles; (stage 7) lateral view of an early and late stage 7 polyp; (stage 8) late stage 8 polyp, the polyp's stalk region becomes reduced and the hypostomal region acquires a bell-like shape; (stage 9) early detached fully metamorphosed medusa with rudiment of the polyps stalk at the tip of the exumbrella (compare Fig. 1F).

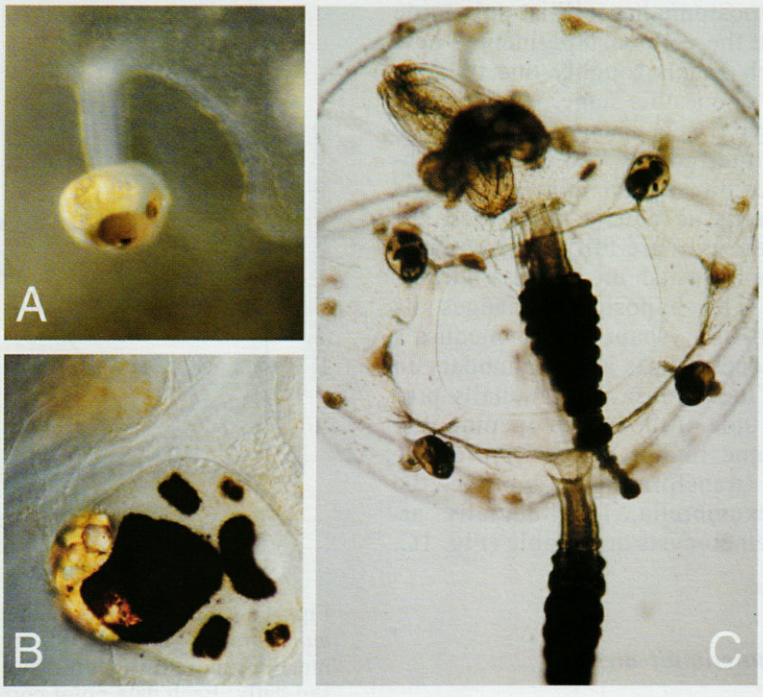


Fig. 3. – Detached, fully differentiated medusa of *Carybdea marsupialis*. (A-B) Rhopalium and lens eyes in a macroscopic (A) and microscopic view (B). Free swimming medusa exhibiting the velarium, two tentacles with thickened pedalia, the four rhopalia and the manubrium with gastral filaments inside (C). (A) X 100; (B) X 150; (C) X 45.

apical region of the polyp, i.e. to the hypostomal and subhypostomal region (Fig. 2). This tissue becomes gradually pigmented and aquires a tetraradial symmetry while the rest of the polyp retains its radial symmetry. During stage 1 (not shown in Fig. 2), a furrow forms in the basal hypostomal region at the boundary to the side of tentacle insertion, so that a disitinct bulge-like ring of subhypostomal tissue forms just below the tentacle base. This furrow progressively invaginates during metamorphosis and represents the shaping force for the formation of gastric cavities and subumbrellar space. During stage 2, the tentacles become displaced into four distinct groups with 4-6 tentacles, which is the first visible step in the transformation of the radial symmetry towards a tetraradial symmetry. Simultaneously, the hypostomal and subhypostomal region expand (Fig. 2, stage 2). In stage 3 a furrow (hypostomal furrow) forms at the base of the lower hypostome thereby separating the hypostomal region from the rest of the polyp (Fig. 2, stage 3). In each of the four tentacle groups, tentacle bases begin to merge and at their common base a pigmented spot appears. This pigmented tissue will finally differentiate into the lens eye of the medusa (Fig. 3A-B). Stage 4 is characterized by the emergence of the first anlage of the medusa tentacles, which differentiate interradially (Fig. 2, stage 4). Only one of the two tentacle pairs grows further during metamorphosis; the second pair completes differentiation not before the young medusa. Additionally, further pigmented spots appear in the eye anlage. Interestingly, also the entire ectodermal tissue of the presumptive medusa is acquiring a yellowish colour, which might reflect an increased tissue turnover or cell differentiation.

Setting up the medusa body plan

During the second phase of metamorphosis (stages 5-7) the redesign of the polyp continuously extends towards a basal direction (Fig. 1B-C). This process includes an invagination of the circular (hypostomal) furrow and the formation of four gastric pockets. By these changes the polyp acquires a tetraradial symmetry. During stage 5 the distal parts of the polyp's tentacles are progressively resorbed and the rhopalia further increase in size (Fig. 1B, Fig. 2, stage 5). Each rhopalium finally differentiates six eyes, and by invagination of the two large central pigment spots the lens eyes are formed. Apically, a statolith differentiates which is easily visible by its crystalline inclusions. The medusa tentacles elongate and form an increasing number of circular nematocyst battery cells. Thereafter (stage 6), the polyp's tentacles are completely absorbed and the eye stalk becomes visible (Fig. 2, stage 6). The medusa's tentacles contract, grow, and differentiate battery cells with mounted

nematocysts. The hypostome has still ist polypoide conus-like shape, but the yellowish pigmented new medusa tissue, which equals roughly one third of the polyp's original size at this stage, is contrasted from the whitish polyp tissue. The gastric filaments which differentiate interradially can be easily observed through the transparent body wall. At stage 7 the medusa tissue equals about half the original tissue, and the eyes are fully differentiated (Fig. 2, stage 7). The brownish medusa has a transparent appearance. The hypostome changes its shape into a bell-like shape, the medusa's manubrium. This process starts at the boundary to the gastric cavity of the medusa and gradually progresses to the apical side of the mouth opening, but notably the hypostome retains its position at the apical end of the transforming polyp. In the ectoderm of the exumbrella, longitudinally arranged clusters of nematocysts are visible (Fig. 1C, Fig. 2, stage 7).

Liberation of the functional medusa

During the final phase of metamorphosis the medusa attains its final shape and becomes functional. In stage 8 the hypostome (manubrium) becomes rapidly translocated into the interior of the medusa, and nematocytes are restricted to the squarelike opening of the manubrium (Fig. 2, stage 8). At the side of the exumbrella the longitudinally arranged nematocyste clusters are visible. Mostly the tentacles are retracted inwards to the subumbrellar cavity so that the velum is visible. The medusa begins to contract regularly, and at the final stage 9 the young, bell-shaped medusa detaches from the peridermal cup (Fig. 2, stage 9). Frequently, one can find at the tip of the exumbrella a pointed piece of the rest of the polyps basal end, which is absorbed within a few hours (Fig. 1F). The umbrella has a diameter of about 2.5 mm depending on the polyp's original size. The early detached medusa lacks pedalia and velar channels, but it can catch and digest prey.

In summary, about three weeks are required from the induction of metamorphosis until the detachment of the medusa. A quantitative analysis of the length of individual developmental stages on an absolute time scale (Fig. 4) revealed that each stage required about 36-42 hours. This progression was roughly linear with a mean variation of only 36 hr, indicating that we selected representative developmental stages for the staging scheme of metamorphosis.

Differentiation of a medusa-specific cnidom

The medusa of *Carybdea* possesses medusa-specific nematocysts (cnidom), which differ from

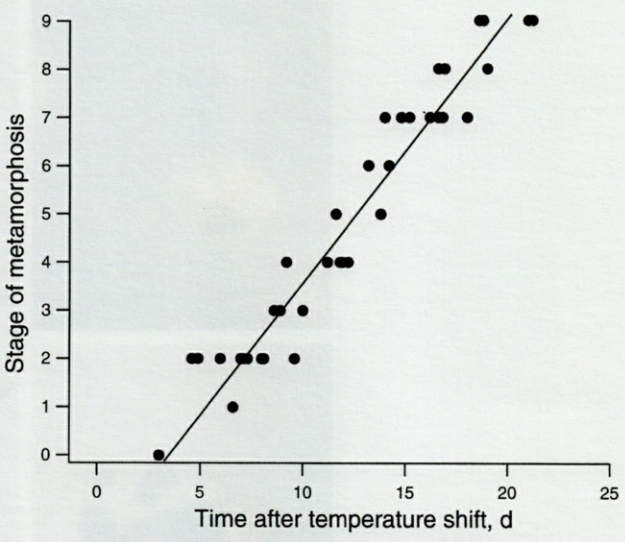


Fig. 4. – Time course of metamorphosis in *Carybdea marsupialis*. Cubopolyps were kept at 20°C, induced to metamorphose by a temperature shift to 28°C and analysed daily. Each data point corresponds to the average of five animals, and ordinate indicates time after temperature shift.

those of the polyp. The polyp has pseudo-stenoteles and microbasic euryteles while the medusa has heterotrichous microbasic euryteles and microbasic mastigophore, atrichous, basitrichous, and holotrichous isorhizas. The medusa-specific nematocysts begin to differentiate during early metamorphosis. Differentiation stages can be visualized by staining with the cationic dye DAPI which binds the polyanioc rich matrix (gamma-polyglutamate) of nematocysts tightly (Szczepanek *et al.* 2002) (Fig. 1D-F). During metamorphosis, the large, polyp-specific pseudo-stenoteles of the polyp (Fig. 1D) rapidly disappear and we presume that they undergo apoptosis. At stage 6 of metamorphosis large (13.3 ± 1.6 μm) and small haplonemes (5.4 ± 0.6 μm) begin to form a highly regular pattern which finally ends in eight bands of five clusters each on the surface of the presumptive exumbrellar ectoderm (compare Figs. 1E and 1F). These nematocyst bands extend from the apex of the presumptive medusa up to the region where tentacles and ropalia are inserted (Fig. 1F). Interestingly however, these nematocyst bands do not colocalize with the site of tentacle insertion, but they rather define the tissue located between the tentacles and rhopalia, i.e. the "interradii". During further growth of the exumbrella the distance between these clusters increases, but neither the number of clusters nor the number of bands increases until the medusa is fully differentiated. In the medusa tentacles ring-like batteries consisting of heterotrichous microbasic euyteles (15.9 μm ± 10.6 μm) and tentacle-specific isorhizas differentiate.

Induction of Metamorphosis

Cubozoan polyps of *Tripedalia cystophora* can spontaneously metamorphose into a medusa (Werner *et al.* 1971). To induce metamorphosis in *Carybdea marsupialis* we explored three environmental parameters: feeding, temperature and population density. We found that the polyps, which were cultured at temperatures varying from 18°C to 28°C, never underwent metamorphosis at temperatures lower than 23°C. However, higher temperatures seemed to be permissive, and sometimes the majority of polyps in a culture dish metamorphosed. We therefore analysed the effect of temperature on metamorphosis first. For that, daily fed polyps were selected. They had an average of 16-17 tentacles (Fig. 1A) and could propagate asexually by budding. These polyps were cultured for a period of two weeks at temperatures of either 20°C or 24°C. Thereafter, the polyps were kept at 28°C without further feeding. Figure 5A shows that both, at a sudden temperature shift from 20°C to 28°C (filled triangles) and a temperature shift from 24°C to 28°C (filled squares) reliably induced metamorphosis in all polyps. Under these conditions the first signs for metamorphosis, e.g. formation of the subhypostomal bulge (stage1) and displacement of the tentacles (stage 2), appeared after 5 and 7 days respectively, and metamorphosis was complete at about 20 days. By comparison, a temperature shift from 20°C to 24°C (filled circles) could also induce metamorphosis, but it was not sufficient for finishing metamorphosis. Metamorphosis was stopped at stage 2 to 3 in such polyps which continued to propagate asexually afterwards. This indicates that not a relative increase in temperature, but rather an increase over a critical threshold temperature of 24°C is required for metamorphosis in *Carybdea marsupialis*.

We also found that the population density had an effect on metamorphosis. When polyps were kept at a high density (1 polyp/ml), progression of metamorphosis was slowed down at late stages (Table I) compared to polyps cultured at low density (1 polyp/30 ml) which finished metamorphosis after 20 days. Interestingly, however, the presence of metamorphosing polyps also exerted a stimulatory effect on metamorphosis. Figure 5B shows a co-culture experiment where an already metamorphosing polyp was placed into a culture dish. In such a co-culture the fraction of polyps starting metamorphosis after a temperature shift from 20 to 24°C (filled circles) significantly increased. While in normal cultures only 40% of all polyps started metamorphosis, in the co-culture 75% started metamorphosis. More dramatically, about 8% of the co-culured polyps reached the medusa stage, while in normal cultures metamorphosis stopped at stage 2-3 (Fig. 5A). Even after a temperature shift to 28°C, which reliably induced metamorphosis (Fig. 5A), we found that the efficiency of metamorphosis was significantly higher as kinetics were shortened by about 2 days (Fig. 5B). This indicates that metamorphosing polyps release a factor stimulating and maintaining metamorphosis.

Table I. – Influence of population density on the induction metamorphosis in *Carybdea marsupialis*. Polyps were cultured at 20°C or 24°C and fed every second day. Metamorphosis was induced by a temperature shift as indicated. In the high density and low density experiment polyps were kept at a density of one polyp per 1 ml and 30 ml seawater, respectively. For the induction and feeding experiment we used high density conditions. The presence of an metamorphosing polyp stimulated metamorphosis (induction), while feeding completely inhibited metamorphosis.

	Stage 0 (polyp)	stages 1-2	stages 3-5	stages 6-8	stage 9 (medusa)
high density					
20°C → 25°C	54%	46%	0%	0%	0%
24°C → 28°C	8%	0%	17%	25%	50%
20°C → 28°C	0%	0%	0%	0%	100%
low density					
24°C → 28°C	0%	0%	0%	10%	90%
induction					
20°C → 25°C	25%	33%	33%	1%	8%
feeding					
20°C → 28°C	100%	0%	0%	0%	0%

Metamorphosis can be blocked by reducing the temperature again down to 20°C. This block is efficient up to metamorphosis stage 7, when polyp tentacles have been completely absorbed and the medusa eyes are already present. Under these conditions the medusa-specific organs regress. After about a week the polyp tentacles have regenerated. This indicates a considerable morphogenetic plasticity of *Carybdea* tissue, which is reminiscent to similar phenomena found in hydrozoans (Hauenschild 1956, Kakinuma 1969, Schmid 1972, 1992, Tardent 1965) and scyphozoans (Kakinuma & Sugiura 1980, Spangenberg 1965).

In a further experiment we tested the effect of feeding on metamorphosis (Table I). In mass cultures that were fed once a week at temperatures of 24°C metamorphosing polyps could be periodically found. However, in cultures which were fed daily over a period of 18 months, we never observed any metamorphosing polyps. Even after a temperature shift from 20°C to 28°C, which is the strongest inducing factor, no polyp started metamorphosis (Table I). This clearly shows that feeding is an efficient inhibitor of metamorphosis in *Carybdea marsupialis*.

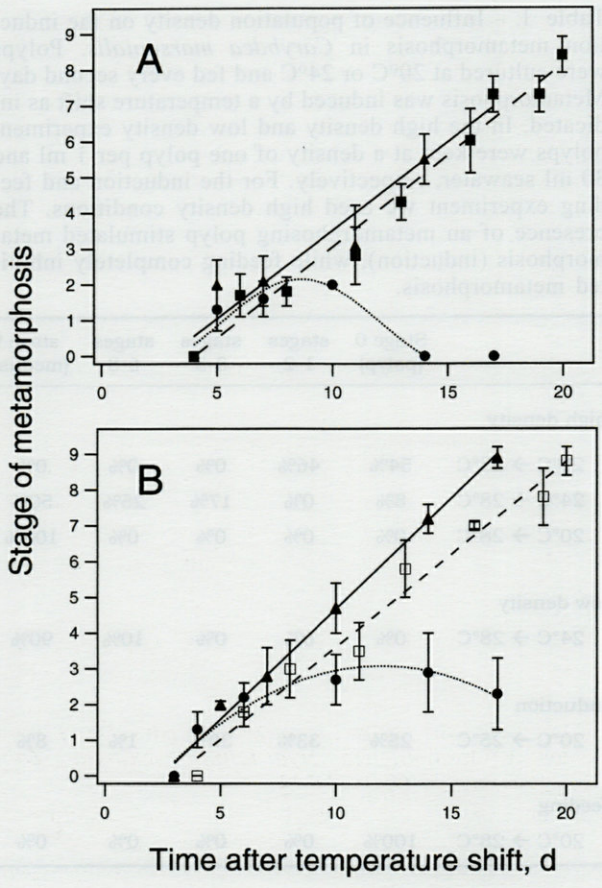


Fig. 5. – The induction and maintenance of metamorphosis requires a shift over a critical threshold temperature. (A) Animals were kept at 20°C or 24°C. At t_0 the temperature was shifted from 20°C to 24°C (filled circles) or to 28°C (filled triangles), and from 24°C to 28°C (filled squares). In animals shifted from 20°C to 24°C metamorphosis is initiated but stops at stage 2-3. (B) Stimulatory effect of a metamorphosing polyp. Animals were kept as in (A), except that a metamorphosing polyp was added to the culture (for details see text).

Medusae represent the gamete producing generation in the cubozoan life cycle. In other cnidarians, the production and release of gametes can be induced by very different external triggers. An increase of temperature can cause the Anthozoa *Anthopleura* to spawn rapidly under laboratory conditions (Siebert 1974), and in *Hydra vulgaris,* which lacks the medusa stage, polyps form gametes in response to starvation within 10-14 days (Hobmayer *et al.* 2001, Martin *et al.* 1997, Miller *et al.* 2000). By comparison light stimuli, which are an important factor for the synchronization of gamete release in hydrozoans (Ballard 1942, Müller 1961) and anthozoans (Baker 1936, Fritzenwanker & Technau 2002, Ryland 1997) have virtually no effects on medusa induction in *Carybdea.*

Medusa formation in Carybdea *is similar to the metamorphosis of the stauromedusa* Stylocoronella

The mode of medusa formation in *Carybdea* shares some similarities with the metamorphosis in Stauromedusae. Polyps of this group do not produce free-swimming medusae by strobilation, as it is typical for other scyphozoans, and adult stauromedusae live attached to the substrate by a stalk. Kikinger & Salvini-Plawen (1995) have shown that the juvenile polyp of *Stylocoronella* develops into a sessile medusa by metamorphosis. The apical half of the metamorphosed medusa bears a number of characters that are similar to adult medusae in other scyphozoans and cubuzoans, e.g. rhopalia, circular coronal muscles gonads and occeli, while the stalk region by comparison retains a polypoid characters such as gastric septa and four longitudinal muscles which are associated with the four peristomal pits surrounding the mouth. This form of metamorphosis is highly reminiscent to the metamorphosis we found in *Carybdea marsupialis*, where the transformation of the polyp was initially restricted to the oral end of the polyp. This is different to *Tripedalia*, where, as in detail described by Werner (1983), the entire polyp appears to undergo a metamorphosis, only leaving the periderm cup of the polyp.

It should be pointed out that in scyphozoans, i.e. in Coronatae, Semaeostomae and Rhizostomae juvenile medusae (ephyrae) are produced by strobilation, i.e. by transverse fission of the ephyra at the oral end of the polyp. While most scyphozoans are characterized by polydisc strobilation, in rhizostomae only a single ephyra develops at the oral end of the polyp. However, monodisc strobilation is different to the stauromedusan and cubozoan mode in medusa formation, since polyps always remain intact after transverse fission of the medusa and continue to propagate asexually.

It has been also proposed that the cubozoan metamorphosis shares some characteristics with medusa formation in the hydrozoan Narcomedusae (Bouillon 1987, Petersen 1979). Similar to Cubozoa the narcopolyps reproduce asexually and subsequently undergo metamorphosis into a single medusa. However, the parasitic narcopolyps are extremely different from other hydrozoan polyps (Bouillon 1987). Both groups are pelagic and have lost their polyp stage, only in some parasitic Narcomedusae a polyp is present, and probably represent a re-evolved polyp-like stage.

ACKNOWLEDGEMENTS. – We would like to thank J Jarms (Hamburg) for his kind gift of *Carybdea marsupialis* polyps and B Hobmayer (Darmstadt) for reading the manuscript critically. Supported by the DFG.

REFERENCES

Arneson AC, Cutress CE 1976. Life history of *Carybdea alata* Reynaud, 1830 (Cubomedusae). *In* Coelenterate Ecology and Behaviour, GO Mackie ed, Plenum Press New York: 227-236.

Baker EGS 1936. Photoperiodicity in the spawning reaction of *Pennaria tiarella. Proc Indiana Acad Sci* 45: 251-252.

Ballard WW 1942. The mechanism for synchronous spawning in *Hydractinia* and *Pennaria. Biol Bull* 82: 329-339.

Berger EW 1898. Histological structure of the eyes of Cubomedusae. *J Compar Neurol* 8: 123-146.

Berger EW 1900. Physiology and histology of the Cubomedusae. *Mem. Biol. Lab.* Johns Hopkins Univ 4: 1-81.

Boero F, Gravili C, Pagliara P, Piraino S, Bouillon J, Schmid V 1998. The cnidarian premises of metazoan evolution: from triploblasty, to coelom formation, to metamery. *Ital J Zool.* 65: 5-9.

Bouillon J 1981. Origine et phylogenèse des Cnidaires et des Hydropolypes-Hydroméduses. *Annals Soc Roy Zool Belg* 111: 45-56.

Bouillon J 1985. Essai de classification des Hydropolypes-Hydroméduses (Hydrozoa-Cnidaria). *Indo-Malayan Zool.* 1: 29-234.

Bouillon J 1987. Considérations sur le développement des Narcoméduses et sur leur position phylogénétique. *Indo-Malayan Zool* 4: 189-278.

Bouillon J, Boero F 2000. Phylogeny and classification of Hydroidomedusae. *Thal Salent* 24: 1-296.

Bridge D, Cunningham CW, Schierwater B, DeSalle R, Buss LW 1992. Class-level relationship in the phylum Cnidaria: evidence from mitochondrial genome structure. *Proc Natl Acad Sci* USA 89: 8750-8753.

Bridge D, Cunningham CW, DeSalle R, Buss LW 1995. Class-level relationship in the phylum Cnidaria: molecular and morphological evidence. *Mol Bio Evol* 12: 679-689.

Brusca RC, Brusca GJ 1990. Invertebrates. Sinauer Association, Sunderland, MA, USA.

Chapman DM 1978. Microanatomy of a cubopolyp, *Tripedalia cystophora* (Class Cubozoa). *Helgol wiss Meeresunters* 31: 128-168.

Claus C 1878. Arbeiten des zoologischen Instituts Wien 2: 221-276.

Conant FS 1897. Notes on the Cubomedusae. Johns Hopkins Univ Circ 132: 8-10.

Conant FS 1898. The Cubomedusae. *Mem Biol Lab* Johns Hopkins Univ 4: 1-61.

Collins AG, Valentine JW 2001. Defining phyla: evolutionary pathways to metazoan body plans. *Evol Devel* 3: 432-442.

Cutress CE, Studebaker JP 1978. Development of the Cubomedusa *Carybdea marsupialis. Proc Ass Isl Mar Labs Caribb* 9: 25.

Fritzenwanker J, Technau U 2002. Induction of gametogenesis in the basal cnidarian *Nematostella vectensis* (Anthozoa). *Dev Genes Evol* 212: 99-103.

Hauenschild C 1956. Experimentelle Untersuchungen über die Entstehung asexueller Klone bei der Hydromeduse Eleutheria dichotoma. *Z Naturforsch* 11b: 394-402.

Hobmayer B, Rentzsch F, Holstein TW 2001. Identification and expression of HySmad1, a member of the R-Smad family of TGF-beta signal transducers, in the diploblastic metazoan Hydra. *Dev Genes Evol* 211: 597-602.

Hyman LH 1940. The Invertebrates, Vol. 1. Protozoa through Ctenophora. McGraw-Hill, New York.

Kakinuma Y, Sugiura Y 1980. Organ differentiation in *Aurelia aurita. In* Developmental and cellular biology of coelenterates (eds P Tardent & R Tardent) Elsevier North Holland, Amsterdam: 257-262.

Kakinuma Y 1969. On the differentiation of isolated medusa buds of the hydrozoans *Cladonema uchidai* and *Cladonema* sp. *Bull Mar Biol Stat Asamuchi* 13: 169-172.

Kikinger R, Salvini-Plawen Lv 1995. Development from polyp to stauromedusa in Stylocoronella (Cnidaria, Scyphozoa). *J Mar Biol Assoc UK* 75: 889-912.

Linnaeus C 1758. Systema naturae, 10th ed., Stockholm.

Martin JV, Littlefield CL, Archer WE & Bode HR 1997. Embryogenesis in *Hydra. Biol Bull* 192: 345-363.

Meglitsch PA, Schram FR 1991. Invertebrate Zoology, 3rd ed. Oxford University Press, New York.

Miller M, Technau U, Smith K, Steele R 2000. Oocyte development in *Hydra* involves selection from competent precursor cells. *Dev Biol 224:* 326-338.

Müller WA 1961. Untersuchungen zur Ablaichrhythmik des Hydroidpolypen *Hydractinia echinata. Zool Jahrb Physiol* 69: 317-324.

Nielsen C 2001. Animal Evolution: Interrelationships of the living phyla. Oxford University Press, New York.

Okada YK 1927. Note sur l'ontogénie de *Charybdea. Bull Biol Fr Belg* 61: 241-249.

Odorico DM, Miller DJ 1997. Internal and external relationship of the Cnidaria: implications of primary and predicted secondary structure of the 5'end of the 23S-like rDNA. *Proc R Soc London* B 264: 77-82.

Petersen KW 1979. Development of coloniality in Hydrozoa. *In* Biology and Systematics of Colonial Organisms, G Larwood & BR Rosen eds, Academic Press, New York: 105-139.

Petersen KW, Eernisse DJ 2001. Animal phylogeny and the ancestry of bilaterians: inferences from morphology and 18S rDNA gene sequences. *Evol Devel* 3: 170-205.

Piraino S, Boero F, Aeschbach B, Schmid V 1996. Reversing the life cycle: Medusae transforming into polyps and cell transdifferentiation in *Turritopsis nutricula* (Cnidaria, Hydrozoa). *Biol Bull* 190: 320–312.

Ryland JS 1997. Reproduction in Zoanthidea (Anthozoa: Hexacorallia). *Invert Reprod Dev* 31: 177-188.

Salvini-Plawen Lv 1978. On the origin and evolution of the lower Metazoa. *Zeitsch zool Syst Evol* 16: 40-88.

Salvini-Plawen Lv 1987. Mesopsamic Cnidaria from Plymouth (with systematic notes). *J Mar Biol Assoc UK* 67: 623-637.

Schmid V 1972 Untersuchungen über Differenzierungsvorgänge bei Medusenknospen und Medusen on *Podocoryne carnea* M.Sars. *Wilhelm Roux's Arch Develop Biol* 169: 281-301.

Schmid V 1992. Transdifferentiation in medusae. *Int Rev Cytol* 142: 213–261.

Schuchert P 1993. Phylogenetic analysis of the Cnidaria. *Zeitsch zool Syst Evol* 31: 161-173.

Siebert AE Jr 1974. A description of the embryology, larval development, and feeding of the sea anemones *Anthopleura elegantissima* and *A. xanthogrammica*. *Can J Zool* 52: 1383-1388.

Spangenberg DB 1965. Rhopalium development in *Aurelia aurita*. *J Exp Zool* 178: 183-194.

Szczepanek S, Cikala M, David CN 2002. Poly-gamma-glutamate synthesis during formation of nematocyst capsules in Hydra. *J Cell Sci* 115: 745-51.

Tardent P 1965. Developmental aspects of regeneration in coelenterates. *In* Regeneration in Animals and Related Problems, V Kiortsis & H A L Trampusch eds, Elsevier North-Holland, Amsterdam: 71–87.

Tardent P 1978. Coelenterata, Cnidaria. *In* Seidel F ed, Morphogenese der Tiere. Fischer Verlag, Jena/Stuttgart.

Uchida T 1929. Studies on the Stauromedusae and Cubomedusae. *Jap J Zoology* (Tokyo) 2: 103-193.

Werner B 1973. New investigations on systematics and evolution of the class Scyphozoa and the phylum Cnidaria. *Pub Seto Mar Biol Lab* 20: 35-61.

Werner B 1975. Bau und Lebensgeschichte des Polypen von *Tripedalia cystophora* (Cubozoa, Class nov., Carybdeidae). *Helg wissensch Meeresunters* 27: 461-504.

Werner B 1983. Die Metamorphose des Polypen von *Tripedalia cystophora* (Cubozoa, Carybdeidae) in die Meduse. *Helgol Wissensch Meeresunters* 36: 257-276.

Werner B 1984. Stamm Cnidaria. *In* A Kaestner, Lehrbuch der Speziellen Zoologie, 4th ed. by HE Gruner I (2) VEB G. Fischer Verlag, Jena: 11-305.

Werner B, Cutress CE, Studebaker JP 1971. Life cycle of *Tripedalia cystophora* Conant (Cubomedusae). *Nature* 232: 582-583.

Werner B, Chapman DM, Cutress CE 1976. Muscular and nervous system of the cubopolyp (Cnidaria). *Experientia* 32: 1047-1048.

Yamaguchi M, Hartwick R 1980. Early life history of the sea wasp *Chironex fleckeri* (Class Cubozoa). *In* Developmental and cellular biology of coelenterates Elsevier (eds P Tardent & R Tardent): 11-16, Amsterdam.

Yamasu T, Yoshida M 1976. Fine structure and complex ocelli of a cubomedusan, *Tamoya bursaria* Haeckel. *Cell Tiss Res* 170: 325-339.

Reçu le 5 juillet 2002; received July 5, 2002
Accepté le 28 août 2002; accepted August 28, 2002

VIE MILIEU, 2002, 52 (4) : 141-147

From Marine Ecology to Developmental Biology
In Honour of Pierre Tardent (1927-1997)

INDUCTION OF LARVAL METAMORPHOSIS IN THE TROPICAL SCYPHOZOAN *MASTIGIAS PAPUA*: STRIKING SIMILARITY WITH UPSIDE DOWN-JELLYFISH *CASSIOPEA* SPP. (WITH NOTES ON RELATED SPECIES)

D.K. HOFMANN[1]*, *G. CROW*[2]

[1]*Ruhr-University-Bochum, Dept. Zoology, Devel. Biol. Unit, D-44780 Bochum, Germany*
[2]*University of Hawai'i, Waikiki Aquarium, Honolulu, Hawai'i 96815, USA*

Dietrich.K.Hofmann@ruhr-uni-bochum.de
* *to whom correspondence should be addressed*

CNIDARIA
MASTIGIAS PAPUA
CASSIOPEA SPP.
PHYLLORHIZA PUNCTATA
CEPHEA CEPHEA
METAMORPHOSIS

ABSTRACT. – Planula larvae of *Mastigias papua* were obtained from egg masses released from a medusa on display at the Waikiki Aquarium, Honolulu, Hawai'i, and were induced to develop into the polyp form in the laboratory. The pattern of metamorphic responses of the larvae to a biogenic substratum, i.e. deteriorating leaves of the Red Mangrove, *Rhizophora mangle*, and to the dissolved, synthetic oligopeptide Z-GPGGPA, coincided strikingly with observations published on *Cassiopea* spp. (Rhizostomea). *M. papua* thus represents the second rhizostomean genus in which experimental induction of larval metamorphosis has been achieved.- Scyphistomae of the rhizostomes *M. papua*, *Cassiopea* spp, *Cephea cephea*, and *Phyllorhiza punctata* show identical modes of asexual reproduction: monodisk strobilation of medusae and budding of larva-like motile buds. Buds of *Cassiopea* spp. responded likewise to metamorphosis-inducing peptidic cues emanating from mangrove leaves (Fleck *et al.* 1999), and buds from all species metamorphosed within 24h when treated with the hexapeptide Z-GPGGPA at micromolar concentrations. Our findings support the view of Siefker *et al.* 2000 that exogenous cues inducing larval metamorphosis in species of the order Semaeostomea differ significantly from those described here, where they act uniformly on propagules of four species belonging to three different families of the order Rhizostomea.

CNIDARIA
MASTIGIAS PAPUA
CASSIOPEA SPP.
PHYLLORHIZA PUNCTATA
CEPHEA CEPHEA
METAMORPHOSE

RÉSUMÉ. – Des larves planula de *Mastigias papua* ont été obtenues à partir d'œufs pondus par une Méduse maintenue en aquarium (Waikiki Aquarium, Honolulu, Hawaii) ; ces larves ont été induites à se développer en polype au laboratoire. L'ensemble des réponses métamorphiques des larves à un substrat biogénique (en l'occurrence des feuilles de mangrove [*Rhizophora mangle*] en décomposition) et à un oligopeptide synthétique dissout le Z-GPGGPA, correspond largement aux observations déjà publiées au sujet de *Cassiopea* spp. (Rhizostomea). *M. papua* représente donc le deuxième cas d'un genre de Rhizostomé permettant l'induction expérimentale de la métamorphose des larves. – Les scyphistomes des Rhizostomés *M. papua*, *Cassiopea* spp., *Cephea cephea* et *Phyllorhiza punctata* présentent des modes de reproduction asexuée identiques : strobilisation en « monodisc » pour la formation de méduses et bourgeonnement de boutons mobiles ressemblant à des larves. Les boutons de *Cassiopea* spp. répondent également aux inducteurs peptidiques de métamorphose émanant des feuilles de mangrove (Fleck *et al* 1999), et les boutons de toutes les espèces étudiées effectuent la métamorphose en 24 heures après exposition à des concentrations micro-molaires de l'hexapeptide Z-GPGGPA. Nos résultats confortent l'hypothèse de Siefker *et al.* (2000) selon laquelle les inducteurs exogènes de métamorphose agissant sur les larves des espèces de l'ordre des Semaeostomea diffèrent fortement de ceux décrits dans ce travail, qui agissent sur les propagules de quatre espèces représentant trois familles de l'ordre des Rhizostomea.

INTRODUCTION

Mastigia papua is a widely distributed rhizostome jellyfish species, recorded e.g. from the Phillipines, the Jellyfish Lake in Palau (Micronesia), and from the coasts of Japan (see Kramp 1970, for review). These conspicuous medusae have also been observed in Hawaiian waters and are displayed frequently at the Waikiki Aquarium in Honolulu (Fig. 1). The life-cycle of *M. papua* has been described in a classic paper by Uchida (1926). Sugiura (1963) contributed further details, and was the first to establish laboratory cultures of this species. Analysing factors controlling asexual reproduction in *M. papua,* this author proved experimentally the indispensability of endosymbiotic zooxanthellae for strobilation of medusae (Sugiura 1964). The present paper aims to report for the first time the experimental induction of larval metamorphosis in *Mastigias papua,* and to compare these findings with published observations on other representatives of the scyphozoan order Rhizostomea: *Cassiopea* spp. It reviews briefly records of rhizostomean polyps and their remarkably uniform mode of asexual reproduction by larva-like, motile buds, and contributes first results on chemical induction of metamorphosis in buds of *M. papua* and of two other rhizostome species: *Cephea cephea* and *Phyllorhiza punctata.*

MATERIALS AND METHODS

On August 23, 2000 one of the *M. papua* L. Agassiz medusae displayed at the Waikiki Aquarium in Honolulu, Hawai'i (originating from the Jellyfish Lake in Palau, Micronesia), released masses of developing eggs when placed in another tank. One batch of eggs was sampled into fingerbowls with seawater that had been passed through a 0.45 µm filter and maintained at 26 °C. A second batch was collected and placed in filtered seawater containing antibiotics (ABS) made up with 100 µg/ml each of penicillin, streptomycin, and neomycin. Several hundred larvae were collected and transferred to seawater containing antibiotics.

To serve as a natural substratum dark, deteriorating leaves of the Red Mangrove (*Rhizophora mangle*) had been collected from a mangrove-sheltered fish pond near Kualoa Point (O'ahu, Hawai'i). Before use the surface biofilm was wiped off with filter paper and the cleaned leaves were thoroughly rinsed in filtered seawater.

Polyps were reared successfully from planula larvae at Kewalo Marine Lab (Honolulu, Hawai'i (see below). Due to the minute size of the newly metamorphosed scyphistomae, rotifers (*Brachionus* sp.) from mass-cultures were used as initial diet. Following appropriate growth the polyps were supplied for several days with chopped *Artemia salina* nauplii, thereafter newly hatched, intact brine-shrimp larvae were provided twice a week. The seawater of the culture dishes was exchanged completely several hours after feeding.

Cultures of scyphopolyps of the rhizostome species *Mastigias papua* L. Agassiz (Lesson 1830), *Phyllorhiza punctata* von Lendenfeld, 1884, and *Cephea cephea* (Forskal 1775) were kindly provided by the Zoo-Aquarium, Berlin. The scyphistomae were reared and propagated asexually in our lab (University of Bochum, Germany) either in fingerbowls with 150-200 ml of pasteurized, natural seawater (from the North Sea), or in rectangular polystyrene containers with about 500 ml seawater at a constant temperature of 25°C. Polyps were fed *Artemia salina* nauplii *ad libitum*, cleaned and provided with fresh sea water twice a week. Polyp cultures of the rhizostome *Cassiopea andromeda*, referred to in this paper, were derived from a strain obtained from the former Löbbecke Museum and Aquarium in Düsseldorf. *C. xamachana* polyps were derived from a stock raised from planula larvae which had been collected on Grassy Key, Florida. Both species were raised in our lab routinely with the above methods.

Scyphistomae of the aforementioned species are known to reproduce asexually by monodisk strobilation of ephyrae and by producing spindle-shaped, larva-like motile buds (see Calder 1982, Table I). To study experimental induction of metamorphosis, planula larvae hatched from egg masses, and larva-like buds propagated in the polyp cultures were collected, washed and stored in ABS. The hexapeptide Carbobenzoxy-Gly-Pro-Gly-Gly-Pro-Ala (Z-GPGGPA) (from BACHEM), known to induce metamorphosis in propagules of *Cassiopea* spp. *in vitro* at micromolecular concentrations (Hofmann *et al.* 1996, for review) was used for the present work. As described by Hofmann & Brand (1987), development of larvae and buds maintained in ABS was compared to those treated continuously in solutions of Z-GPGGPA in ABS at concentrations ranging from 2-15 µg/ml (equaling 0.29 to 2.20 × 10^{-5} mol/l). Ten individuals were introduced into each well of sterile 24-well tissue-culture plates in 1 ml solution; the experiments were performed in two to six replicates. An additional metamorphosis experiment was run in duplicate using glass Petridishes into each of which a glass slide was inserted in oblique position so that both sides were accessible for the larvae. Approximately 100-150 planula larvae were introduced in 28 ml solution of 15 µg Z-GPGGPA/ml ABS.

Three batches of approximately 200 planula larvae were exposed to cleaned and rinsed deteriorating leaves of *Rhizophora mangle* floating in fingerbowls in about 250 ml filtered seawater.

To perform metamorphosis experiments on asexual buds of *Mastigias papua*, *Phyllorhiza punctata*, and *Cephea cephea*, propagules not older than 1-3 days were collected from the polyp cultures, rinsed and stored in ABS, and then assayed in Z-GPGGPA solutions (10 µg/ml) in 24-well tissue-culture plates as detailed above.

Settlement and morphogenesis of larvae and buds were scored after 24 and 48 h according to the stages proposed by Gohar & Eisawy (1960) for *Cassiopea andromeda* planulae, and by Curtis & Cowden (1971), as modified by Hofmann *et al.* (1978), for *C. andromeda* larva-like buds. Subsequent development of newly formed scyphistomae was followed in some cases for up to 120h.

Photomicrographs were taken using a Zeiss Axiophot compound microscope with Kodak T320 Ectachrome

film, and using an Olympus OM2N camera mounted on a flash-equipped Olympus dissecting microscope with Kodak E100S film.

RESULTS

Induction of metamorphosis in planula larvae

Hatching of planula larvae from *Mastigias papua* egg masses maintained in ABS was readily observed within about 48h. On the other hand, the yield from eggs stored in filtered seawater without antibiotics was unexpectedly low, with most embryos abortive. Planula larvae run as controls in the experiments (Fig. 2, Table I, top) continued swimming around with the blunt end ahead, and all remained in the larval state throughout the observation period. However solutions of the hexapeptide Z-GPGGPA elicited settlement and metamorphosis in a dose-dependent manner. Whereas no reaction whatsoever was observed at 2 µg/ml, the percentage of larvae undergoing attachment at the anterior, blunt end, and subsequent polyp formation in the tissue-culture wells increased significantly at higher concentrations (Table I, top). Part of the individuals had attached to the walls, others hanging upside down from the water surface pellicle. The latter frequently showed a strong, irregular spreading of cells of the pedal disk area, which could be due to particular surface tension conditions in the wells of the tissue culture plates. Within 48h, segregation of the pedal disk, the stalk and the calyx portion with the first tentacle anlagen at its rim had taken place (illustrated by Figs 3a,b,c).

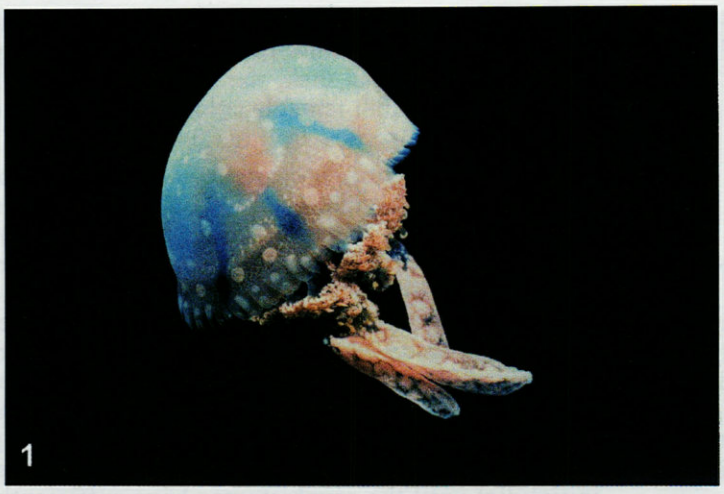
Of the two sets of larvae assayed in larger numbers in glass Petri dishes in Z-GPGGPA solution (15 µg/ml) almost 100 % in one set and about 70 % in the other set had already undergone metamorphosis within 24h. The majority had attached to the glass surfaces of the dish and of the inserted slide; some were observed hanging from the water surface, but without particular aberration or even enlargement of the pedal disk. Feeding trials were undertaken at the end of the experiments, showing that rotifers (*Branchionus* sp.) provide a suitable initial diet until the scyphistomae have sufficiently grown to ingest *Artemia salina* nauplii, the food organism most commonly used for culturing cnidarians.

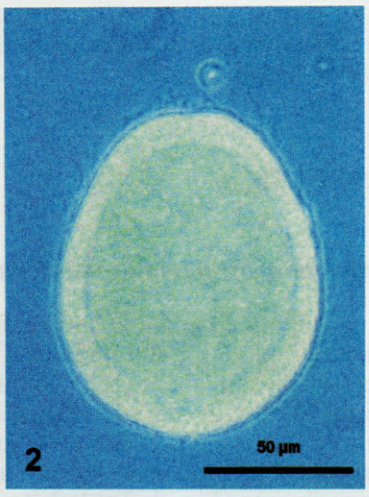
Deteriorating leaves of *Rhizophora mangle* proved to offer a very attractive biogenic substratum for *M. papua* larvae to settle on: in the three replicates of mangrove leaves to which planulae in filtered seawater (without any chemicals added) had been transferred, the majority of the larvae had settled and already metamorphosed within 24h. The planulae attached exclusively to the leaf surfaces (Fig. 4), mostly on the shaded side. None were found fixed to the glass walls, or hanging from the water surface pellicle.

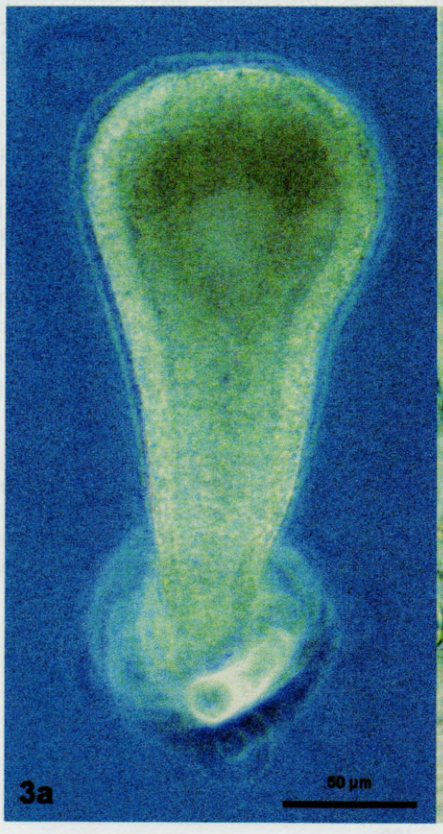
Table I. – Top, induction of metamorphosis in planula larvae of *Mastigias papua* by the hexapeptide Z-Gly-Pro-Gly-Gly-Pro-Ala. For each percentage value n=60, representing the mean of six replicates with 10 larvae each. Larvae were classified as metamorphosed when reaching at least the stage shown in Fig. 3b. Bottom, induction of metamorphosis in larva-like buds by the hexapeptide Z-GPGGPA (10 µg/ml), and in controls in ABS. Buds were classified as metamorphosed when reaching at least a stage comparable to that shown in Fig. 3a for planula larvae. Percentage values represent the mean of either two or three replicates each with 5 (*) or 10 buds.

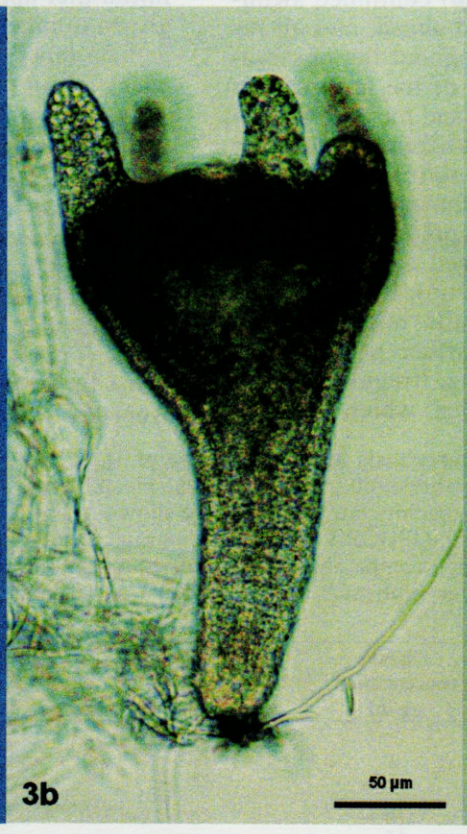
Peptide concentration µg/ml	% Metamorphosis after 48 h
2	0
5	23
10	98
15	100
ABS (control)	0

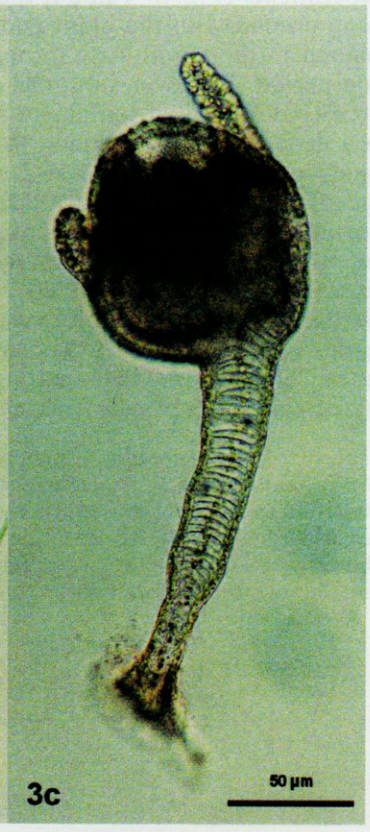
		% Metamorphosis after 24 h		
	n	Z-GPGGPA	n	ABS
Mastigias papua	30	100 %	30	0 %
Cephea cephea	30	100 %	15*	0 %
Phyllorhiza punctata	20	100 %	15*	0 %
Cassiopea xamachana	30	100 %	30	0 %


1

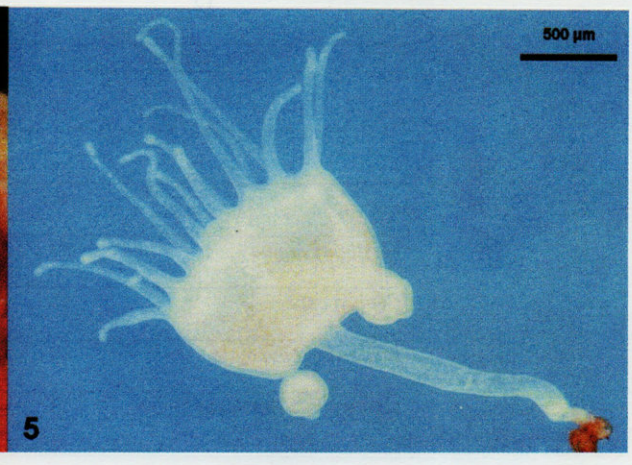

2
50 µm


3a
50 µm


3b
50 µm


3c
50 µm


4


5
500 µm

The metamorphic responses of *M. papua* planulae both to treatment with the synthetic peptide and to the natural substrate were strikingly similar to results of previously published experiments with *Cassiopea xamachana* larvae (summarized in Hofmann *et al.* 1996). The latter metamorphosed at 100 % within 24h when treated with Z-GPGGPA at concentrations ranging from 1.3×10^{-5} to 3.4×10^{-6} mol/l, which are very close to the effective concentrations found here for *M. papua.* Above all, deteriorating mangrove leaves were reported to be the most important natural substrate to settle on in the habitat of *C. xamachana* in the Florida Keys.

Induction of metamorphosis in motile buds

Mastigias papua polyps (Figs 4, 5) propagate asexually by strobilation and by lateral budding of motile, larva-like buds, arising at one or more sites from the lower portion of the polyp's calyx. *Phyllorhiza punctata, Cephea cephea,* and *Cassiopea xamachana* scyphystomae share this type of bud formation (see discussion).

Induction of metamorphosis studied subsequently in the planula-like buds of *M. papua, P. punctata, C. cephea* and, for comparison with previous results, in *C. xamachana*, turned out to be remarkably similar in all four species. No tendency of spontaneous attachment and polyp formation at all was observed in replicates with buds maintained in ABS. But the synthetic peptide Z-GPGGPA at 10 µg/ml (= $1{,}47 \times 10^{-5}$ mol/l) elicited settlement and metamorphosis in 100 % of the propagules within 24 h (Table I, bottom). As seen in planula larvae, buds metamorphosed either attached to the walls of the culture plate wells, or hanging upside down from the water surface pellicle. These results coroborate all observations made previously on *Casssiopea* spp.

DISCUSSION

Scyphopolyps of the Rhizostomea

Scyphomedusae, reaching bell-diameters of up to 225 cm (Arctic form of *Cyanea capillata*) belong to the most impressive members of the marine plankton community (Arai 1997, Goy & Toulemont 1997). On the other hand scyphopolyps, or Scyphistomae, the asexually reproducing form within the metagenetic life-cycle typical of this class, "are inconspicious and small, never reaching the high level of structural diversity observed in polyps of the Hydrozoa" (Tardent 1978, p.109). Within the order Rhizostomea the polyp form is known only from a minority of recorded species of medusae, mostly from laboratory observations starting from embryonic or larval stages (Hofmann *et al.* 1978, Calder 1982, Arai 1997). Scyphistomae have been described from the natural habitat only in *Cassiopea xamachana* (Bigelow 1900, Hofmann *et al.* 1996), in a *Cassiopea* species referred to as *C. andromeda* (Hofmann & Hadfield 2002), in *Rhopilema verrilli* (Cargo 1971), *Cotylorhiza tuberculata* (Kikinger 1992), and tentatively in *Mastigias papua* (Fitt personal comm.).

Asexual reproduction of Scyphistomae: bud formation

Three modes of asexual reproduction of Scyphistomae have been reported (see Calder 1982 for review): i: strobilation of medusae which is usually monodisk, but also polydisk in *Rhopilema verrilli* and *Stomolophus meleagris* (Cargo 1971, Calder 1973), ii: budding from the lower part of the calyx of spindle-shaped, larva-like, motile propagules which, following settlement and metamorphosis, develop into scyphopolyps, iii: formation of podocysts from the pedal disk as dormant propagules, so far described only for *R. verrilli* and *S. meleagris* (Cargo 1971, Calder 1973).

Budding of motile, larva-like buds (sometimes described as frustules, pseudoplanulae, or planuloids, e.g. Tardent 1978, p. 319) is an almost general trait of rhizostome polyps. Subtitled "Monstrositäten und Knospungserscheinungen der Scypistomen (in transl.: monstrosities and budding phenomena in scyphistomae) Goette (1887: 24-25, figs 14-16) described, and illustrated with wood carvings lateral budding in a *Cotylorhiza tuberculata* polyp. He noted that, compared to bud formation in other cnidarians, polarity of buds was reversed in as much as the distal part of the bud would give rise to the future polyp's aboral structures, i.e. pedal disk and stalk, whilst the proximal portion would form calyx, hypostome, and tentacles. All subsequent accounts of budding have confirmed that development of motile buds endowed with this unusual type of polarity is a synapomorphic trait of rhizostome polyps (*Cotylorhiza tuberculata*: Claus

Plate I. – Fig. 1, adult *Mastigias papua* medusa, bell diameter appr. 15 cm. Photograph reproduced with permission of the Waikiki Aquarium. Fig. 2, newly hatched planula larva of *Mastigias papua.* The larva will attach with the anterior, blunt end, which is pointing downwards in the photomicrograph. Fig. 3a, b & c, early and advanced stages of planula larvae of *Mastigias papua* undergoing settlement and polyp metamorphosis (the fibrous material in Fig. 3b has been trapped accidentially in mucus secreted by the settling individual). Fig. 4, *Mastigias papua* polyps raised from planula larvae which had been induced to settle on a degrading *Rhizophora mangle* leaf. Hight with expanded tentacles: 3 to 4 mm. Fig. 5, *Mastigias papua* polyp showing spindle-shaped buds prior to release from two sites at the calyx.

1890 & 93; *Cassiopea xamachana*: Bigelow 1900, Fitt 1991, van Lieshout & Martin 1992; *C. andromeda*: Gohar & Eisawy 1960; *C. frondosa*: Hofmann, unpubl. observations; *Mastigias papua*: Sugiura 1963, and this paper; *Cephea cephea*: Sugiura 1966, and this paper; *Phyllorhiza punctata*: this paper). Except for *Cephea cephea* and *Cotylorhiza tuberculata*, buds separate from the scyphistomae with no visible anlagen of polyp structures such as hypostome or tentacle rudiments. In *C. cephea* however, tentacle buds are already present before the propagules detach from the parent (Sugiura 1966). Whereas Claus 1893 (Plate I) has depicted budding and release of motile buds in *Cotylorhiza tuberculata*, according to Goette (1887, figs 14-16) buds of this species develop even further towards the polyp form before separating: the proximal part forms tentacle anlagen, and the distal end starts elongating as a stalk rudiment with the adhesive cells of the future pedal disk at its tip. Buds at this advanced stage of morphogenesis may attach to the substratum either prior to or after detaching from their parent polyps (Goette 1887, *loc. cit.,* and personal observations). Sugiura (1966) noted that scyphistomae of *Cephea cephea* in his laboratory cultures also showed the separation of buds at different stages of morphogenesis.

Induction of metamorphosis

Summarizing published results and personal observations, Curtis & Cowden (1971, p. 245) noted: "for development to proceed beyond the swimming bud (to be added: and planula) stage, the buds (and planulae) *must* settle on some form of substrate. However, despite the critical importance (of) settling factors affecting *selection* of the substrate by the buds (and larvae) remain obscure and were consequently difficult to control". Studying the life-cycle of the semi-sessile, upside-down jellyfish *Casssiopea xamachana* in the Florida Keys, Fitt (1991), Hofmann *et al.* (1996) and Fleck & Fitt (1999) reported that scyphistomae of this species occurred in the natural habitat in the mangrove-sheltered lagoons almost exclusively on the shaded side of floating, deteriorating leaves of the Red Mangrove, *Rhizophora mangle*. Fleck & Fitt (1999) demonstrated that a natural settlement cue for planula larvae, supposedly at least one peptidic product, is released from such leaves through microbial degrading activity. Further separation steps performed on leaf homogenates revealed several biologically active fractions the most effective of which was characterized as a particularly proline-rich, 5.8 KD peptide (Fleck *et al.* 1999). Deteriorating mangrove leaves where meanwhile observed to induce metamorphosis also in the asexual offspring of several *Cassiopea* species: in the motile buds of *C. andromeda* from the Red Sea, *C. frondosa* and *C. xamachana* from the Caribbean (Hofmann, personal observations; Fitt (1991), and in a *Cassiopea* species introduced to the Hawaiian Islands, referred to as *C. andromeda* (Hofmann & Hadfield 2002). We show in this paper that planula larvae from *Mastigias papua*, representative of another rhizostomean family, settle and metamorphose exclusively on the leaf surfaces when exposed to *Rhizophora mangle* leaves in the laboratory. This result corroborates with observations of polyps settled on submerged leaves in the socalled Jellyfish Lake in Palau, Micronesia (Fitt, personal comm.), the habitat from which the medusa was collected that finally provided the planula larvae for our experiments (record of the Waikiki Aquarium). Submerged mangrove leaves aquire a high diversity biofilm on the surfaces and degrade over a period of several months. They tend to remain afloat on top of even dense bottom layers of detritus in calm water lagoons, and thus represent a unique substratum for scyphopolyps and also for other sessile animal species to settle on.

We showed furthermore that both planula larvae and motile buds of *Mastigias papua* settle and metamophose in response to treatment with the synthetic oligopeptide Z-GPGGPA, the same substance which has been extensively used along with other members of the same peptide family as an artificial cue for studies on metamorphosis induction in planula larvae and buds of *Cassiopea andromeda* and *C. xamachana* (see Hofmann *et al.* 1996, for review). The present study revealed that this hexapeptide, applied at the same range of concentrations as used in experiments with *Cassiopea* species, acts as a metamorphic cue also on the larva-like buds of the other two rhizostome species: *Cephea cephea* and *Phyllorhiza punctata.*

We emphasize that both the rhizostome species investigated in the present work, and the *Cassiopea* species referred to have several important features in common: all of them are symbiotic and harbor intracellular zooxanthellae, *Symbiodinium microadriaticum*; they share the same mode of budding and, finally, sexually as well as asexually produced propagules respond to the same oligopeptidic metamorphic cue. One may speculate, therefore, about common mechanisms controlling the transition from a developmentally quiescent larval state to a physiologically and morphogenetically active condition. Is has been shown so far only in *C. andromeda* that peptide induced metamorphosis is receptor-mediated (Fleck & Hofmann 1995) and that activation of protein kinase C (PKC) is a significant step within the signal cascade leading to polyp formation (Hofmann *et al.* 1996, for review).

The results of the present work support the view of Siefker *et al.* (2000) who demonstrated that the exogenous cues found to induce larval metamorphosis in species of the scyphozoan order Semaeostomea differ significantly from those acting on propagules of the Rhizostomea. Quite sur-

prisingly the former react rather to cues shown to be effective in some representatives of the class Hydrozoa (Leitz 1997, for review). The only synthetic triggers of metamorphosis in common to both Scyphozoa and Hydrozoa are activators of PKC: diacylglycerol and phorbolesters. Considering the fact that medusae and scyphistomae of the Semaeostomea do not harbor zooxanthellae and that their scyphopolyps show very different patterns of budding phenomena compared to those of the Rhizostomea, it should be a fascinating future task to study aspects of the evolution of the divergence of Rhizostomea and Semaeostomea at both the developmental and molecular-genetic levels.

ACKNOWLEDGEMENTS. – The work has mainly been performed at the Waikiki Aquarium and the PBRC Marine Lab, University of Hawai'i, in Honolulu. DKH. is indebted to Dr MG Hadfield for his generous hospitality and support at Kewalo Marine Lab. We appreciate the permission by the Waikiki Aquarium to reproduce the photograph of an adult *Mastigias* medusa (Fig. 1). Particular thanks are due to M Rudschewski, B Simmes and A Fisher, Bochum, for help with polyp cultures and experiments with buds, and A Bonafede for performing the electronic artwork. Furthermore, we wish to extend our thanks to two anonymous reviewers for their very helpful comments and to S Boletzky for the French translation of the abstract.

REFERENCES

Arai MN 1997. A Functional Biology of Scyphozoa. Chapman & Hall, London, 316 p.

Bigelow RP 1900. The anatomy and development of *Cassiopea xamachana*. *Mem Boston Soc Nat Hist* 5: 193-236.

Calder DR 1973. Laboratory observations of the life history of *Rhopilema verrilli* (Scyphozoa:Rhizostomeae). *Mar Biol* 21: 109-114.

Calder DR 1982. Life history of the cannonball jellyfish, *Stomolophus meleagris* L.Agassiz, 1860 (Scyphozoa, Rhizostomida). *Biol Bull* 162: 149-162.

Cargo DG 1971. The sessile stages of a scyphozoan identified as *Rhopilema verrilli*. *Tul Stud Zool Bot* 17: 31-34.

Claus C 1890 & 1893. Über die Entwicklung des Scyphistoma von *Cotylorhiza*, *Aurelia Chrysaora*. *Arb Zool Inst Wien* 9: 85-128 & 10: 1-70.

Curtis SK, Cowden RR 1971. Normal and experimentally modified development of buds in *Cassiopea* (Phylum Coelenterata; class Scyphozoa). *Acta Embryol Exp* 3: 239-259.

Fleck J, Hofmann DK 1995. In vivo binding of a biologically active oligopeptide in vegetative buds of the scyphozoan *Cassiopea andromeda*: demonstration of receptor mediated induction of metamorphosis. *Mar Biol* 122: 447-451.

Fleck J, Fitt WK 1999. Degrading leaves of *Rhizophora mangle* Linné provide a natural cue for settlement and metamorphosis of the upside down jellyfish *Cassiopea xamachana*. *J exp Mar Biol Ecol* 234: 83-94.

Fleck J, Fitt WK, Hahn MG 1999. A proline-rich peptide originating from decomposing mangrove leaves is one natural metamorphic cue of the tropical jellyfish *Cassiopea xamachana*. *Mar Ecol Progr Ser* 193: 115-124.

Fitt WK 1991. Natural metamorphic cues of larvae of a tropical jellyfish. *Am Zool* 31: 106.

Goette A 1887. Entwicklungsgeschichte der *Aurelia aurita* und *Cotylorhiza tuberculata*. Hamburg & Leipzig, Verlag v. Leopold Voss.

Gohar HAF, Eisawy AM 1960. The development of *Cassiopea andromeda* (Scyphomedusae). *Publ Mar Biol Stn Ghardaga* 11: 148-190.

Goy J, Toulemont A 1997. Méduses. Collection Abysses N° 5. Musée Océanographique Monaco, 159 p.

Hofmann DK, Brand U 1987. Induction of metamorphosis in the symbiotic scyphozoan *Cassiopea andromeda*: role of marine bacteria and of biochemicals. *Symbiosis* 4: 99-116.

Hofmann DK, Hadfield MG 2002. Hermaphroditism, gonochorism, and asexual reproduction in *Cassiopea* sp.: an immigrant in the Islands of Hawai'i. *Invert Reprod Devel* 41(1-3): 215-221.

Hofmann DK, Fitt WK, Fleck J 1996 Checkpoints in the life-cycle of *Cassiopea* spp.: control of metagenesis and metamorphosis in a tropical jellyfish. *Int J Devel Biol* 40: 331-338.

Hofmann DK, Neumann R, Henne K 1978. Strobilation, budding and initiation of scyphistoma morphogenesis in the rhizostome *Cassiopea andromeda* (Cnidaria: Scyphozoa). *Mar Biol* 47: 161-176.

Kikinger R 1992. *Cotylorhiza tuberculata* (Cnidaria: Scyphozoa) – Life history of a stationary population. *PSZNI Marine Ecology* 13(4): 333-362.

Kramp PL 1970. Zoogeographical studies on Rhizostomeae (Scyphozoa). *Vidensk Meddr dansk naturh Foren* 133: 7-30.

Leitz T 1997. Induction of settlement and metamorphosis of cnidarian larvae: signals and signal transduction. *Invert Reprod Devel* 31(1-3): 109-122.

Lieshout van JS, Martin VJ 1992. Development of planuloid buds of *Cassiopea xamachana* (Cnidaria: Scyphozoa). *Trans Am Microsc Soc* 111: 89-110.

Siefker B, Kroiher M, Berking S 2000. Induction of metamorphosis from the larval to the polyp stage is similar in Hydrozoa and a subgroup of Scyphozoa (Cnidaria: Semaeostomeae). *Helgol Mar Res* 54: 230-236.

Sugiura Y 1963. On the life-history of rhizostome medusae I. *Mastigias papua* L. Agassiz. *Annot Zool Jap* 36: 194-202.

Sugiura Y 1964. On the life-cycle of rhizostome medusae II. Indispensability of zoo-xanthellae for strobilation in *Mastigias papua*. *Embryologia* 8: 223-233.

Sugiura Y 1966. On the life-history of rhizostome medusae IV. *Cephea cephea*. *Embryologia* 9: 105-122.

Tardent P 1978. Coelenterata, Cnidaria. *In* Morphogenese der Tiere (Erste Reihe: Deskriptive Morphogenese, Lieferung 1: A-1), F Seidel, Herausg Gustav Fischer Verlag, Stuttgart, 415 p.

Uchida T 1926. The anatomy and development of a rhizostome medusa, *Mastigias papua* L. Agassiz, with observations on the phylogeny of Rhizostomeae. *J Fac Sci Imp Univ Tokyo Sect IV Zool.* 1: 45-95, pl. 6.

Reçu le 22 mai 2002, received May 22, 2002
Accepté le 14 juin 2002, accepted June 14, 2002

VIE MILIEU, 2002, 52 (4) : 149-166

From Marine Ecology to Developmental Biology
In Honour of Pierre Tardent (1927-1997)

DEVELOPMENTAL BIOLOGY OF ANIMAL MODELS UNDER VARIED GRAVITY CONDITIONS: A REVIEW

H.-J. MARTHY

UMR CNRS 7628 Observatoire Océanologique, 66650 Banyuls-sur-Mer, France

marthy@obs-banyuls.fr

DEVELOPMENTAL BIOLOGY
SPACE BIOLOGY
DEVELOPMENTAL PROCESSES
ALTERED GRAVITY
MICROGRAVITY
HYPERGRAVITY
AMPHIBIANS
FISH
INSECTS
SEA URCHINS

ABSTRACT. – Once the International Space Station ISS will be fully functional, a new era of research in space will open. Awaiting this event, it seems timely to review the various experiments in developmental biology using suitable animal models, performed over the last 17 years under varied gravity conditions and in particular under real microgravity conditions in space. In selected vertebrate and invertebrate models (amphibians, fish, insects, sea urchins) various developmental processes have been studied, e. g. (micro-) gravity effects on fertilization, on overall embryogenesis, on larval development, on organogenesis, on behavior and/or on the differentiation and functional development of neurological and gravity sensitive structures. From the results now available one basic conclusion can be drawn: whatever the morphological or physiological microgravity effects observed in samples developing in space, or in post-flight samples on the ground, these effects become regulated, and development ultimately leads to viable juveniles. In other terms: gravity doesn't seem required for a normal issue of onto- and embryogenesis in Vertebrates and Invertebrates.

BIOLOGIE DU DÉVELOPPEMENT
BIOLOGIE SPATIALE
PROCESSUS DU DÉVELOPPEMENT
CONDITIONS GRAVITATIONNELLES VARIÉES
MICROGRAVITÉ
HYPERGRAVITÉ
BATRACIENS
POISSONS
INSECTES
OURSINS

RÉSUMÉ. – Dès que la Station Spatiale Internationale ISS sera pleinement fonctionnelle, une ère nouvelle de recherches dans l'Espace s'ouvrira. Dans l'attente de cet événement imminent, il paraît opportun et utile de dresser le bilan des expériences diverses en biologie du développement, menées avec différents modèles animaux au cours les 17 dernières années dans des conditions gravitationnelles variées et particulièrement dans des conditions d'apesanteur réelle. Divers processus du développement ont été étudiés chez des modèles sélectionnés de Vertébrés et d'Invertébrés (Batraciens, Poissons, Insectes, Oursins), tels que le déroulement en microgravité de la fécondation, de l'embryogenèse, du développement larvaire, du comportement et/ou de la différentiation et du développement fonctionnel de structures neurologiques et sensibles à des variations gravitationnelles. Les résultats disponibles pour l'instant amènent à une conclusion essentielle: quels que soient les effets morphologiques ou physiologiques provoqués chez des individus se développant dans l'Espace ou en « post-vol » sur la Terre, ces effets sont régulés et, en fin de compte, le développement aboutit à des juvéniles viables. En d'autres termes : la gravité ne semble pas exigée pour garantir une issue normale de l'onto- et de l'embryogenèse des Vertébrés et des Invertébrés.

DEVELOPMENTAL BIOLOGY RESEARCH IN SPACE ?

As modern "Molecular Developmental Biology" became possible only on the basis of an "Experimental Embryology", which evolved over more than 100 years (e.g. Fraser & Harland 2000), morphological, physiological and behavioural phenomena in organisms developing *in Space*, had also to be identified first, before it could make any sense to envisage studies on mechanisms underlying these phenomena. This exploratory, phenomenological phase of Space biology research is coming to some end. A few organisms only – selected for scientific and practical reasons (particularly on the basis of results obtained in the past and for their suitability for creating multi-generation experiments) – will further serve as "models" when redirecting descriptive research towards causal, molecular and genetic analyses. Nevertheless, exploratory studies with potentially useful organisms have to be still fol-

lowed as well. A decisive step in this direction will be made as soon as the "International Space Station" (ISS) is fully functional. Since this era is imminent, a review on experiments in Developmental Biology with suitable animal models in Space over the last 17 years appears useful and opportune.

ANIMAL DEVELOPMENTAL BIOLOGY EXPERIMENTS IN SPACE

So far, experiments performed on eggs, embryos, larvae or juveniles, in a space environment, had to be designed to be as simple as possible, that is as technically feasible and, in comparison to sophisticated "modern" laboratory work, could be interpreted therefore as "old fashioned". However, such relative simple, yet well defined experiments, performed in Space with suitable animal models, have been shown to be the right way to get more insight into the significance of gravity for ordered ontogenetic processes. Studying developmental modes under real weightlessness conditions is seen as a most promising approach to answering the fundamental question of whether gravity is a determining epigenetic factor for whether an egg develops correctly into a viable organism or whether the genetic program, established throughout the evolution of life on Earth – in the permanent presence of the gravity force! – is alone informative enough.

From automated and man-tended Spaceflight missions (parabolic flights, sounding rockets, satellites, American space shuttle, Russian MIR station) as well as from ground-based gravity research, results or clues became available as to how weightlessness conditions are "sensed" by individual cells, compact tissues and whole plant and animal organisms and how they "respond" to such an unusual environment (for reviews e.g.: Halstead & Dutcher 1987, Mesland & Brillouet *et al.* 1987, Planel 1988, Oser & Battrick 1989, Malacinski *et al.* 1989, Ubbels & Oser *et al.* 1990d, ESA SP-publications on symposia, 1990, 94, 96, 99; Dutcher *et al.* 1994, Claasen & Spooner 1994, Brillouet *et al.* 1995, Souza *et al.* 1995, Moore *et al.* 1996, Cogoli 1996, Demets 1996, Cogoli *et al.* 1997, Sievers *et al.* 1997, Snyder 1997, Brinckmann *et al.* 1999 and others). Among these experiments are several which focus exclusively on developmental aspects, that is which were conceived to reveal whether and how weightlessness does have an effect on (virgin and fertilized) eggs, proliferating embryonic cells and on intact developing embryos or larvae (Marthy 2003). Against strong arguments that the gravity force plays a role on Earth in developmental processes (such as in egg polarisation and symmetrisation in amphibians for example; Ubbels 1997b for review), it is intriguing on a first view, to learn that weightlessness induces apparently *no spectacular* negative effects on embryogenesis. Has this to be interpreted, that the role of gravity is actually minor or negligible for an ordered embryonic and/or larval development? In the following overview, different experiments on different animal models will be presented to explore whether reported "microgravity effects" are effectively significant enough to evaluate the actual role of gravity in developmental processes.

MICROGRAVITY EFFECTS ON FERTILIZATION AND DEVELOPMENT

A first generation of developmental biology studies in Space had to be "look and see"-experiments. One had to know firstly the behaviour (in a broad sense) under altered gravity conditions of the biological material considered as a potential model system. Thus, a vertebrate (early stages of the anuran amphibian *Xenopus laevis*) and invertebrates (different stages of the insects *Carausius morosus* and *Drosophila melanogaster*) were exposed to weigthlessness for a few days and then examined on the Ground ("D1 mission 1985": Mesland & Brillouet *et al.* 1988). From about 1990 onwards, other "models" for developmental research were successfully explored during parabolic and orbiting space flight missions (urodelan amphibians: newt (*Cynops pyrrhogaster*) and salamander (*Pleurodeles waltl*); fish: Medaka (*Oryzias latipes*); sea urchins: (*Paracentrotus lividus*, *Sphaerechinus granularis, Lytechinus pictus*)). Such experiments dealing with more or less general aspects of egg fertilization and embryonic development will be considered later. For specifically neurobiological experiments under altered gravity conditions, one is referred to recent literature (Anken & Rahmann 1999 (review) and Rahmann *et al.* 1992, Slenzka *et al.* 1994 (for synaptic plasticity); Pronych *et al.* 1996 (for optomotor behaviour), Horn *et al.* 1995, 96, 98 and Sebastian *et al.* 1996 (for vestibulo-ocular reflex development)).

Vertebrates

A. The Amphibian Models

Anurans: "frogs"

The first experiments in space for studying potential effects of weightlessness on vertebrate embryogenesis has been done with embryos of the frog *Rana pipiens*. Exposure of embryos from the 2-cell stage onwards to microgravity for a few days revealed no developmental alterations (Young *et al.* 1968, 70; review: Souza 1986). The basic

question whether gravity might be really required for a normal embryonic development had apparently to be approached using eggs fertilized in space. The readily available eggs of another well known "laboratory frog" were chosen for this purpose.

In this "frog", the South African clawed toad *Xenopus laevis*, fertilization occurs *in vitro*. That is, males and females shed their gametes into the water, where egg insemination, fertilization and embryonic development occur. Under experimental conditions, an *in vitro* fertilization can easily be achieved by simply mixing the gametes taken separately from the two sexes. A pioneer work on its development under space flight conditions is due to the dutch scientist GA Ubbels and her collaborators (for review: Ubbels 1997a & b).

In vitro -fertilization and early developmental events could be successfully achieved on sounding rockets (Texus-17, Maser-3 and -6) as well as during space shuttle missions (IML-1 and IML-2) by means of a fully automated experiment device (Huijser *et al.* 1990, Ubbels *et al.* 1990c, Willemsen 1994), functional on the following basis. In an AEC (Automated Experiment Container, 79.5 × 19.0 × 33.1 mm, fitting into a Biorack Type I/E container) virgin eggs and testes are loaded separately. In flight, the gametes are mixed by a pre-programmed sequential activation of a "plunger system". In sounding rocket experiments, some samples of freshly fertilized eggs were fixed within 5 minutes, whereas others were not and were recovered alive for further investigations on Earth. In the case of space shuttle experiments (particularly successful: IML-1 mission, 1992), the developing embryos were fixed after about 90 minutes (3rd cleavage stage) and 12 hours (gastrula stage) post-fertilization respectively, for postflight analyses by classical histology and confocal laser scanning microscopy.

It turned out that egg fertilization (a real first in Space!) and symmetrisation in microgravity do occur normally, that is fertilization is monospermic with a sperm impact towards the animal pole and the spatial bilateral symmetrical body pattern gets well established (Ubbels *et al.* 1989a, 90a,b, 92, 94, de Mazière *et al.* 1996). Furthermore, the tempo of cleavage divisions is not perturbed by microgravity conditions (Ubbels 1997b). In Sounding Rocket experiments, some embryos which were recovered alive continued their development on Earth and developed abnormal axes (Maser-3: Ubbels *et al.* 1989; 90a,b; 92); however, experiments on Maser-6 and ground-based simulation experiments showed that such abnormalities were presumably not due to variations of the g-level (Ubbels 1997a & b).

That gravity is effectively not required for a successful fertilization of *Xenopus* eggs was confirmed by Souza *et al.* (1995b) and Black *et al.* (1996) by means of experiments performed during a space shuttle flight (in Spacelab-J). In their experiment, ovulation in frog females (maintained on-board in the NASA "Frog Environmental Unit (FEU)" was induced by injecting human chorionic gonadotropin (hCG). Collected eggs were then fertilized *in vitro* with a sperm suspension and incubated under microgravity conditions and on the 1xg FEU centrifuge. The embryos were fixed at various developmental stages for postflight analyses. In addition, also embryos were recovered alive postflight for further development on Earth. The results show that frogs ovulate correctly in the absence of gravity, that the eggs become fertilized at a high rate (> 80 %), that development proceeds synchronously with 1×g controls and that embryos apparently are able to regulate some developmental abnormalities, capable to differentiate in (nearly) normal larvae, which can be raised through metamorphosis up to reproducing adult stages (with normal F1 tadpoles).

So far, gravity appears clearly not necessary for ordered fertilization and subsequent embryogenesis. However, early embryos show some morphological and histological characteristics which are not harmful for overall development but which are also not seen during development on Earth, namely:

a) In embryos fixed in-flight at the two-cell stage, the cleavage furrow is found in its normal position (at 0×g and at 1×g), the mitotic asters in microgravity samples, however, are found at a more vegetal position than normal (Fig. 1; Black *et al.* 1996).

b) In embryos fixed in-flight at gastrula stages, a high percentage of microgravity samples has a more or less reduced blastocoelic volume (Ubbels *et al.* 1994, 1995, Souza *et al.* 1995b, Black *et al.* 1996, de Mazière *et al.* 1996). When compared with 1xg controls, these embryos show also a remarkably thicker blastocoel roof and a slight shift of the blastopore towards a more vegetal latitude (Fig. 2; Souza *et al.* 1995b). A disturbance in the epibolic process of the cells, the so-called "radial interdigitation" (Keller 1980), is one of the possible explanations of the phenomenon. The morphology of neurula stages appears normal (in contrast to urodeles; see below).

c) Some abnormalities became manifest also during organogenesis. Thus, histology performed on tadpoles fixed shortly before landing showed significantly smaller lungs. Postflight observations revealed that tadpoles developed under microgravity swam in a water column at a lower position than tadpoles developed on the 1×g onboard centrifuge. On the other hand, video recordings taken of swimming tadpoles in microgravity, and taken within 4.5 hours after landing, showed essentially a normal swimming behavior (Souza *et al.* 1995b). In contrast to studies on tadpoles after parabolic flights, almost no "looping" was observed. In microgravity samples the optomotor re-

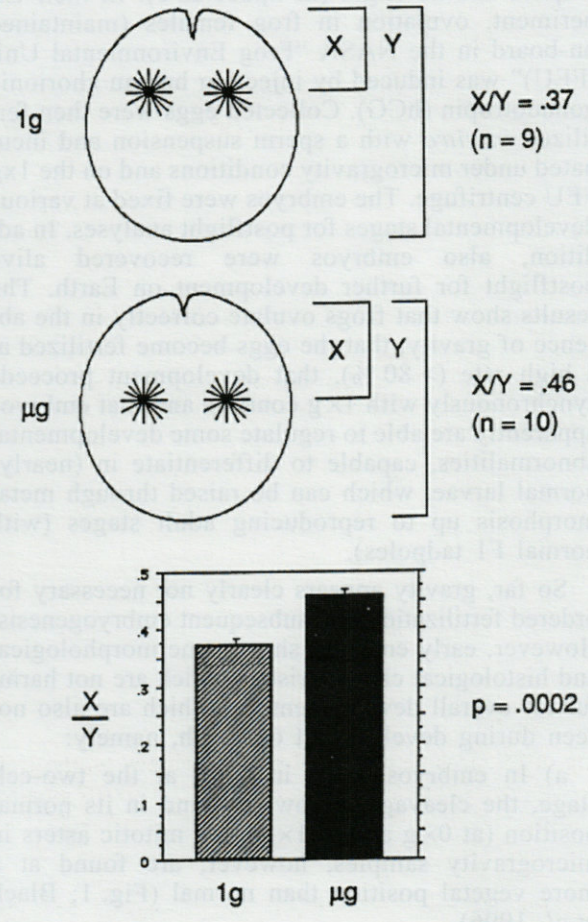


Fig. 1. – Two-cell mitotic asters are displaced toward the vegetal pole in *Xenopus laevis* embryos, kept in microgravity conditions from fertilization onwards. The position of the asters was measured relative to animal-vegetal height. The graph shows the means and 95 % confidence intervals. From Black *et al.* 1996 (Copyright 1996 – Elsevier Science).

sponse appeared slightly stronger. Behavioral differences disappeared progressively within 2 days.

Various studies on the functional development of sensory organ systems, in particular the visual and vestibular systems, as well as on the (exaggerated) optomotor behaviour (Pronych *et al.* 1996) in microgravity were performed with tadpole stages of *Xenopus laevis*. Thus, the influence of microgravity on the development of the static and the roll-induced vestibuloocular reflex (rVOR), reflecting well the efficiency of the vestibular system, could be evaluated in quantitative terms (D-2 and S/MM-06 missions: Horn *et al.* 1995, 99, Sebastian *et al.* 1996). Long-term periods of exposure to microgravity affect the rVOR significantly in a long-term manner whereas short periods are almost ineffective (Sebastian *et al.* 1998). Tadpoles exposed to microgravity for more than 3 days developed a still enigmatic anatomical malformation, "upwards curved tails" (Horn *et al.* 1999).

Urodeles: newts and salamanders

In contrast to anurans, fertilization in newts and salamanders occurs *in vivo,* that is, sperm is stored in the cloacal tract of the females weeks or even months prior to spawning. During egg laying, whether naturally or artificially induced by hCG, the spermatozoa become active and inseminate the eggs. This behaviour is of considerable practical interest for developmental biology studies under space flight conditions. Only females need to be used and by means of automatic or manual injections of hormones, at a well defined moment of the experiment flow, egg maturation can be induced and egg laying timed. Fertilized eggs are then laid and embryonic development is initiated.

The first newts which spawned in Space ("AstroNewts") were the japanese red-bellied *Cynops pyrrhogaster* (Mogami *et al.* 1996a,b, Yamashita 1997). In this case, ovulation was induced by release of hCG from implanted minipellets. Two space missions (IML-2 on STS-65 of NASA, July 1994; unmanned Japanese Space Flyer Unit "SFU" on H-II rocket of NASDA, March 1995) provided evidence, in physiological (inflight video recordings), morphological and chronological terms, that eggs become normally fertilized in Space and that embryonic development did proceed normally as well (Mogami *et al.* 1996b). The presence of gravity appears again not to be required for the determination of the embryonic axes (however, the two recovered adult newts from the IML-2 mission revealed particularly severe pathological damages in the lungs (Pfeiffer *et al.* 1995)).

Information on fertilization processes and embryonic development at 0×g conditions of another urodele, the salamander *Pleurodeles waltl*, was obtained from the French-Russian missions "Cassiopée" (August 1996), "Pégase" (1998) and "Perséus" (1999) on the Russian space station MIR. Female animals were kept in a complex housing and experiment facility, developed under contract by CNES, termed FERTILE ("Fécondation et Embryogenèse Réalisées chez le Triton In vivo dans l'Espace"; for technical aspects see Chaput *et al.* 1994; Husson 1998). Ovulation was triggered by hCG. As seen on video-recordings, spawning and embryonic and larval development seemed to occur normally in microgravity. Postflight studies on larvae fixed in space as well as recovered alive, brought quite a number of reliable results (Husson 1998, Husson *et al.* 1998, Duprat *et al.* 1998, Aimar *et al.* 2000). These are basically in line with the observations made in frogs and newts, but new insights are obtained on the following (Husson 1998, Aimar *et al.* 2000):

– Hormone-induced spawning is normal.

– Natural *in vivo* fertilization (by sperm differentiated at 1xg on Earth) does occur and initiation

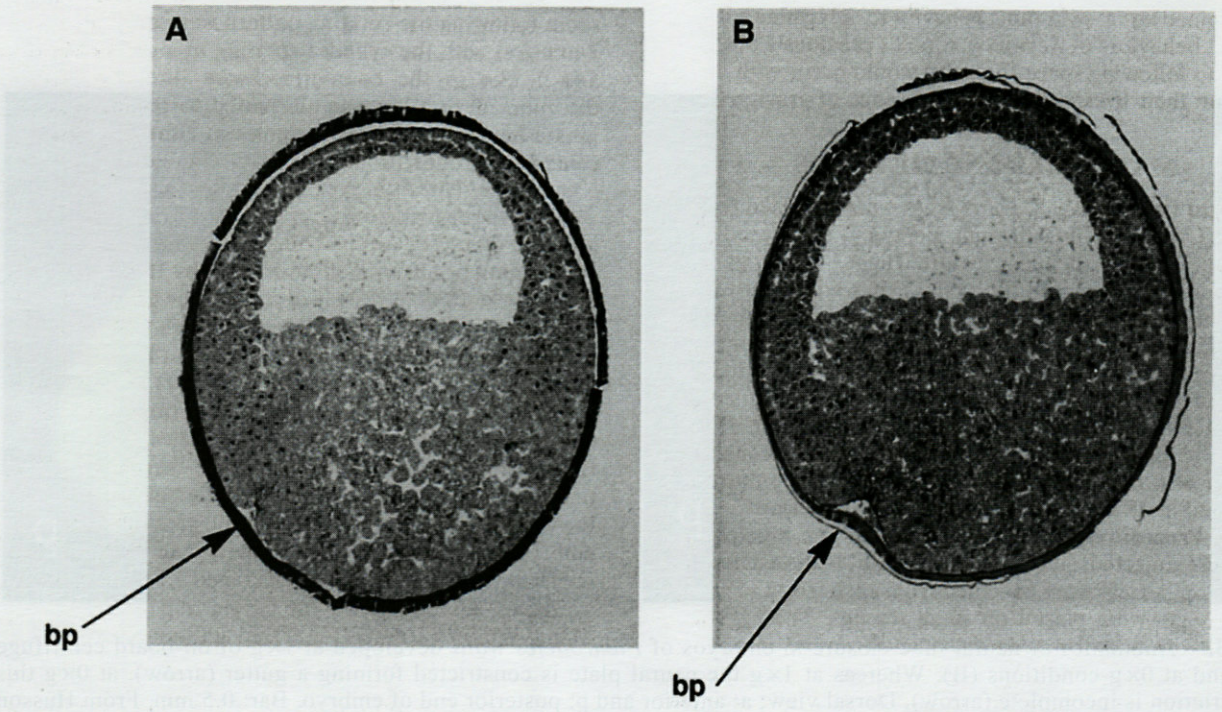


Fig. 2. – Sagittal section of gastrulae of *Xenopus laevis,* showing differences between embryos developed on the 1×g centrifuge (A) and those developed at microgravity (B). The microgravity sample show thicker blastocoel roofs and the blastopore (pb) formed nearer the vegetal pole than in the 1×g controls. From Souza *et al.* 1995 (Copyright 1995 National Academy of Sciences, USA).

of development appears normal; however, microvilli elongation on fertilized eggs at 0xg appears reduced (reflecting the dynamic state of actin?) and some pigment concentration towards the animal pole, not seen in 1xg controls, seems to occur (reflecting the gravity sensitivity of the microtubular system (?)).

– At cleavage stages, the cohesion between blastomeres appears reduced (reflecting disturbances in the cytoskeleton and/or the expression of adhesion molecules (?)).

– At blastula stage, a thicker blastocelic roof is noticed (thickening seems due to looser packed epidermal cells whereas in *Xenopus* enlarged cells are seen).

– At gastrula stage, the blastopore appears in the normal position (in contrast to the observation in *Xenopus* embryos).

– At neurula stage (early organogenesis), the closure of the neural tube is occasionally retarded or absent and micro- or acephalic larvae differentiate (Fig. 3 and 4).

– Later embryonic and larval development is judged as normal. That is, later organogenesis becomes essentially normal and the embryos/larvae show no pronounced external malformations (reflecting a regulation capacity of the larvae). Nervous and muscular differentiation processes reveal to be normal as well (expression of the neurotransmitters Cholin Acetyl-Transferase "Chat" and Gamma Amino-Butric Acid "GABA");

– the overall chronology of development appears (nearly) identical in 0×g and 1×g samples.

The phenomenon of a reduced or even absent cohesion between the cells in embryos at cleavage, gastrula and early neurula stages is particularly noteworthy. It is likely to be a real causal microgravity effect. On the other hand, at the caudal bud stage, the cells appear normally adhering, a possible clue that microgravity effects are stronger in early embryonic stages, that is at stages where cellular adhesion is relatively weak and morphogenetic movements intense. The most frequent and most intense disturbances are observed at the neurula stage, that is at a stage, where the cellular adhesion molecules are particulary required. The plausible conclusion is that microgravity conditions affect the subtle dynamic state of the cytoskeleton (Tabony *et al.* 2002) and can alter microvillar transformations, intercellular contacts and adhesion mechanisms as well (Aimar *et al.* 2000, Husson 1998).

One has to keep in mind, however, that the *majority* of embryos under microgravity conditions develop normally up to advanced stages and that some of the disturbances observed prior to and during neurogenesis are transient. This is an additional

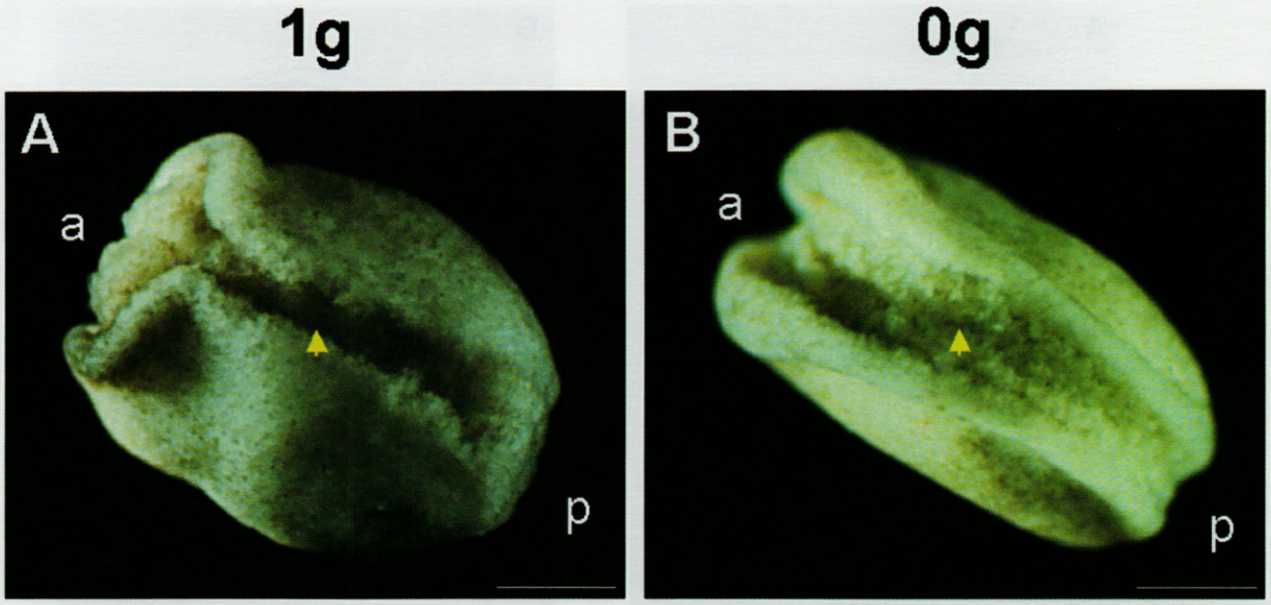


Fig. 3. – Anomalies of neural tube closure in embryos of *Pleurodeles waltl* developed at 1×g of on-board centrifuge (A) and at 0×g conditions (B). Whereas at 1×g the neural plate is constricted forming a gutter (arrow), at 0×g this constriction is incomplete (arrow). Dorsal view; a: anterior and p: posterior end of embryo. Bar: 0.5 mm. From Husson 1998 (courtesy of the author).

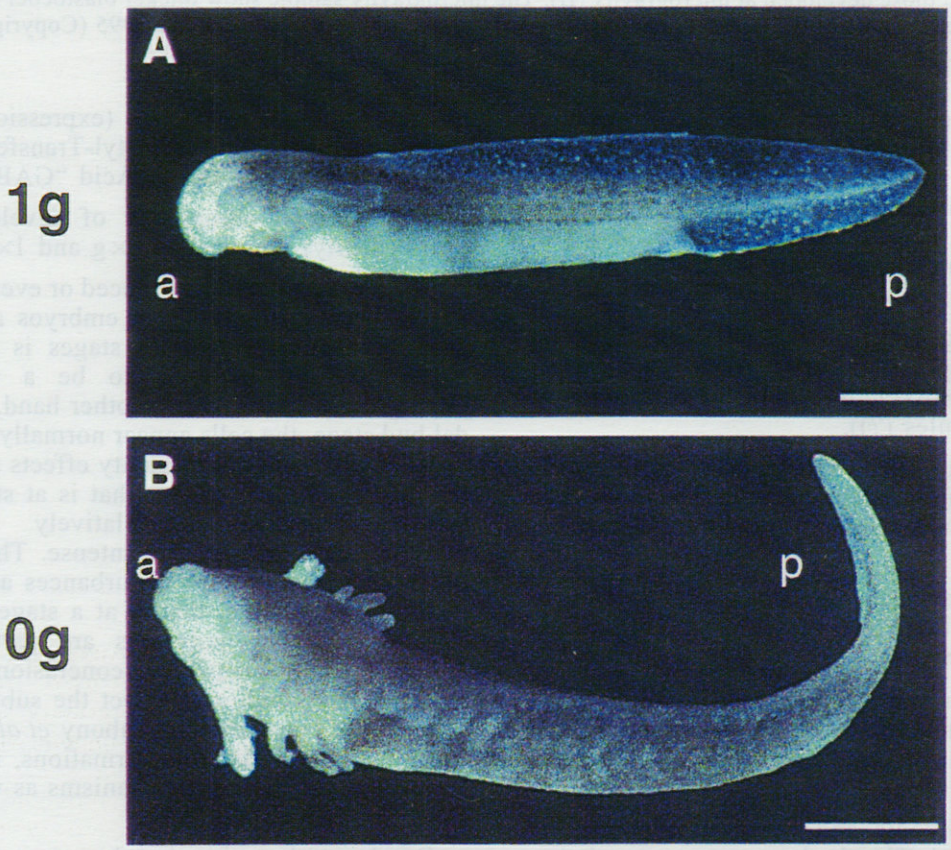


Fig. 4. – Anomalies in cephalic morphogenesis in embryos of *Pleurodeles waltl* developed at 1×g of onboard centrifuge (A) and at 0×g conditions (B). Whereas at 1×g cephalic organogenesis is normal, at 0×g a head atrophy is noticed in an embryo otherwise normal. Lateral view; a: anterior and p: posterior end of embryo. Bar: 1 mm. From Husson 1998 (courtesy of the author).

hint that the eggs and then the embryos and larvae of amphibians are well able to adapt to an unusual environment due to their well known high regulation capacity. The behaviour of the urodelan models, and in particular of the salamander, supports therefore the view that gravity actually plays some role in embryonic and larval development. Even if it's known that altered gravity conditions can disrupt the process of embryonic axis specification in *Xenopus* eggs, resulting in altered axis polarity or in twinning (Black & Gerhart 1985, 1986, Black 1990), the morphogenetic role of the gravity force doesn't seem primordial, however.

The development of gravity-sensing organs in altered gravity conditions was first studied in newts (Wiederhold *et al.* 1997). It turned out that the size of the saccular otolith and the volume of the otoconia were considerably enlarged in newts developing at 0×g, with lasting functional consequences (abnormal "head upward" posture) in animals subsequently reared on Earth.

From the MIR station missions, *Pleurodeles* embryo and larva samples were also recovered, fixed and alive, for identifying possible microgravity effects on the particular differentiation of the inner ear. Preflight on-ground studies on otolith differentiation (Oukda *et al.* 1999a, b, c) document the crystallographic and chemical nature of the otoconia and serve at present as a solid basis for studying the flight samples (Dournon pers. comm.). Studies of the differentiation *in vivo* of the inner ear under microgravity conditions as well as the differentiation *in vitro* of embryonic muscle and neuronal cells of *Pleurodeles* were initiated by means of the French experiment facility IBIS-1 (Chaput *et al.* 1994, Husson 1998) during the 14-day flight of the Russian satellite "Foton 10" (1995). Unfortunately, after a successful flight, the retrieval of the satellite failed and the biological material was almost entirely lost. The analysis of the few intact samples of the experiments "Celimène" (Cellule en Impesanteur, Muscles et Neurones Embryonnaires) and "Torcoll" (Triton en Orbite: Recherche Concernant l'Oreille Interne et la Ligne Latérale) respectively is fragmentary only. As to the "Celimène" experiment (Husson 1998, Husson *et al.* 1998), all embryos were lost. The recovered 60 % of cell cultures (0xg and 1xg inflight samples) show an essential normal differentiation. Morphological aberrances (blebs) in neurites might just be due to incomplete adherence of the cells to the substrate. There is also a clue that at 0xg cultured cells used their yolk reserve more rapidly (accelerated metabolism). The problem as to whether and to what extent gravity might have a role in neurogenesis of amphibians has been addressed by Duprat *et al.* (1998). As to the "Torcoll" experiment, 2 embryos (1 at 0×g and 1 at 1×g) were recovered alive and lived on Earth surprisingly without metamorphosis for 8 and 14 months respectively (Bautz *et al.* 1996, 1997). Only new experiments will reveal whether this has to be considered as an artifact.

B. The Fish Models

After a rigorous selection process on hundreds of Medaka fish specimen (*Oryzias latipes*), four microgravity tolerant individuals (ccT strain, no "looping" behavior, strongly eye-dependent, Ijiri 1995c), two males and two females, were sent to space during the "IML-2 mission" (STS-65, 1994) by the Japanese scientist Ijiri and his collaborators (Iijri 1994 95a,b,c, 98, Imamizo *et al.* 1993, 94). In-flight videorecordings showed that the fish were able to mate under microgravity conditions – the first mating in Space! – and, after about 24 hours the first 3 eggs were found in the "egg compartement" of the AAEU (Animal Aquatic Experimental Unit; Iijri 1995a). Throughout the 15 days mission, the fish continued regularly to mate and lay eggs (in total 43 eggs). Most eggs developed normally at 0×g. The first fry was observed at the end of day 12. In contrast to the adult fish, which had great difficulty to adapt to 1xg gravity conditions when returned to Earth (they swam normally only after four days), the eight space-born fry showed no difference in behaviour and swam normally. These fry have grown up normally and produced normal offspring (Fig. 5). Other eggs, also laid in Space, and at various developmental stages at the end of the mission, developed further on the Ground and hatched normally.

Since the Medaka ricefish were able to mate from the beginning of the mission and produce normal offspring, microgravity conditions don't seem to disturb them much. It suggests that vertebrates adapt quite easy to a weightlessness environment. Furthermore, the production of normal offspring shows that gametogenesis is not susceptible to be influenced by microgravity (Ijiri 1998). The Medaka experiment is not least an encouraging preparatory experiment for multi-generation breeding experiments to be performed on the ISS and, perhaps one day, on a lunar base (Ijiri 1995c) or elsewhere.

Other fish species such as the cichlids *Oreochromis mossambicus* (Anken *et al.* 1992) and *Astronotus ocellatus* are used for specific neurobiological research on gravity sensations in lower vertebrates (e.g. Rahmann *et al.* 1992, Neubert *et al.* 1994, Slenzka *et al.* 1994, Horn *et al.* 1995 and others). They appear well appropriate to studying the influence of a transient sensory deprivation by gravity (lack of neurotrophic effects) on the functional development of sensory organ systems such as the visual and/or vestibular systems and to investigating the neuronal basis of particular behavioural adaptations (e.g. postural

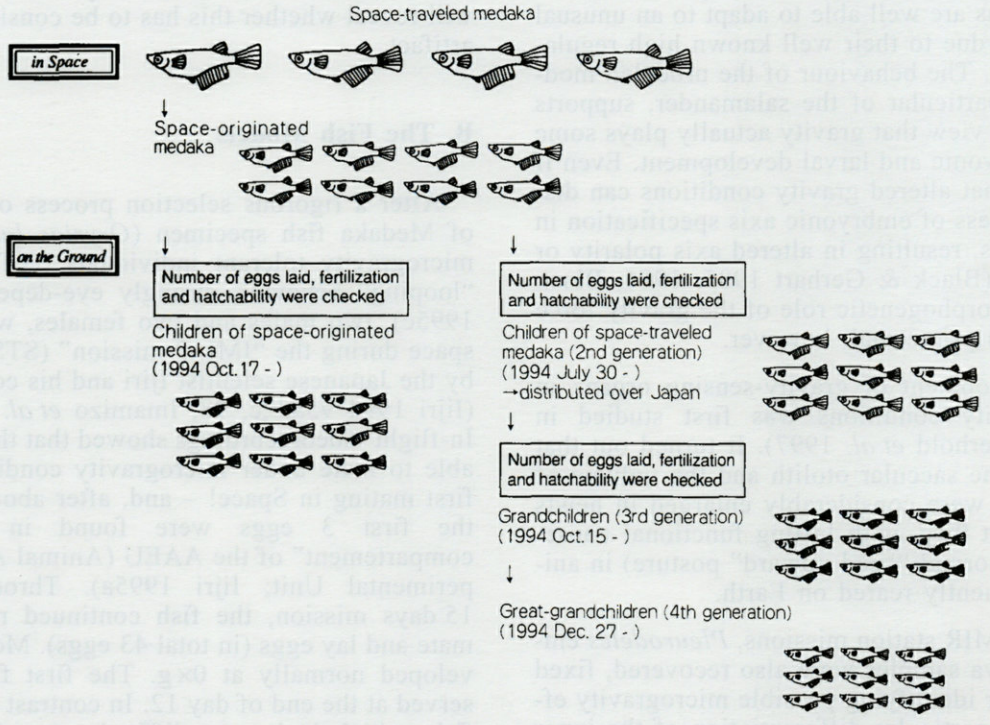


Fig. 5. – Space-travelled adult Medaka fish (*Oryzias latipes*) and their offspring. The date in parentheses shows when each generation was produced for the first time, i.e. the first day their parent generation started to lay eggs. From Ijiri 1995.

control) to altered gravity conditions (for review: Anken *et al.* 1999).

Invertebrates

A. The Arthropod Models

Insects

Carausius morosus

Information on embryogenesis and organogenesis of the stick insect *Carausius morosus* under spaceflight conditions was obtained during the D-1 mission (Bücker *et al.* 1988). Embryonic and larval development of these eggs lasts approximately 100 days (at + 20°C). Eggs (1.8 mm × 2.7 mm) at five different stages of development were used in the experiment and exposed in Type I and II containers of ESA Biorack for 7 days to microgravity conditions and to cosmic radiation (HZE particles). Whereas undeveloped eggs (stage I) and embryos beyond 43 days (stages IV and V) were revealed to be resistant to radiation, eggs between 1-14 and 15-43 days after oviposition (stages II and III respectively) were revealed both to be sensitive to radiation, the latter being unable to regulate. The hatching rate appeared clearly related to a phase-specific exposure to spaceflight conditions (Fig. 6). Embryos older than 43 days showed a hatching rate similar to ground-controls (about 80 %). The hatching rate in younger embryos, however, decreased significantly to 68 % (stage I) and 47 % (stage II) and even to 36 % and 56 % (stages II and III) where a synergistic effect of microgravity and of HZE hits has to be assumed. The most sensitive phases are the stages II and III. This particular sensitivity between days 16 and 36 of development may be correlated to the germ layer formation and to characteristic movements of the embryos (Koch 1964, Reitz *et al.* 1995). In contrast, and whether or not they were hit by an HZE particle, no significant reduction in the hatching frequency was observed for eggs that were kept on the 1xg reference centrifuge. A substantial portion of larvae hatched from 0xg and 1xg flight samples showed various body anomalies, in particular deformations of abdominal segments, of extremities or antennae.

The experiment is particularly worthy of note since it shows by the range of abnormalities the potentiation of the biological response, that is the effect of HZE particle hits on the "system" *under* microgravity conditions. The question "whether microgravity disturbs certain processes, such as the repair of radiation damage, in the radiobiological chain of events" (Bücker *et al.* 1988) is unanswered.

Drosphophila melanogaster

During the same D-1 mission, fruitflies (*Drosophila melanogaster*) were also exposed to

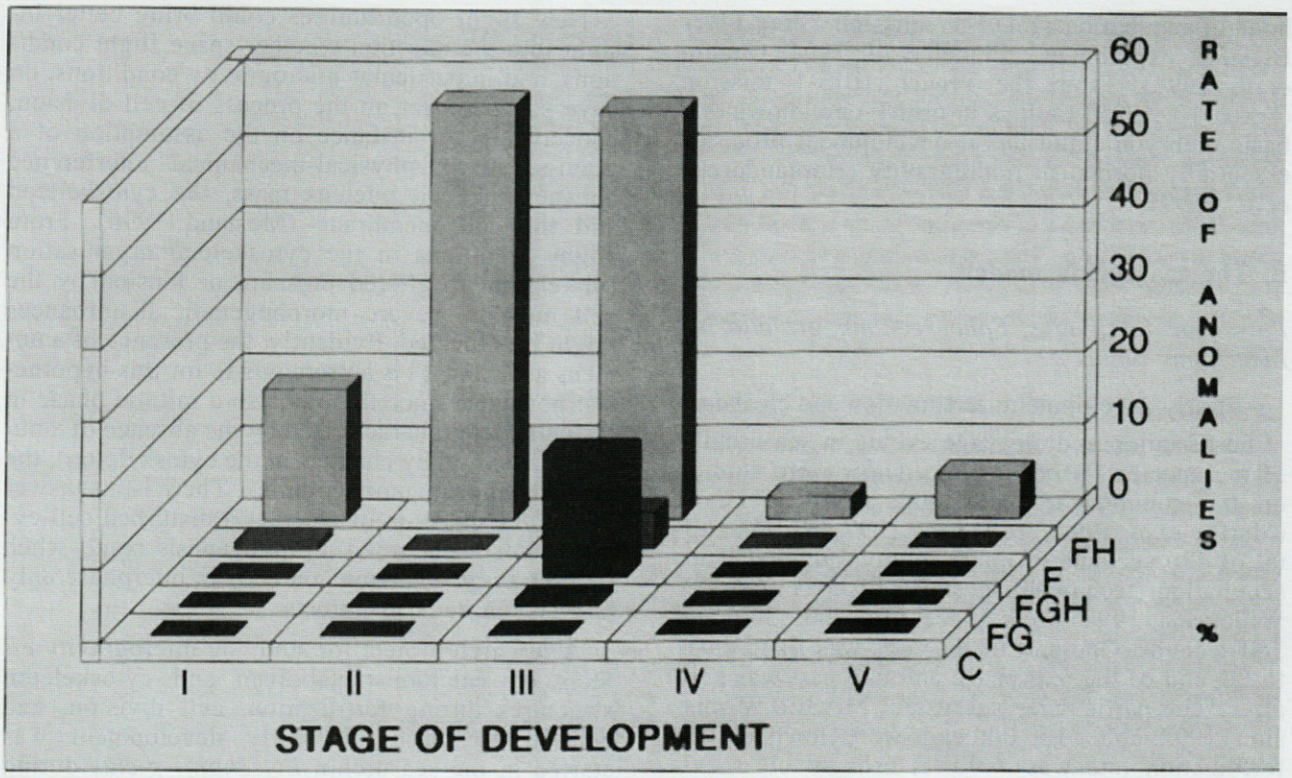


Fig. 6. – Rate of anomalies (%) of larvae hatched from eggs of the stick insect *Carausius morosus*, that were exposed to different spaceflight conditions in different stages of development (I – V), compared to ground controls (C). F: flight samples in microgravity; FH: flight samples in microgravity, hit by an HZE particle; FG: flight samples on the 1xg centrifuge; FGH: flight samples on the 1xg centrifuge, hit by an HZE particle. From Bücker *et al.* 1988.

spaceflight conditions. Their eggs are much smaller (1,5 mm × 0,3 mm) and their development much faster (22h at +25°C) than in the case of the stick insect. From this mission as well as from the "Biocosmos 9" mission (1992), the essential results concerning ovoposition, early embryonic development and life span can be summarized as follows (Vernos *et al.* 1988, 1989, Marco *et al.* 1992, 95, 96, 99a):

– development is feasible in the absence of gravity

– size and number of eggs are increased under continued microgravity conditions

– life span in microgravity is decreased in males, but not in female flies and also not in males kept at 1xg on the on-board centrifuge.

In relation to evolutionary developmental questions (e.g. how living systems may change in an environment very different from that in which they evolved), the *Drosophila* model is likely to play once more a prime role as soon as long-term experiments on the future ISS (International Space Station) can be done (Marco *et al.* 1999b).

Acheta domesticus

From a first successful experiment in space (STS 90: "Neurolab mission", 1998), the cricket *Acheta domesticus* is likely to become another remarkable insect model for developmental (neuro-) biology research in space (Horn 2000, Horn *et al.* 96, 98). In this first experiment, different developmental stages were exposed to microgravity for 16 days and, after the flight, the mean head response characteristics were recorded between day 1 and day 4. Since no difference could be observed in comparison to ground controls (Förster *et al.* 1999), crickets appear capable of normalising very quickly the development of their multi-channel gravity sensory system (Horn & Föller 1985).

Crustacean

Artemia salina and Artemia franciscana

Among crustaceans, the brine shrimps *Artemiae* have characteristics which make of them useful models for studies on development and life span in a space environment. Dormant gastrulae (cysts) appear particularly useful for studying effects of an outer space environment. In fact, *Artemia* cysts survived 437 days of space flight. A stimulatory effect was observed in the first steps of development (increase of metabolic activity and thus accelerated development within the first 20 hours of post-flight incubation). The outer space-exposed cysts showed higher enzyme activities involved in metabolic pathways of sugar and lipid degradation after one

hour of incubation (L.D.E.F. mission "Free Flyer Biostack experiment": Planel *et al.* 1994, Gaubin *et al.* 1996). From the French IBIS-1 mission (Foton-10, 1995; 14 days in orbit), one knows that basic embryonic and larval development proceeds essentially normal in microgravity (Hernandorena *et al.* 1999).

B. The sea urchin models

Paracentrotus lividus, Sphaerechinus granularis, Lytechinus pictus

– Early development: fertilization and cleavage

Fertilization and cleavage events in sea urchins of the species *Paracentrotus lividus* were studied on the sounding rocket flights MASER IV-VI (Marthy *et al.* 1994, 95, Marthy 1997). Within a total of 420 seconds of microgravity, automated insemination (technical aspects: Huijser *et al.* 1990, Willemsen 1994) took place after 60, 300 and 360 seconds. One part of the eggs was fixed close to the end of the 0xg phase and one part was kept alive. The particularly successful MASER V mission (1992; about 120 000 eggs were flown and the fertilization rate was > 90 %) brought clear evidence that fertilization was monospermic and correct in microgravity. Live samples continued postflight their larval development up to 40 days post-fertilization. Scanning and transmission electron microscope studies on fixed virgin and freshly fertilized eggs revealed normal structures. Flown sperm maintained its complete fertilization ability on fresh virgin eggs. Surprisingly however, flown virgin eggs could not be fertilized on the ground with fresh sperm. This unspectacular result (normal larvae developed on Earth from eggs fertilized in space) is in line with the normal issue of development in amphibians, fish and arthropods as reported above. It's a clue again, that gravity has apparently no primordial role in onto- and embryogenesis. However, there are observations from the Maser VI mission (1993), that microgravity is "sensed" by embryonic cells (Marthy 1997). During this Sounding Rocket flight, cleaving eggs of *Sphaerechinus granularis* were exposed for 6 minutes to space flight conditions at 0xg and at 1xg in-flight (both synchronously (1-4 embryonic cells) as well as asynchronously dividing eggs (> 16 embryonic cells)). A quantitative evaluation, done on fixed flight samples, of the total cell number reached during the experiment phase, revealed some delay in cell divisions of eggs maintained on the 1×g centrifuge in comparison to those at 0×g and to ground controls. Embryos at later cleavage stages (> 16 cells) did not show such a difference. Cleaving eggs of any stage, recovered alive from space, developed to a high percentage (> 75 %) into normal feeding pluteus larvae, which were capable metamorphosis (Marthy 1997).

New flight opportunities could bring better insight into the question whether space flight conditions, and in particular microgravity conditions, do have some impact on the process of cell division, conceivable for instance on the assumption of a gravi-sensitive, physical-mechanical interference between the cell nucleus mass, the cytoskeleton and the cell membrane (Mesland 1990). From slight deviations in the cytoskeletal organisation (in relation to altered pressure or tension by the cell nucleus) severe morphogenetic disturbances might be expected. Evidently, the presence of a nucleus as a "mass" is a prerequisite for this hypothesis; however, since there is also a mitotic phase in the cell cycle (characterised by the absence of a nucleus mass and by changes in the cytoskeleton), the problem appears more complex. There is no answer yet on the question of whether a disturbed cell cycle and/or a disturbed morphogenesis result when microgravity is "acting" on cells in interphase only and not on cells in mitosis.

A research project for studying microgravity effects on calcium metabolism and cytoskeleton structures during fertilization, cell division, and cell differentiation in early development was started in the sea urchin *Lytechinus pictus* during the STS-77 space shuttle flight (Steffen *et al.* 1992, Schatten *et al.* 1996, 98, 99a,b, Chakrabarti *et al.* 1995). In agreement with the result obtained in the sounding rocket experiments reported above, fertilization in microgravity did also occur in this species. Detailed structural analyses revealed however that the sperm-induced calcium release of cortical granule contents was occasionally incomplete. Fertilization-associated microvillar elongation appeared reduced (reduced polymerization of actin ?) and the cytoskeletal organisation altered. Partially abnormal cell divisions (only 4 % of all dividing cells) might indicate that the centriole separation and orientation were affected in microgravity (Schatten *et al.* 1998, 99b, pers comm).

– Later embryonic development: Skeletogenesis

The sea urchin model (species *Sphaerechinus granularis*) has been successfully studied for determining a potential role of gravity on the particular organogenetic event of *skeletogenesis*. In fact, the structure and composition of the internal larval skeleton, a "biocrystal" consisting of calcium carbonate crystallized in the calcite form with a small amount of magnesium and an organic matrix, is very well known (Okazaki & Inoué 1976, Inoué & Okazaki 1977 and others). When a larva dies or is killed, the crystalline skeleton persists as a solid structure and its morphology, shape, crystallography and chemical composition can be analysed. The whole process of skeletogenesis is, in physiological, cellular and molecular terms, equally well known (e.g. Hörstadius 1973, Czihak 1975, Decker *et al.* 1988, Ettensohn *et al.* 1993, Guss *et al.* 1997). From blastula stage onwards the primary

mesenchyme cells, descendants of the micromeres, differentiate into skeletogenic cells which arrange typically in the blastocel and which produce the skeletal spicules. It has been shown experimentally that the interactions of the primary mesenchyme cells with the basal lamina of the ectoderm wall provides "spatial, temporal and scalar information that regulates skeleton production" (Hardin *et al.* 1992, Ettensohn 1992, Armstrong *et al.* 1993, Wilt 1997 and others).

Based on such knowledge, the problem of whether the process of skeletogenesis might be gravi-dependent has been approached during the "IML-2 mission" (ESA Biorack in Spacelab of "Columbia" space shuttle flight STS-65, 1994), the French "IBIS-1 mission" (Russian satellite Foton-10, 1995) and the "Shuttle-to-Mir mission" S/MM-03 (ESA Biorack in Spacehab of "Atlantis" space shuttle flight STS-76, 1996). The experimental design was essentially identical for all three missions: embryos from the blastula stage onwards (prior to onset of skeletogenesis) were exposed for several days to microgravity conditions as were 1×g controls on the on-board centrifuge. Arrest of larval development was obtained by heat (transfer of samples for 2 hours to +37°C). After return of the samples to Earth, the architecture, shape and dimension of the preserved spicules and skeletons were examined. Mean values of spicule diameters from 0×, 1× and 1.4×g samples allowed an estimation of the amount of spicule mineralisation. Additionally, live recovered larvae were further cultured. As to potential microgravity effects, the essential observations are as follows (Marthy *et al.* 1996, 98, 99).

In microgravity, the sea urchin embryos develop into viable four-armed plutei with more or less complete skeletons. This means that the skeletogenic cells differentiated correctly and generated calcareous spicules. The process of "mineralisation" is evidently independent of gravity.

On the other hand, spicules which differentiated under space flight conditions were slightly thinner than those obtained on Earth (Fig. 7). It means that skeletogenesis "in Space" is slightly retarded. This latter phenomenon reflects ultimately a *retardation of the overall development* of the larvae. It's still an open question of whether this retardation is a microgravity effect or just a transient problem of adaptation of the embryo to the flight and unusual gravity conditions. The difference between flight samples and ground controls is greatest after 2 days and diminishes then progressively. One may assume that the embryos and larvae do "sense" the unusual environment (weightlessness condition is a part of it) and do "react" correspondingly. No demineralization of skeletons and spicules seemed to occur under space flight conditions (Marthy *et al.* 1996, 99).

A great problem is the evaluation of potential microgravity effects on the skeleton and/or spicule architecture. Larvae under normal gravity conditions develop more or less pronounced individual morphological characteristics. Thus viable *Sphaerechinus* larvae may produce skeletons with

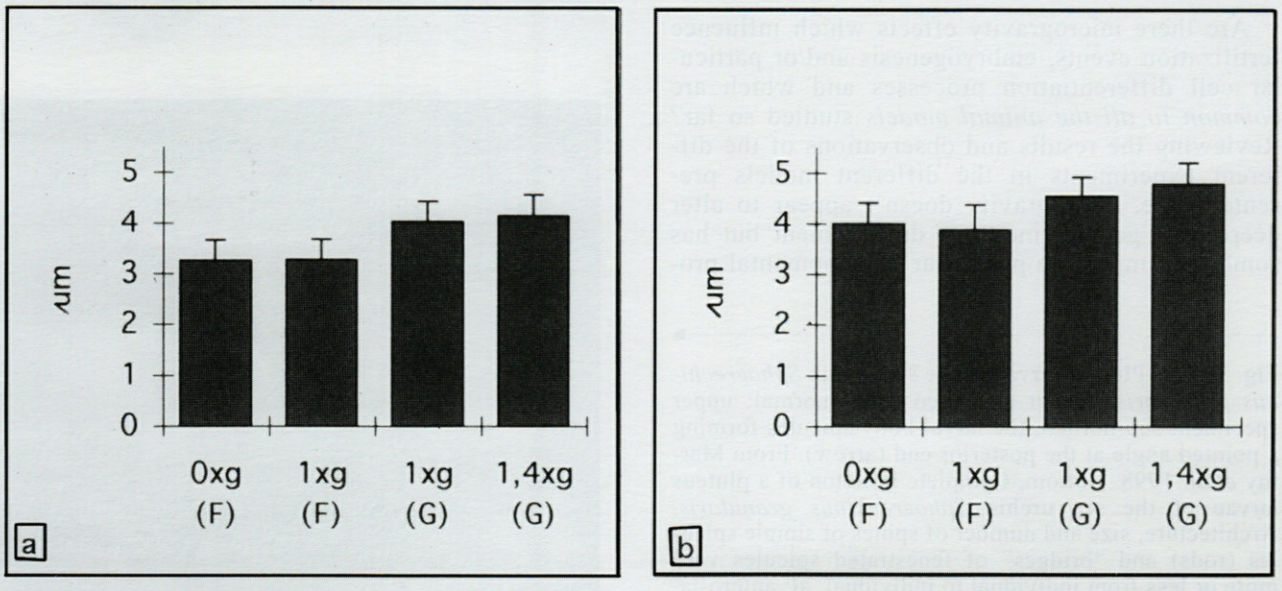


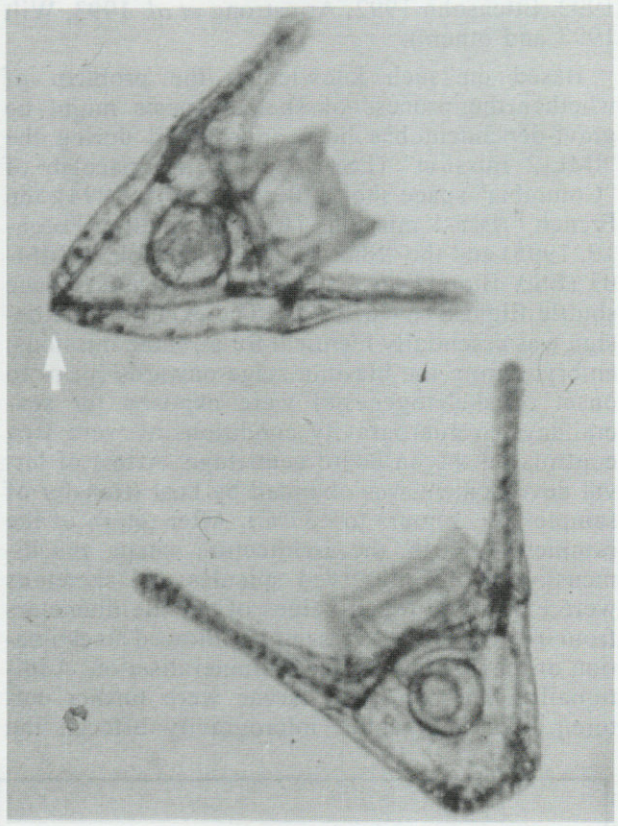
Fig. 7. – Comparison of the mean values of spicule thickness in larvae samples (a,b) of the sea urchin *Sphaerechinus granularis*, developed under various gravity conditions in-flight (F) and on-ground (G). Mean thickness of skeleton spicules after 2 days of skeletogenesis (degree of mineralisation) is shown in (a) and after nearly 6 days of skeletogenesis in (b). Note particularly the difference between F and G samples in (a), less pronounced in (b). F 0×g: microgravity; F 1×g: on-board centrifuge; G 1×g: ground control; G 1.4×g: centrifuge on-ground. S/MM-03 mission, STS-76 space shuttle flight. From Marthy *et al.* 1999.

pointed instead of rounded posterior ends, and simple or fenestrated spicules regularly show a great variability in spines and bridges (Fig. 8:1 & 2). An identical situation is found in skeletons and spicules of larvae from Space. Therefore, whether gravity is involved in the particular process of *positioning* of the skeletogenic cells inside the blastocoel of the developing larvae, via an interaction with the informative ectodermal wall (Hardin *et al.* 1992, Armstrong *et al.* 1993) has to be studied in the future. A causal relationship may exist between skeleton differentiation and the simultaneous development of the geonegative swimming pattern of the larvae. There is also a hint that spiculogenesis *in vitro*, that is the differentiation *in vitro* of isolated spiculogenic primary mesenchyme cells, could well serve to study skeletogenesis and biomineralization processes under simplified conditions (Marthy & Bacchieri 1999). In fact, it appears that spiculogenic cells, which adhere *in vitro* to the substrate, tend to differentiate into bi-polar spicules whereas those cells, which form non-adhering clusters, form much more complicated skeletal structures. One may speculate that particularly the latter forms will differentiate under microgravity conditions. The ISS will offer excellent experimental conditions for approaching such problems.

WHAT DO MICROGRAVITY EFFECTS TELL US ON THE ROLE OF GRAVITY IN DEVELOPMENTAL PROCESSES?

Are there microgravity effects which influence fertilization events, embryogenesis and/or particular cell differentiation processes and which are *common to all the animal models* studied so far? Reviewing the results and observations of the different experiments in the different models presented here, microgravity doesn't appear to alter deeply the general mode of development but has some real impact on particular developmental processes. Thus, it appears that under microgravity conditions the following are true.

– *Fertilization sensu stricto does occur* and occurs essentially in a correct manner. However reduced microvilli (in salamander and sea urchin eggs) suggest that the process of actin polymerisation is altered (indicating the gravi-sensitivity of



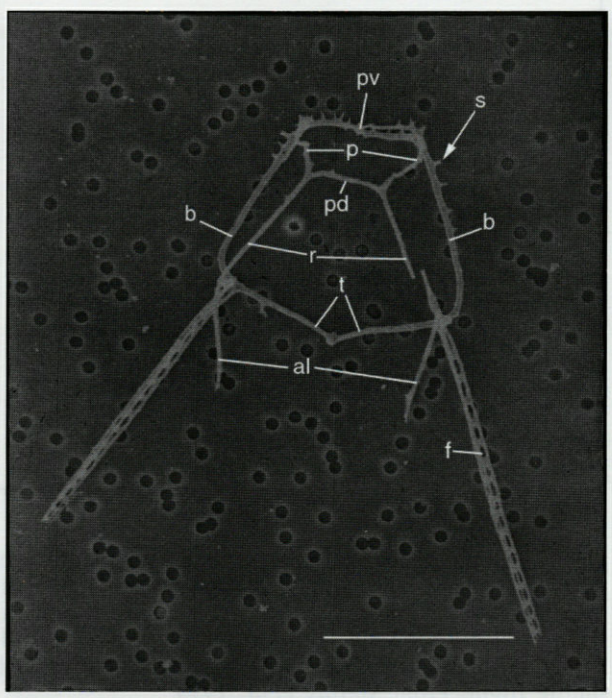


Fig. 8. Top, Pluteus larvae of the sea urchin *Sphaerechinus granularis*. Lower specimen: fully normal; upper specimen: sub-normal, the larval body spicules forming a pointed angle at the posterior end (arrow). From Marthy *et al.* 1998. Bottom, Complete skeleton of a pluteus larvae of the sea urchin *Sphaerechinus granularis*. Architecture, size and number of spines of simple spicules (rods) and "bridges" of fenestrated spicules vary more or less from individual to individual. al: antero-lateral rods; b: body rods; f: fenestrated postoral or anal rods; p: posterior dorso-ventral rods; pd: posterior dorsal rod; pv: posterior ventral rod; r: recurrent rod; s: spines; t: fused transverse rod. Dorsal view, Scanning electron micrograph (holes in substrate denote filter pores). Bar 120 µm, ×250x. From Marthy *et al.*1998, 1999.

the process (?)). Experiments with other egg types are necessary to determine whether it is a general phenomenon (0× g-effects on cell division or cell cycle regulation need also to be confirmed during new space flight opportunities; Marthy 1999, Schatten *et al.* 1999).

– *Embryogenesis sensu stricto does occur.* However, temporary, stage-specific or transient physiological and morphological alterations are observed in the course of embryogenesis (disturbed germ layer formation, reduced adhesiveness between cells; reduced consistency of embryonic tissues, slight modification of developmental chronology). The alterations appear clearly species-specific, indicating the gravi-sensitivity of particular developmental events. No far-reaching generalization appears allowed, except that embryonic cells actually sense altering gravity conditions (as do single cells in culture) and react in a species- and stage-specific manner. Early developmental stages appear more sensitive to altering gravity conditions then more advanced ones. Embryos developing in microgravity may encounter an adaptation problem to the unusual environment and express this by a physiological or morphological "response"; however, such a response might well mask some real microgravity effects too.

– *Organo- and histogenesis do essentially occur.* However, transient physiological deviations in behavioural patterns and morphological alterations in the underlying structures are reported for vertebrates and invertebrates (e.g. modified reflexes, altered modes of moving or swimming, altered differentiation of visual and/or vestibular systems, altered skeletogenesis, slight modification of organogenetic chronology). Such different phenomena indicate again that embryonic cells in differentiating organ anlagen "sense" altering gravity conditions and respond correspondingly. This is particularly true for organ systems, whose correct differentiation is dependent on gravi-stimulation. Serious malformations as reported for anuran and urodelan amphibians (distored tails; lethal a- or microcephalic individuals) might include artifacts due to culture conditions. A direct relationship between an organogenetic negative phenomenon and the particular developmental stage exposed to 0xg conditions appears established. Finally,

– *Transient or continued* ***embryonic development in Space*** (or, in post-flight samples on the Ground) ***leads in all cases ultimately to viable juveniles***. Whatever the microgravity effects are, they become regulated throughout the course of embryonic and larval development!

The regulation capacity makes a fundamental difference between the multi-cellular entity as is an embryo and a single cell system. On the one hand, the former appears less gravi-sensitive than individual cells in culture (reviews for cells: Cogoli *et al.* 1984, Claasen & Spooner 1994, Cogoli 1997, 2002). On the other hand, the embryo is composed of a number of cells and each one of these cells may behave similar as do single cells in the *in vitro* cultures. However, they do not and the question remains open why they do not. The regulation capacity in embryogenesis appears as a pronounced characteristic of an entity of *interacting cells* whose final destiny is – against the effects of an unusual environment – the creation of a *viable* organism.

Remembering the strong "classic effects" obtained in anuran amphibians by hypergravity or egg inversion experiments (twinning of anterior body axes; e.g. Black *et al.*1985, 86, Black 1990), the *weak* microgravity effects do surprise but have to be accepted as such. Even if "weak", they contribute to identifying the significance of gravity in embryonic development.

There seems to be no doubt: **Gravity is not required for a normal issue of ontogenesis and embryogenesis** in Vertebrates and Invertebrates. It appears, however, that the normal course of development gets somehow "de-stabilized" in the absence of gravity (transient alterations without vital consequences). Whether a tendency towards an increasing de-stabilisation will manifest in long-term, multi-generation experiments under continued weightlessness conditions is at present completely unknown.

IS THERE A FUTURE FOR DEVELOPMENTAL BIOLOGY RESEARCH IN SPACE?

So far, one essential insight from Developmental Biology Research in Space on selected animal models is gained*: gravity doesn't have a primordial epigenetic role in embryonic and larval development of vertebrates and invertebrates*. The question is whether this conclusion has to be considered as a "negative result" (as occasionally heard from people opposed to research activities in space) or, on the contrary, an encouraging notion in view of long duration manned spaceflights ? If one knows now that viable organisms differentiate from eggs developing in Space, why should one continue developmental biology research programs in Space?

Well, considering fundamental *scientific objectives* only, an answer is easy and clear: any chance for advance in scientific knowledge is a challenge which has to be taken up. "Space and weightlessness conditions", as available, should be used without hesitation as an unique "research laboratory" with an environment very different from that on Earth. From the few developmental biology experiments done in the past and presented here, the actual role of gravity in basically gene-controlled processes, is certainly not yet sufficient identified.

Only long-term experiments performed during space flight under real microgravity conditions will learn us of how "living systems may change if transferred permanently into environments very different from those in which they have evolved" (Marco *et al.* 1999). Again, nobody knows at present whether the regulation capacities of developing organisms on Earth will progressively diminish under continued microgravity conditions – a problem which can be solved indeed only in Space by an ambitious research program lasting certainly for many more years. Not least, the *molecular* mechanisms underlying the different phenomena already identified, have to be unravelled, which logically require the continuation of scientific research in Space. It should also not be overlooked that the mode of development in microgravity has been studied in a few species only. There are many other candidates as, for example, the large, yolk-rich, telolecithal eggs of readily available birds, squids (cephalopods) or dogfish (chondrichthyes), whose developmental modes are still unexplored in Space but well worth to be considered (Marthy 1994). National and International Space Agencies are well aware of the needs of the scientific community. The possibilities of the ISS will open a complete new era for gravitational biological research (Wassersug 1999).

Developmental Biology in Space is more than just a fascinating research activity in an unusual environment. The scientific insights through space research gained so far into the significance of the gravity force in animal development are a preliminary, yet decisive step to approaching the problem of the precise role of gravity in developmental processes on the one hand and, on the other hand, of the physiological and/or genetic plasticity or instability (Barlow 1998) of organisms developing over generations in weightlessness. Such stimulating perspectives may hopefully help to attract an increasing number of young scientists for an engagement in this field, thus assuring a successful future of Developmental Biology Research under altered gravity conditions and in particular"in Space".

ACKNOWLEDGEMENTS. – Substantial support is given to the author's developmental space biology research program on sea urchin eggs and embryos by the French Space Agency CNES and the national research organism CNRS and is greatly acknowledged. Thanks are also addressed to scientific and administrative projects managers and engineers and their teams of ESA and NASA for flight opportunities and wide-ranging assistance.

REFERENCES

Aimar C, Bautz A, Durand D, Membre H, Chardard D, Gualandris-Parisot L, Husson D, Dournon C 2000. Microgravity and hypergravity effects on fertilization of the salamander *Pleurodeles waltl* (Urodele Amphibian). *Biol Reprod* 63: 551-558.

Anken R, Kappel T, Slenzka K, Rahmann H 1992. The early morphogenetic development of the cichlid fish *Oreochromis mossambicus* (Periciformes, Teleostei). *Zool Anz* 230: 1-10.

Anken RH, Rahmann H 1999. Effect of altered gravity on the neurobiology of fish. *Naturwissenschaften* 86: 155-167 (review).

Armstrong N, Hardin J, McClay DR 1993. Cell-cell interactions regulate skeleton formation in the sea urchin embryo. *Development* 119: 833-840.

Barlow PW 1998. Gravity and developmental plasticity. *Adv Space Res* 21 (8/9): 1097-1102.

Bautz A, Durand D, Oukda M, Tankosic C, Dournon C 1996. Les missions spatiales 1995 et 1996 du Laboratoire de Biologie Expérimentale-Immunologie de l'Université Henri Poincaré de Nancy. *Bull Acad Soc Lorr Sci* 35: 195-201.

Bautz A, Oukda M, Durand D, Membre H, Aimar C, Dournon C 1997. Differentiation in microgravity of the inner ear in the amphibian urodele *Pleurodeles waltl* embarked on a Foton satellite. *Int J Dev Biol* 41: 9S (abstract).

Benguria A, Grande de Juan E, Ugalde C, Miquel J, Garesse R, Marco R 1996. Microgravity effects on *Drosophila melanogaster* behavior and aging. Implications of the IML-2 experiment. *J Biotechnol* 47: 191-201.

Beniash EL, Addadi, Weiner S 1999. Cellular control over spicule formation in sea urchin embryos: a structural approach. *J Structural Biol* 125: 50-62.

Black S 1990. Experimental control of axis polarity in amphibian embryos. *In* Guyenne TD ed Microgravity as a tool in developmental biology. ESA SP-1123: 43-48.

Black S, Gerhart J 1985. Experimental control of the site of embryonic axis formation in *Xenopus laevis* eggs centrifuged before first cleavage. *Dev Biol* 108: 308-324.

Black S, Gerhart J 1986. High-frequency twinning of *Xenopus laevis* embryos centrifuged before first cleavage. *Dev Biol* 116: 228-240.

Black S, Larkin K, Jacqmotte N, Wassersug R, Pronych S, Souza KA 1996. Regulative development of *Xenopus laevis* in microgravity. *Adv Space Res* 17 (6/7): 209-217.

Brillouet C (sc. coord.), C Mattok ed 1995. Biorack on Spacelab IML-1. ESA Publi Division, Noordwijk ESA SP-1162: 231.

Brinckmann E, Brillouet C (sc. coords), Perry M ed 1999. Biorack on Spacehab, ESA Publi Divison, Noordwijk ESA SP-1222.

Bücker H, Facius R, Horneck G, Reitz G, Graul EH, Berger H, Höffken H, Rüther W, Heinrich W, Beaujean R, Enge W 1988. Embryogenesis and organogenesis of *Carausius morosus* under spaceflight conditions. *In* Mesland D, Brillouet C (sc.coord.) Biorack on Spacelab *D1*. ESA SP-1091: 135-145.

Chakrabarti A, Stoecker A, Schatten H 1995. Modification of experimental protocols for a space shuttle flight and applications for the analysis of cytoskeletal structures during fertilization, cell division, and development in sea urchin embryos. *Amer Inst Aeronautics Astronautics* 95-1095: 1-10.

Chaput D, Bouzouklian H 1994. Fertile: An instrument to study developmental processes of amphibians in microgravity. IAF G.2.138.

Claassen DE, Spooner BS 1994. Impact of altered gravity on aspects of cell biology. *Int Review Cytology* 156: 301-373.

Cogoli A guest ed 1996. Biology Under Microgravity Conditions in Spacelab IML-2. *J Biotechnology* 47 (2/3): 403. Sp Issue, Elsevier.

Cogoli A ed 2002. Cell biology and biotechnology in Space. ASBM Ser 8: 1-252, Elsevier.

Cogoli A, Tschopp A, Fuchs-Bislin P 1984. Cell sensitivity to gravity. *Science* 225: 228-230.

Cogoli A, Friedrich U, Mesland D, Demets R eds 1997. Life Sciences Experiments performed on Sounding Rockets (1985-1994). ESA Publi Division, Noordwijk., ESA SP-1206: 117.

Czihak G ed 1975. The sea urchin embryo. Biochemistry and Morphogenesis. Springer Verlag Berlin, Heidelberg, NewYork.

De Mazière A, Gonzales-Jurado J, Reijnen M, Narraway JM, Ubbels GA 1996. Transient effects of microgravity on early embryos of *Xenopus laevis*. *Adv Space Res* 17: 219-223.

Decker GL, Lennarz WJ 1988. Skeletogenesis in the sea urchin embryo. *Development* 103: 231-247.

Demets R 1996. Biological Experiments on Bion-8 and Bion-9. ESA Publi Division, ESA SP-1190: 45.

Duprat A-M, Husson D, Gualandris-Parisot L 1998. Does gravity influence the early stages of the development of the nervous system in an amphibian? *Brain Res Reviews* 28 (1-2): 19-24.

Dutcher FR, Hess EL, Halstead TW 1994. Progress in plant research in space. *Adv Space Res* 14 (8): 159-171.

Ettensohn CA 1992. Cell interactions and mesodermal cell fates in the sea urchin embryo. *In* Stern C, Ingham P eds, Gastrulation. *Development* Suppl: 43-51.

Ettensohn CA, Malinda KM 1993. Size regulation and morphogenesis: a cellular analysis of skeletogenesis in the sea urchin embryo. *Development* 119: 155-167.

ESA Life Sciences Research in Space: Proceedings of the 4th-7th European Symposium, ESA SP-307 (1990), -366 (1994), -390 (1996), ESA-Abstractbook (1999), ESA Publications Division, Noordwijk, NL.

Förster S, Sebastian C, Horn ER 1999. Functional regeneration of the cercal gravity sensory system of crickets (*Acheta domesticus*) in the absence of gravity. *In* Elsner N *et al* eds, Göttingen Neurobiology Report 1999, Thieme, Stuttgart, New York,Vol II: 800.

Fraser SE, Harland RM 2000. The molecular metamorphosis of experimental embryology. *Cell* 100: 41-55.

Gaubin Y, Prévost MC, Cariven, Pianezzi B, Planel H, Soleilhavoup JP 1996. Enzyme activities and membrane lipids in *Artemia* cysts after a long duration space flight. *Adv Space Res* 18 (12): 221-227.

Guss KA, Ettensohn CA 1997. Skeletal morphogenesis in the sea urchin embryo: regulation of primary mesenchyme gene expression and skeletal rod growth by ectoderm-derived cues. *Development* 124: 2355-2364.

Halstead TW, Dutcher FR 1987. Plants in Space. *Ann Rev Plant Physiol* 38: 317-345.

Hardin T, Coffman JA, Black SD, McClay DR 1992. Commitment along the dorsoventral axis of the sea urchin embryo is altered in response to NiCL. *Development* 116: 671-685.

Hernandorena A, Scherer H, Villa A, Diaz C, Mateos J, Reitz G, Marco R 1999. A crustacean, another useful arthropod model system for space biology: *Artemia* dormant gastrulae. A system for studying the effects of the direct exposure of complex organisms to the space environment. *In* Wilson A ed, 2nd Symposium on Utilisation of the International Space Station. ESA SP-433: 521-525, ESA Publi Division, Noordwijk.

Horn ER, Föller W 1985. Tonic and modulatory subsystems of the complex gravity receptor system in crickets. *J Insect Physiol* 31: 937-946.

Horn ER, Sebastian CE, Esseling K, Neubert J 1995. The static vestibulo-ocular reflex in lower vertebrates after a transient gravity deprivation during an early period of life. *Naturwissenschaften* 82: 289-291.

Horn ER, Sebastian CE, Kämper G 1996. Why crickets offer an ideal model system to analyse effects of altered gravitational forces on the development of gravity sensitive neuronal networks. *ASGSB Bull* 10 (1): 58 (abstract).

Horn ER, Föller W 1998. Induction of a gravity-related response by a single receptor cell in an insect. *Naturwissenschaften* 85: 121-123.

Horn ER, Sebastian CE 1999. A comparison of normal vestibulo-ocular reflex development under gravity and in the absence of gravity. *In* Brinckmann E, Brillouet C authors & Perry ed, Biorack on Spacehab (Biological experiments on three Shuttle-to-Mir missions), ESA SP-1222: 127-138.

Horn ER 2000. It was a first class start which laid the basis for a promising future. *In* El-Gerk MS ed, Space Technology and Applications International Forum. Amer Inst Phys Conf Publ Melville NY: 376-382.

Hörstadius S 1973. Experimental Embryology of Echinoderms. Clarendon Press, London.

Huijser R, Aartman L, Willemsen H 1990. Cells in Space: sounding rocket facilities for cell biology and biotechnology in microgravity. *In* David V ed, Life Sciences Research in Space. ESA SP-307: 455-466.

Husson D 1998. Etude du rôle de la pesanteur dans la fécondation et l'embryogenèse de l'amphibien urodèle *Pleurodeles* waltl. Thèse Univ P Sabatier Toulouse III.

Husson D, Gualandris-Parisot, Foulquier F, Grinfeld S, Kan P, Duprat AM 1998. Differentiation in microgravity of neural and muscle cells of a vertebrate (amphibian). *Adv Space Res* 22 (2): 303-308.

Ijiri K 1994. A preliminary report on IML-2 Medaka experiment: mating behavior of the fish Medaka and development of their eggs in Space. *Biol Sciences Space* 8 (4): 231-233.

Ijiri K 1995a. The first Vertebrate mating in Space – a fish story, RICUT, Japan (request to author for book, free of charge). 57 p.

Ijiri K 1995b. Fish mating experiment in space – what it aimed at and how it was prepared. *Biol Sciences Space* 9 (1): 3-16.

Ijiri K 1995c. Medaka fish had the honor to perform the first successful vertebrate mating in space. *Fish Biol J MEDAKA* 7: 1-10.

Ijiri K 1998. Development of space-fertilized eggs and formation of primordial germ cells in the embryos of Medaka fish. *Adv Space Res* 21 (8/9): 1555-1558.

Imamizo M, Koike H, Mogami Y, Izumi-Kurotani A, Yamashita M, Nagaoka S, Yoshida M, Asashima M 1993. Egg laying of newt in space. 10th ISAS Space Utilization Symposium, July 1993, Tokyo: 49-52.

Imamizo M, Yoshida M. Fujioka K, Takada Y, Hisada A, Izumi-Kurotani A, Yamashita M, 1994. Sustained release of hCG minipellet for newt experiment in Space. *Biol Sci Space* 8: 226-230.

Inoué S, Okazaki K 1977. Biocrystals. *Sci Amer* 236: 82-85.

Keller RE 1980. The cellular basis of epiboly: a SEM study of deep-cell rearrangement during gastrulation in *Xenopus laevis*. *JEEM* 60: 201-234.

Koch P 1964. *In vitro*-Kultur und entwicklungsphysiologische Ergebnisse an Embryonen der Stabheuschrecke *Carausius morosus* Br. Roux. *Arch Entwicklungsmech* 155: 549-593.

Malacinski GM, Neff AW, Alberts JR, Souza KA 1989. Developmental Biology in outer space. *BioScience* 39: 314-320.

Marco R, Gonzalez-Jurado J, Calleja M, Garesse R, Maroto M 1992. Microgravity effects on *Drosophila melanogaster* development and aging: comparative analysis of the results of the fly experiment in the Biokosmos 9 biosatelllite flight. *Adv Space Res* 12 (1): 157-166.

Marco R, Gonzalez-Jurado J, Calleja M, Manzanares, Maroto M, de Juan E, Miquel J 1995. Effects of microgravity on *Drosophila melanogaster*. Development and Aging. *In* Brillouet C sc. coord. Biorack on Spacelab IML-1. ESA SP-1162: 199-203. ESA Publications Division, Noordwijk NL.

Marco R, Benguria A, Sachez J, Juan E 1996. Effects of the space environment on *Drosophila melanogaster* development. Implications of the IML-2 experiment. *J Biotechnol* 47: 179-189.

Marco R, Diaz C, Benguria A, Mateos J, Mas J, de Juan E 1999a. The role of gravity in the evolutionary emergence of multicellular complexity. The effects of microgravity on arthropod development and aging. *Adv Space Res* 23 (12): 2075-2082.

Marco R, Diaz C, Benguria A, Mateos J, de Juan E 1999b. *Drosophila melanogaster*, a key arthropod model in the study of the evolutionary long term adaptation of multicellular organisms to the space environment. *In* Wilson A ed, Utilisation of the International Space Station (2nd Europ Symp), ESA Publi Division, Noordwijk ESA SP-433: 433-440.

Marthy H-J 1994. Telolecithal eggs need attention for evaluating the morphogenetic role of gravity in embryogenesis. Proc 5th Europ Symp Life Sc Res In Space, ESA Publications Division, Noordwijk ESA SP-366: 177-180.

Marthy H-J 1997. Sea urchin eggs under microgravity conditions. *In* A Cogoli, U Friedrich, D Mesland, R Demets eds, Life Sciences Experiments performed on Sounding Rockets (1985-1994)-Texus 11-32, Maser 3-6 and Maxus 1. ESA Publications Division, Noordwijk, ESA SP-1206: 81-91.

Marthy H-J ed 2003. Developmental Biology Research in Space. ASBM ser 9, Elsevier (in press).

Marthy H-J, Schatt P, Santella L 1994. Fertilization of sea urchin eggs in space and subsequent development under normal conditions. *Adv Space Res* 14: 197-208.

Marthy H-J, Schatt P, Marthy U 1995. Development of sea urchin eggs after exposure to microgravity during cleavage stages. *ELGRA News* 19: 77.

Marthy H-J, Gasset G, Tixador R, Schatt P, Eche B, Dessommes A, Giacomini T, Tap G, Gorand D 1996. The sea urchin larva, a suitable model for biomineralisation studies in space (IML-2 ESA Biorack experiment "24-F urchin"). *J Biotechnol* 47: 167-177.

Marthy H-J, Gasset G, Tixador R, Schatt P, Eche B, Dessommes A, Marthy U, Bacchieri R 1998. Skeletogenesis in sea urchin larvae under modified gravity conditions. *Adv Space Res* 21: 1151-1154.

Marthy H-J, Gasset G, Eche B, Bacchieri R, Gorand D 1999. Sea urchin development in space as revealed by skeletogenesis. *In* Brinckmann E, Brillouet C authors, Perry M ed, Biorack on Spacehab. ESA Publications Division, Noordwijk, ESA SP-1222: 139-148.

Marthy H-J, Bacchieri R 1999. *In vitro* spiculogenesis of sea urchins: an *in vitro* model system for studying biomineralization processes. *ELGRA News* 22: 135-136.

Mesland D, Brillouet C sc. coords, Longdon N, David V 1987. Biorack on Spacelab D1. ESA SP ESA Publications Division, Noordwijk, 1091: 182.

Mesland D 1990. Gravity effects on cells. *In* David V ed, Life Sciences Research in Space, ESA SP-307: 221-225, ESA Publications Division, Noordwijk, NL.

Mogami Y, Imamizo M, Yamashita M, Izumi-Kurotani A, Wiederhold ML, Koike H, Asahima M 1996a. "Astro-Newt": early development of newt in space. *Adv Space Res* 17 (6/7): 257-263.

Mogami Y, Koike H, Yamashita M, Izumi-Kurotani A, Asahima M 1996b. Early embryogenesis of amphibians in space: AstroNewt for the space embryology in IML-2 and SFU. *In* Bainum PM *et al.* eds, Advances in the Astronautical Sciences, 19 (Strengthening Cooperation in the 21st Century), Amer Astron Soc Publication.

Moore D, Cogoli A 1996. Gravitational and space biology at the cellular level. *In* Moore D, Bie P, Oser H eds, Biological and Medical Research in Space, Springer Verlag, Chapter 1: 1-106.

Moore D, Bie P, Oser H eds 1996. Biological and Medical Research in Space, ISBN 3-540-60636-X Springer Verlag Berlin Heidelberg New York, 1996: 569.

Neubert J, Briegleb W, Schatz A, Bromeis B, Linke-Hommes, Rahmann H, Slenzka K, Horn E, Esseling K, Sebastian C 1994. Spacelab mission D-2 experiment STATEX "Gravity Perception and Neuronal Plasticity". *In* Oser H & Guyenne TD comp. *Life Sciences Research in Space* (Symposium), ESA Publications Division, ESA SP-366: 77-81.

Okazaki K, Inoué S 1976. Crystal property of the larval sea urchin spicule. *Dev Growth Diff* 18: 413-434.

Oser H, Battrick B 1989. Life Sciences Research in Space. ESA Publications Division, ESA SP-1105:135.

Oukda M, Membre H, Bautz A, Aimar C, Dournon C 1999a. Saccular otoconia during the inner ear development of an urodele amphibian. Ground study before the space mission Perséus. 7th European Symp Life Sciences Res in Space. Abstract book.

Oukda M, Michel F, Membre H, Bautz A, Dournon C 1999b. Crystallographic and chemical composition of otoconia in the salamander *Pleurodeles waltl*. *Hearing Res* 132, 85-93.

Oukda M, Bautz A, Membre H, Ghanbaja Michel F, Dournon C 1999. Appearance and evolution of calcitic and aragonitic otoconia during *Pleurodeles waltl* development. *Hearing Res* 136: 114-126.

Pfeiffer CJ, Yamashita M, Izumi-Kurotani A, Koike H, Asahima M 1995. Cytopathologic observations of the lung of adult newts (*Cynops pyrrhogaster*) on-board the Space Shuttle Columbia during the Second International Microgravity Laboratory experiments. *J Submicrosc Cytol Pathol* 27 (4): 501-509).

Planel H 1988. L'Espace et la Vie. Larousse, Paris, ISBN 2-03-505205-X.

Planel H, Gaubin Y, Pianezzi B, Delpoux M, Payonove J, Bes JC, Heilmann C, Gasset G 1994. Influence of long duration exposure, 69 months, to the space flight factors in *Artemia* cysts, tobacco and rice seeds. *Adv Space Res* 14 (10): 21-32.

Pronych SP, Souza KA, Neff AW, Wassersug RJ 1996. Optomotor behaviour in *Xenopus laevis* tadpoles as a measure of the effect of gravity on visual and vestibular neural integration. *J Exp Biol* 199: 2689-2701.

Rahmann H, Slenzka K, Körtje KH, Hilbig R 1992. Synaptic plasticity and gravity: ultrastructural, biochemical and physico-chemical fundamentals. *Adv Space Res* 12: 63-72.

Reitz G, Beaujean R, Hiendl OC, Rüther W, Pross HD, Kost M, Kiefer J 1995. Radiation and microgravity effects observed in the IML-1 MOROSUS experiment. *In* Brillouet C sc. coord. Biorack on Spacelab IML-1 ESA Publications Division, Noordwijk, ESA SP-1162: 187-197.

Schatten H, Chakrabarti A 1996. Culture of sea urchin embryos in the new aquatic research facility (ARF) and first results from its maiden space voyage on STS-77. *Grav Space Biol Bull* 10 (1): 46 (abstract n° 83).

Schatten H, Taylor M, Chakrabarti A, Kemp R, Crosser M 1998. Scanning Electron microscopy of sea urchin eggs and embryos exposed to microgravity on a space shuttle flight. *Scanning* 20: 223-224.

Schatten H, Chakrabarti A, Levine HG, Anderson K 1999a. Utilization of the aquatic research facility and fertilization syringe unit to study sea urchin development in space. *J Grav Physiol* 6 (2): 43-54.

Schatten H, Chakrabarti A, Taylor M, Sommer L, Levine H, Anderson K, Runco M, Kemp R 1999b. Effects of spaceflight conditions on fertilization and embryogenesis in the sea urchin *Lytechinus pictus*. *Cell Biol Intern* 23 (6): 407-415.

Sebastian C, Eberling K, Horn ER 1996. Altered gravitational experience during early periods of life affects the static vestibulo-ocular reflex of tadpoles of the southern clawed toad *Xenopus laevis* Daudin. *Exp Brain Res* 112: 213-222.

Sebastian C, Horn E 1998. The minimum duration of microgravity existence during space flight which affects the development of the roll-induced vestibulo-ocular reflex in an amphibian. *Neurosci Letter* 253: 1-4.

Sievers A, Buchen B, Scott TK eds 1997. Plant Biology in Space. Proceed of the Internat Workshop, Springer. *Planta*, Suppl 203: 219.

Slenzka K, Appel R, Hilbig R, Kappel T, Vetter S, Freischutz B, Rahmann H 1994. Behavioral and biochemical investigations of the influence of altered gravity on the CNS of aquatic vertebrates during ontogeny. *Adv Space Res* 14: 309-312.

Snyder RS comp 1997. Second International Microgravity Laboratory (IML-2) Final Report. NASA MSFC, Alabama. NASA Reference Publication 1405: 217.

Souza KA 1986. Amphibian development in Microgravity. *In* Watanabe S ed, *Biol Sc Space*: 61-68 Nagoya, Japan.

Souza KA, Hogan R, Ballard R eds 1995a. Life into Space. Space Life Sciences Experiments NASA Ames Research Center 1965-1990. NASA Ref Publ 1372: 606.

Souza K A, Black SD, Wassersug RJ 1995b. Amphibian development in the virtual absence of gravity. *Proc Natl Acad Sci* 92: 1975-1978.

Steffen S, Fiser R, Simerly C, Schatten H 1992. Microgravity effects on sea urchin fertilization and development. *Adv Space Res* 12(1): 167-173.

Tabony J, Glade N, Papaseit C, Demongeot J 2002. Microtubule self-organization and its gravity dependence. *Adv Space Biol Med* 8 (in press).

Ubbels GA 1992. Developmental biology in unmanned spacecraft. *Adv Space Res* 12(1): 117-122.

Ubbels GA 1997a. Fertilisation and development of *Xenopus* eggs on sounding rockets and in a clinostat. *In* Cogoli A, Friedrich U, Mesland D, Demets R ed: Wilson A Life Sciences Experiments performed on Sounding Rockets (1985-1994), ESA SP-1206: 25-36.

Ubbels GA 1997b. Establishment of polarities in the oocyte of *Xenopus laevis*: the provisional axial symmetry of the full-grown oocyte of *Xenopus laevis*. CMLS, Cellular and Molecular Life Sciences, Birkhäuser Verlag Basel (Review of her basic research and space experiments). 53: 382-409.

Ubbels GA, Brom TG 1984. Cytoskeleton and gravity at work in the establishment of the dorso-ventral polarity in the egg of *Xenopus laevis*. *Adv Space Res* 45(12): 9-18, 1984.

Ubbels GA, Berendsen W, Narraway JM 1989. Fertilization of frog eggs on a sounding rocket in space. *Adv Space Res* 9: 187-197.

Ubbels GA, Berendsen W, Kerkvliet S, Narraway JM 1990a. The first seven minutes of a Xenopus egg fertilised on a Sounding Rocket in Space. *In* Guyenne TD ed. Microgravity as a tool in developmental biology. ESA SP-1123: 49-58.

Ubbels GA, Berendsen W, Kerkvliet S, Narraway JM 1990b. Fertilization of *Xenopus* eggs in Space. *In* David V ed., Life Sciences Research in Space, 4th Europ. Symp., ESA SP-307: 249-254.

Ubbels GA, Willemsen HP 1990c. Automatic experiment container (AEC) for fertilisation of frog eggs in space – a multi-purpose piece of classified space hardware. *In* Guyenne TD ed. Microgravity as a tool in developmental biology. ESA SP-1123: 75-78.

Ubbels GA, Oser H sc. coord., Duc Guyenne T ed 1990d. Microgravity as a tool in developmental biology. ESA Publications Division, Noordwijk, ESA SP-1123, 1-93.

Ubbels G, Berendsen W, Kerkvliet S, Narraway JM 1992. Fertilization and development of eggs of the South African clawed toad *Xenopus laevis*, on soun-

ding rockets in Space. *Adv Space Res* 12 (1): 181-194.

Ubbels GA, Reijnen M, Meijerink J, Narraway JM 1994. *Xenopus laevis* embryos can establish their spatial bilateral symmetrical body pattern without gravity. *Adv Space Res* 14/8: 257-269.

Ubbels GA, Reijnen M, Meijerink J, Narraway JM 1995. The role of gravity in the establishment of the dorsoventral axis in the amphibian embryo. *In* Mattoc C ed, Biorack on Spacelab IML-1, ESA SP-1162: 175-185.

Vernos I, Gonzalez-Jurado J, Calleja M, Carratala M, Marco R 1988. Effects of short spaceflights on *Drosophila melanogaster* embryogenesis and life span. *In* Mesland D & C Brillouet sc.coord. Biorack on Spacelab D1. ESA SP-1091: 121-133.

Vernos I, Gonzalez-Jurado J, Calleja M, Marco R 1989. Microgravity effects on the oogenesis and development of embryos of *Drosophila melanogaster* laid in the Space Shuttle during the Biorack experiment. *Int J Dev Biol* 33: 213-226.

Wassersug RJ 1999. Life without gravity. *Nature* 40: 758.

Wiederhold ML, Gao WY, Harrison JL, Hejl R 1997. Development of gravity-sensing organs in altered gravity. *ASGSB Bull* 10 (2): 91-96, 1997.

Willemsen HP 1994. Automatic hardware for biological experiments in microgravity. *In* Oser H, Guyenne TD eds, Life Sciences Research in Space, ESA SP-366: 461-465.

Wilt FH 1997. Looking into the sea urchin embryo you can see local interactions regulate morphogenesis. *Bioessays* 19 (8): 665-668.

Yamashita M 1997. Fertilization and embryonic development of japanese newt in Space. *In* Snyder R compl, Second International Microgravity Laboratory (IML-2), Final Report, NASA Ref Publ 1405: 106-107.

Young RS, Tremor JW 1968. The effect of weightlessness on the dividing egg of *Rana pipiens*. *Bioscience* 18: 609-615.

Young RS, Deal PH, Souza KA, Whitfield O 1970. Altered gravitational field effects on the fertilized frog egg. *Exp Cell Res* 59: 267-271.

Reçu le 5 août 2002, received August 5, 2002
Accepté le 18 septembre 2002, accepted September 18, 2002

VIE MILIEU, 2002, 52 (4) : 167-175

From Marine Ecology to Developmental Biology
In Honour of Pierre Tardent (1927-1997)

NEW DATA ON MATING IN AN AUTOTROPHIC DINOFLAGELLATE, *PROROCENTRUM MICANS* EHRENBERG

M.O. SOYER-GOBILLARD, Y. BHAUD, D. SAINT HILAIRE

Observatoire Océanologique de Banyuls-sur-Mer, Laboratoire Arago,
UMR 7628 "Modèles en Biologie Cellulaire et Evolutive",
Université P. et M. Curie, 66651 Banyuls-sur-Mer, France
mosg@obs-banyuls.fr

DINOFLAGELLATE
MATING
SEXUAL REPRODUCTION
TEMPERATURE EFFECT
SEM
TEM
PROROCENTRUM MICANS

ABSTRACT. – Cultures of the autotrophic dinoflagellate *Prorocentrum micans* Ehr. were submitted to 4°C temperature in darkness. This provokes the appearance of cell matings followed by steps of the meiotic process which were observed until it became blocked after the first meiotic division. We also observed, for the first time in *Prorocentrum micans*, the rotation of the chromatin within the nucleus of the zygote and thus can discuss its role. Profound modifications of the chromosome architecture were also observed in TEM: stretching and partial unwinding during injection of the donor cell nucleus into the receiver cell through a fertilization tube, total unwinding and crossing over during the conjugation step, unwinding and peripheral loops in the zygote, slight compaction but not supercoiling during the first meiotic division. These chromatin transformations were analyzed in the light of previous biochemical and structural indications about maintenance of dinoflagellate chromosome architecture.

DINOFLAGELLÉ
ACCOUPLEMENT
REPRODUCTION SEXUÉE
EFFET DE LA TEMPÉRATURE
MEB
MET
PROROCENTRUM MICANS

RÉSUMÉ. – Des cultures du Dinoflagellé autotrophe *Prorocentrum micans* Ehr. ont été soumises à une température de 4°C dans l'obscurité pendant plusieurs jours. Des appariements par accolement d'isogamètes, dont les noyaux contiennent q ADN, au niveau des deux épines apicales, s'effectuent au bout de 24 heures. Ce phénomène est suivi par les différentes phases du processus méiotique: formation d'un tube de fertilisation qui permet l'injection du noyau de la cellule donneuse dans la cellule receveuse, conjugaison des deux noyaux à q ADN dans le planozygote, doublement de la masse d'ADN (4q ADN). A ce stade, nous avons également pu observer la rotation rapide de la chromatine, précédant le début de la première division méiotique (cellules à 2 noyaux contenant 2q ADN). Le rôle de cette cyclose chromatique nucléaire est discuté. La seconde division méiotique ne s'effectue pas et les cellules restent bloquées en fin de première mitose. De profondes modifications de l'architecture chromosomique se produisent tout au long de ces phénomènes méiotiques: élongation chromosomique, détorsion partielle durant l'accouplement, déroulement total permettant le crossing-over durant la phase de conjugaison, détorsion et apparition de boucles latérales dans le planozygote, légère recompaction lors de la mitose de première division méiotique. Ces transformations structurales des chromosomes sont analysées à la lumière des résultats biochimiques et structuraux antérieurs concernant la maintenance de l'architecture chromosomique des Dinoflagellés.

A. INTRODUCTION

Dinoflagellates are eukaryotic protists showing great diversity: they can be autotrophic, heterotrophic, mixotrophic, parasitic or symbiotic and are widely distributed throughout the world in the sea and in fresh water. Cells are generally haploid and can display either vegetative division and/or sexuality with postzygotic meiosis. One of the most interesting characteristics of dinoflagellates is the composition of their DNA and the organization of their chromosomes as well as the course of their mitosis (dinomitosis) (for a review, see Soyer-Gobillard & Moreau 2000).

Chatton & Biecheler (1934) were the first to observe sexual reproduction in the dinoflagellate *Coccidinium mesnilii*, and later several authors observed many species having the potentiality to have two strategies of multiplication: – vegetative repro-

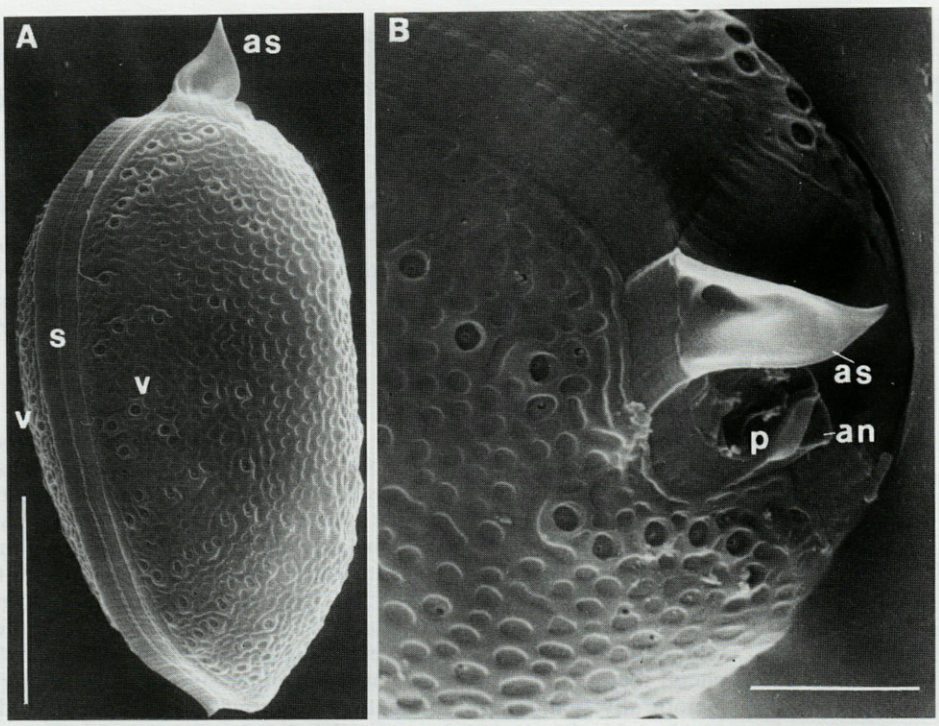


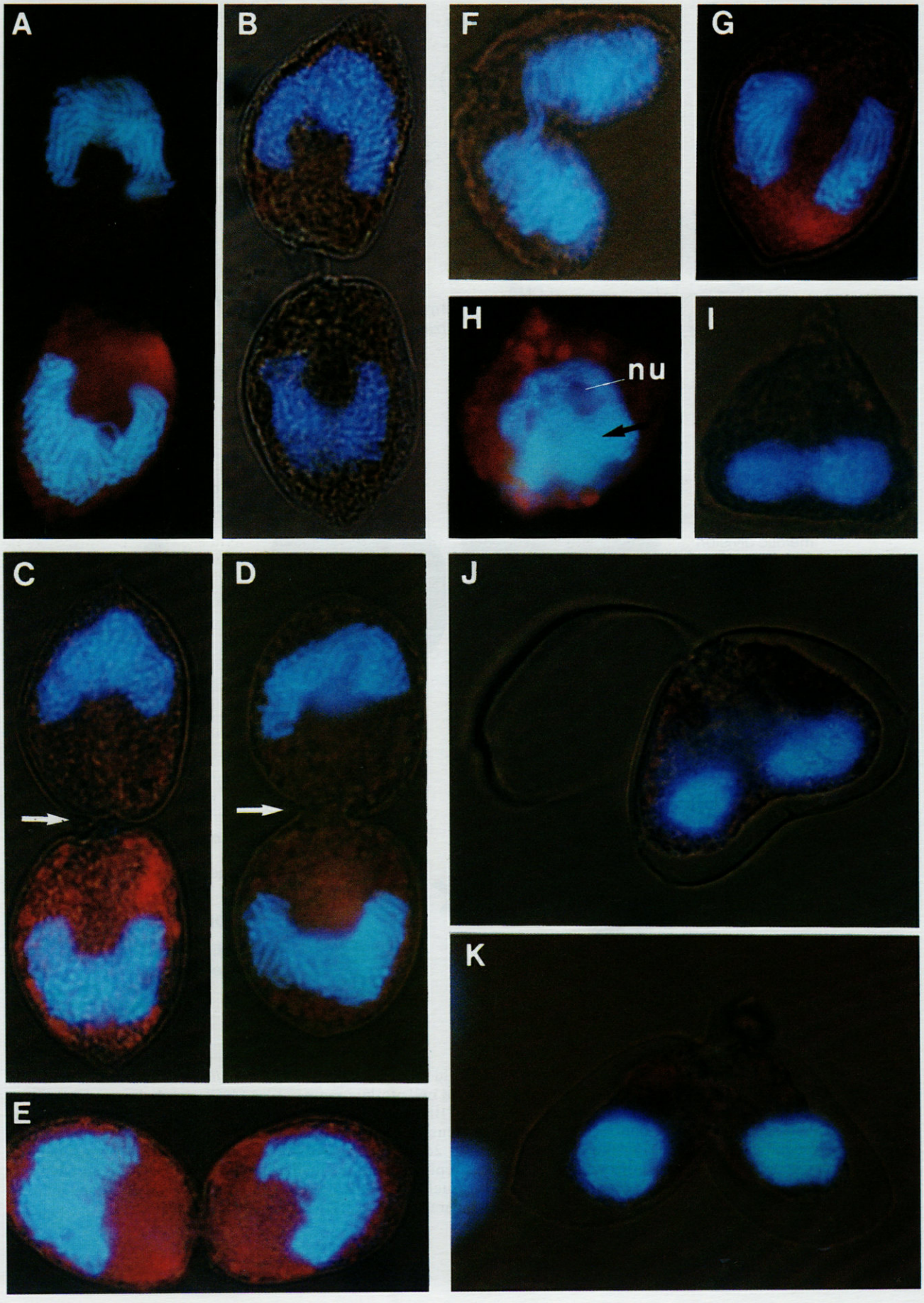
Fig. 1. – A, B. Scanning electron microscope observations of *Prorocentrum micans* Ehrenberg. A. View of the whole cell showing the two valves (v) separated by a longitudinal suture (s). Note the apical spine (as) at the anterior part of the cell. X 3,000 (Scale bar= 10 µm). B. Detail of the anterior part of the cell. Observe the little pores distributed on the valves, the apical pore (p) and an apical notch (an) close to the apical spine (as). X 7,200 (Scale bar = 10 µm).

duction and/or – sexual reproduction, the latter essentially appearing when conditions in the culture medium become unfavourable (Bhaud *et al.* 1988, Pfiester 1989, Faust 1991, 1993).

We previously observed (Bhaud *et al.* 1988) that in the dinoflagellate *Prorocentrum micans* Ehr., changing the culture medium (switching from Erd-Schreiber's to Provasoli's medium) triggered sexual reproduction. Our light microscopy observations and microspectrofluorimetric measurements made *in vivo* demonstrated that cells playing the role of isogametes (haploid and containing q DNA) paired and formed a fertilization tube through which a donor cell (male) injects its nucleus into a recipient cell (female). After conjugation, the zygote containing 2q DNA replicates and two successive meiotic divisions lead to the formation of a tetrad in which each nucleus contains q DNA. Cells released from the tetrads seem to be adapted to the new medium and vegetative divisions occur again.

Induction of sexual reproduction in dinoflagellates was thoroughly studied in the last decades (for reviews see Bhaud *et al.* 1988, Pfiester 1989). Anderson *et al.* (1985) have shown that in *Gyrodinium uncatenatum* nitrogen deficiency of the medium was not sufficient to trigger sexuality but that an unfavourable temperature (too low or too high) was also required. Also Faust (1992) observed that sexual process occurred either in *Coolia monotis* when culture medium was modified by using mangrove soil extract substituting garden soil of Erd-Schreiber's medium and in mangrove populations of *Prorocentrum lima* cultured in natural medium in which Erd-Schreiber's sea-

Fig. 2. – Course of pairing (A-C), mating (D-F) and beginning of sexual reproduction (G-K) of *Prorocentrum micans* as observed *in vivo* after staining of the nuclei with DAPI and fluorescence coupled with Nomarski interference light microscope observations. During pairing and conjugation, several cell pairs presented a bright (red) autofluorescence (A, C, E, G, H). A-C. During pairing the cells are disposed with the anterior part of one cell in front of the other anterior part, apical spine against apical spine. The two nuclei are distally disposed. D, Note the fixation of one of the apical spines anchored into the partner cell (arrow) at the beginning of mating. E-G, After the formation of a fertilization canal (Bhaud *et al.* 1988), and injection of one nucleus of the donor cell into the receiver (F), the two nuclei are located in the same cell (G). H, After conjugation of the nuclei, replication occurs. The nucleus containing 4q DNA presents very thin and punctuated chromosomes (arrow). An intense rotation movement (cyclosis) is also observable in the nucleus. The outline of the nucleus is wavy and three nucleoli (nu) are visible. I-K, The first nuclear meiotic division takes place but cytoplasmic segregation does not occur. Meiosis is incomplete.


A
B
F
G
H
nu
I
C
D
J
E
K

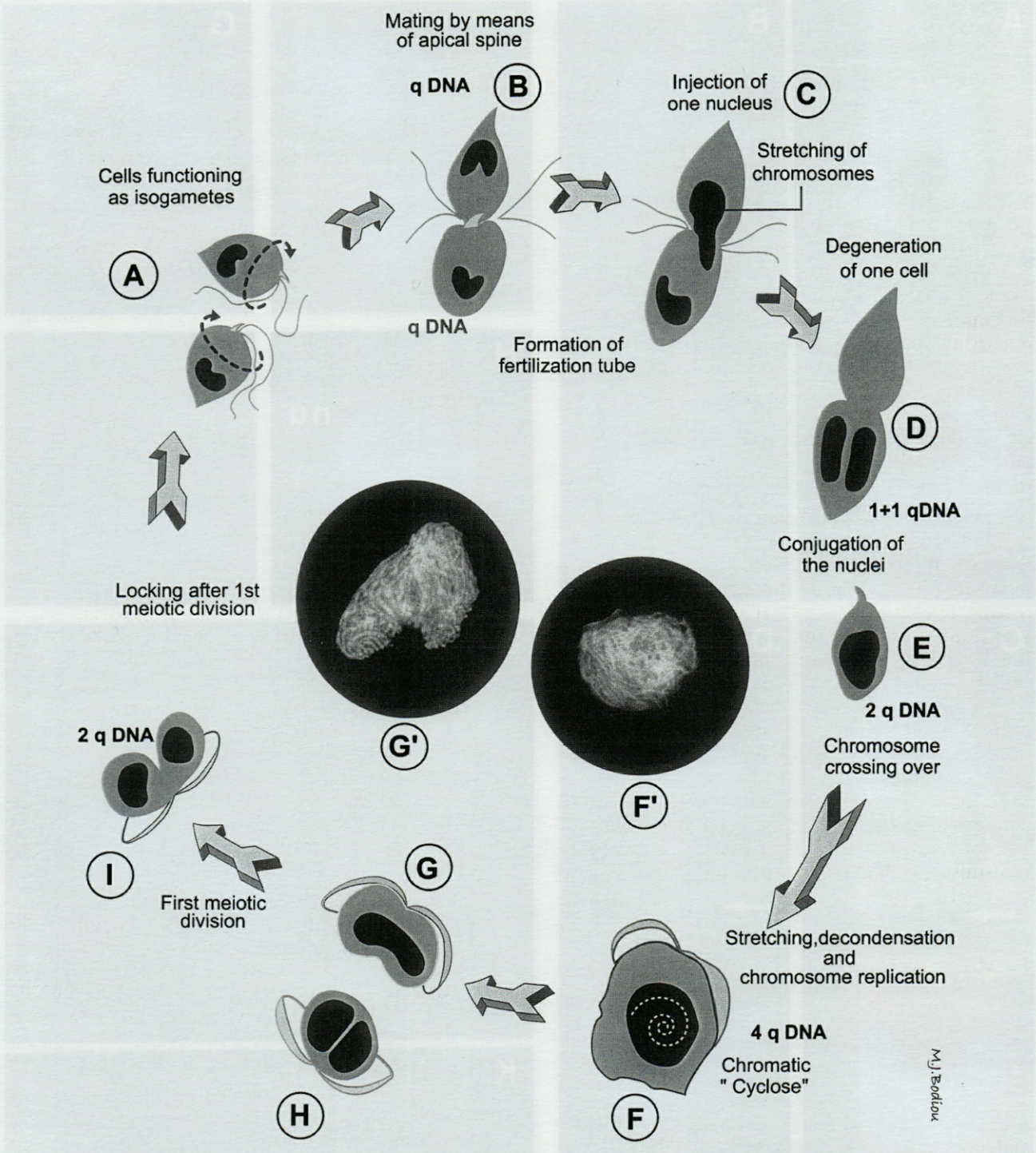


Fig. 3. – A-I. Diagram of the partial course of sexual reproduction phases of *Prorocentrum micans* from *in vivo* and TEM observations of the nuclei after exposure of the cultured cells to low temperature (4°C) and darkness. Vegetative cells functioning as isogametes (A) and containing q DNA (Bhaud *et al.* 1988) become paired by means of their respective apical spines (B), then the donor cell injects its nucleus with stretched chromosomes into the receiver cell (C). After the two nuclei conjugate (D) in the zygote containing 2q DNA (E), crossing over of the chromosomes occurs. Chromosomes become completely unwound, stretched and replicated. In the nucleus containing therefore 4q DNA (F) chromatin begins to spin round giving a round shape to the nucleus as shown on the photograph (F'). Only one meiotic division occurred (G, G', H) leading to an incomplete separation of the 2q DNA containing cells (I).

water medium was supplemented with mangrove sediment extract (Faust 1993). In *Prorocentrum micans*, induction of sexuality by changing the culture medium led to the complete process of meiosis, the second meiotic division leading to the formation of a tetrad in which nuclei contain q DNA (Bhaud *et al.* 1988). Addition of a cofactor such as high or low temperature was not necessary.

From 1883 and 1885, Pouchet was the first to observe the process of "cyclose nucléaire" in the nucleus of two marine dinoflagellates *Ceratium fusus* and *Ceratium tripos*. Next Biecheler (1935, 1938, 1952) brought an important contribution to the understanding of this phenomenon generally occurring just before prophase of dinomitosis in the six species of *Peridinium* studied by her. Later Von Stosch (1972, 1973) observed that nuclear cyclosis occurred during sexual life of *Gymnodinium excavatum*, *G. pseudopalustre* and *Woloszynskia apiculata*, specifically just before the meiotic prophase.

In this work, we observed *in vivo* stages of mating and sexual reproduction induced by poor conditions of culture, in this case, exposure to low temperature (4°C) without illumination. Pairing and mating followed by onset of meiosis occurred but was stopped after the first meiotic division. The behaviour of the mating couples and especially the role of the apical spine in mating as well as for the first time the rotation of the *Prorocentrum micans* chromatin preceding the first meiotic division were observed *in vivo* with a fluorescence light microscope coupled with an interference contrast system. Modifications of the chromosome structure and condensation during the mating phenomenon and part of meiosis were also observed by means of transmission electron microscopy.

B. MATERIAL AND METHODS

Culture of Prorocentrum micans cells: Procentrum micans Ehrenberg, 1834 (Order Prorocentrales) cells, originating from the Botany School of Cambridge, were routinely grown in Erd-Schreiber's medium (Bhaud *et al.* 1988) in an alterning 12h-light (2000 lux)/12h-dark cycle at 20°C. This autotrophic dinoflagellate has a large number of chromosomes (100, see Herzog & Soyer 1981) and a large DNA content (42 pg/nucleus, see Haapala & Soyer 1974). The vegetative cell cycle is rather long, 5 days, and discontinuous, with a typical eukaryotic interval of DNA synthesis (Bhaud & Soyer-Gobillard 1986).

Light microscopy: The observations were performed *in vivo* after penetration of 0.1 µg/ml DAPI (4-diamidino-2-phenyl indole) from SIGMA, which stains the whole DNA, either using epifluorescence with a 330-380 nm/LP418 nm filter alone or coupled with an interference contrast system on a Reichert Polyvar (Leica) photomicroscope.

Scanning electron microscopy: Specimens were collected from the culture medium by gentle centrifugation (1, 200 rev/min). The pellet was fixed in a phosphate-buffered (0.2M; pH7.0) 4 % glutaraldehyde solution for 30 min at 20°C, then dehydrated with acetone. Each step was preceded by a gentle centrifugation, so the peripheral mucopolysaccharidic coat was removed, allowing a better observation of the theca and especially of the apical structures.

Cells were then prepared with a critical-point drying method of liquid CO^2, coated with gold by sputtering and observed at 20 kV with a JEOL JSM 35 scanning electron microscope at the Center of Microscopy of Lausanne University (Fig. 1A, B).

Transmission electron microscopy: Fast Freeze Fixation (FFF): A drop with highly concentrated *Prorocentrum micans* cells was put on filter paper (10 mm^2) and mounted on a specimen holder. The sample was slammed onto a metal mirror block of pure copper cooled with liquid helium at –269°C on a cryovacublock (Reichert-Jung, Leica) and stored in liquid nitrogen prior to freeze substitution. Freeze substitution according to Nicolas (1991) was done in acetone and 2% OsO_4 in the presence of molecular sieves to absorb the water extracted from the sample and then carried out in a Cryocool apparatus (RUA) for 3 days at –80°C. The temperature was then gradually raised to –30°C and kept there for 2 h. The samples were thawed at room temperature for 1 h, washed successively in pure acetone, absolute ethanol, then propylene oxide and embedded in Epon. Ultrathin sections were cut and observed with an HITACHI H 7500 transmission electron microscope from the Laboratoire Arago, after staining with alcoholic uranyle acetate and post staining with lead citrate according to Reynolds (1963).

C. RESULTS

Cells of *Prorocentrum micans* measure between 50 and 100 µm, they are flattened laterally. The theca is formed of two lateral valves with numerous pores, separated by a longitudinal suture (Plate IA). The apex of the cell bears an apical spine and an apical notch in the vicinity of the two apical pores (Fig. IA, B), through which emerge the two flagella, one longitudinal and one transverse. The gentle centrifugation carried out during the preparation for scanning electron microscopy was responsible for the removal of the mucopolysaccaride epitheca which surrounds the theca (Soyer *et al.* 1982). The undulating "transverse" flagellum is fixed on this epitheca, whose removal allowed us to better observe the apical spine region, the apical notch and the pores.

Cultures of *Prorocentrum micans* cells routinely maintained in Erd-Schreiber medium were transferred from a culture chamber at 20°C to a refrigerator at 4°C and held there during several days without any illumination. Pairing of swimming cells were observed after 24 h in these unfavourable conditions. After 48 h, almost all the cells were

paired and we were able to observe mating and partial meiosis.

Pairing swimming cells (Fig. 2A), containing q DNA (Bhaud *et al.* 1988), began to turn towards each other, apical spine against apical spine (Fig. 2B, C). Often, one or two pairing cells presented a bright cytoplasmic autofluorescent red color (Fig. 2A, C, E). In all the cases, both nuclei were symetrically oriented at the distal part of the conjugating cells. When the apical spine of one of the pairing cells was well anchored in the second cell (Fig. 2D), they formed the fertilization tube (Fig. 2E) by which the donor cell injects its nucleus into the receiver cell (Fig. 2F) as previously described by Bhaud *et al.* (1988). Then two nuclei were present in the planozygote which contains 2q DNA (Fig. 2G). In the planozygote, replication of the chromatin occurred leading the cell to contain 4q DNA and was followed by a rapid clockwise rotation of the chromatin within the nuclear envelope (nuclear cyclosis). At this stage, chromatin is organized in thin and punctuated chromosomes (Fig. 2H, Fig. 3F'). Nucleoli were present at this stage and the nucleus contained 4q DNA (Bhaud *et al.* 1988) while the cytoplasm showed a bright autofluorescent red color. The outline of the nucleus appeared wavy. The first meiotic division occurred (Fig. 2I, J) but the separation of the two daughter cells remained incomplete (Fig. 2K) and under these conditions will never lead to the second meiotic division nor tetrad formation. This set of *in vivo* observations is schematised in Figure 3.

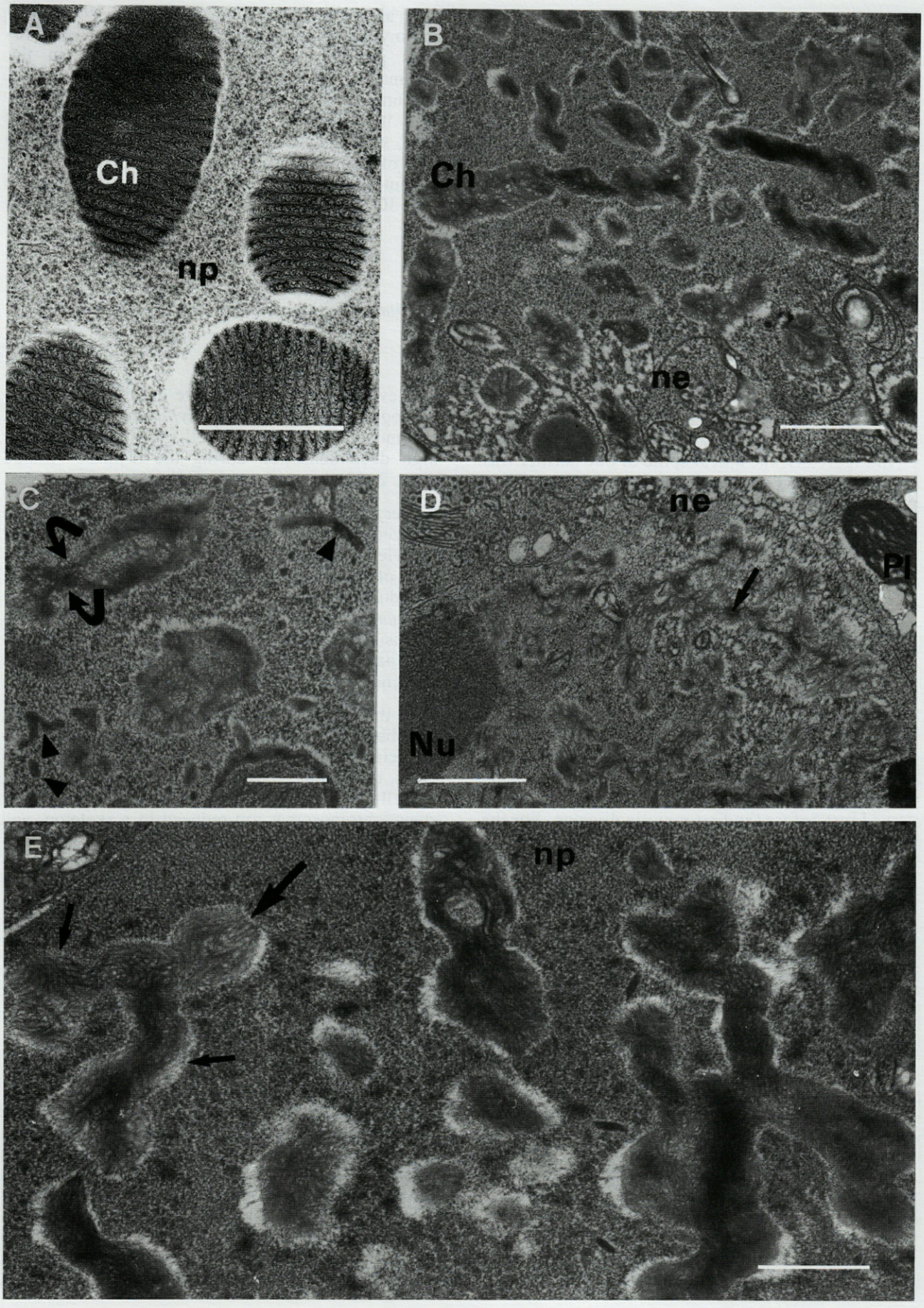
We have completed these data by selecting several cells at different stages of mating and meiosis and observing them in transmission electron microscopy after slam-freeze preparation of the cells (Fig. 4A-E). In the vegetative cells (Fig. 3A) here playing the role of isogametes (Bhaud *et al.* 1988), nuclei contained chromosomes showing the architecture usually described for dinoflagellates: a compacted right handed superhelix of nucleofilaments (Haapala & Soyer 1973), ultrathin sections observed in TEM presenting an arch-shaped organization (Fig. 4A). The mean chromosome diameter was about 1 µm. When the nucleus of the donor cell began to be injected in the receiver cell (Fig. 3C), the chromosomes were stretched, so their diameter decreased to a mean diameter ranging from 0.3 to 0.5 µm. Chromosomes were unwound and the arch-shaped structure disappeared (Fig. 4B). The nuclear envelope was always present but very indented (Fig. 4 B). During the conjugation of the two nuclei (see E in Fig. 3), figures of crossing over can be observed as well as the reduction of part of the chromatin into small pieces, widespread in the nucleoplasm (Fig. 4 C). When the replication was effected in the zygote, the rotation of the chromatin occurred and the nucleus was recognizable by its round shape and thin stretched chromosomes (Fig. 2H, Fig. 3F'). In TEM, chromosomes appeared as very stretched and showed essentially a more compact axial structure (Fig. 4D, arrow) surrounded by loops of chromatin. Their mean diameter was about 0.2 µm. The nucleolus was functional at this stage and the nuclear envelope was present. During the first meiotic division, chromosome nucleofilaments remained loose although showing a more compact organization; they still lacked the super-helical right handed organization and thus their arch-shaped aspect. Nucleofilaments were grouped in chromosomic masses which present splitting figures (Fig. 4E).

D. DISCUSSION AND GENERAL CONCLUSION

Induction of the sexuality by unfavourable ecological conditions

We have tried to use unfavourable cold temperature together with darkness and we observed the complete process of meiosis except for the second meiotic division. This new experiment allowed us to complete the description of the different stages of mating and meiosis in *P. micans*. Nevertheless we have benefited from our very careful measurements of the DNA contents at the different stages of meiosis given in our previous work (Bhaud *et al.* 1988). Von Stosch (1964, 1965) induced sexuality in *Ceratium cornutum* by decreasing temperature, day length and light intensity. For the same author (1973) gamete formation can also occur when cultures of *Gymnodinium pseudopalustre* and

Fig. 4. – A-E. TEM observations of nuclei of vegetative and pairing cells during mating. Note the modifications of the chromosome architecture at the different stages. A. In the nucleus of this vegetative cell in G1 phase (Fig. 3A), chromosomes (Ch) are compact and nucleofilaments arch-shaped. np. nucleoplasm. X 20,000 (scale bar = 1 µm) B. When the donor cell injected its nucleus into the receiver cell (Fig. 3, C), the injected nucleus presented stretched chromosomes (Ch) and a complete but indented nuclear envelope (ne). X 10,500 (scale bar= 2 µm) C. After the fusion of the two nuclei in the zygote (Fig. 3E), figures of crossing over were visible (arrows). Chromatin is also reduced to small fragments (arrowheads). X 14,700 (scale bar = 1 µm) D. During the rotation of the chromatin (Fig. 3F, F'), chromosomes are very stretched, keeping a visible axis (arrow) and showing multiple loops. The nucleolus (Nu) is present as well as plastids (Pl). X 10,500 (scale bar = 2 µm) E. During the first meiotic division (Fig. 3G), relaxed chromosomes with individualized nucleofilaments began to divide. Note the splitting of a chromosome (arrows). X 21,000 (scale bar = 1 µm).


A
Ch
np
B
Ch
ne
C
D
ne
Pl
Nu
E
np

Woloszynskia apiculata were submitted to a temperature variation from 21°C to 15°C. Then sexual process can occur. In the contrary, for Anderson (1985) studying *Gyrodinium uncatenatum,* two cofactors are necessary to release sexual process, nitrogen deficiency and unfavourable temperature. Pfiester (1989) concluded that temperature alone is not sufficient to induce sexuality directly but that the process is initiated first by nutrient depletion. In the case of *P. micans*, low temperature alone was sufficient to induce sexual process. These differences of reactions of dinoflagellate cells towards the environmental factors to induce sexuality seems highly specific, life cycles and way of life of these protists being extremely diverse, directly related with their physiology (autotrophy or not).

Role of the apical spine during mating

In Figure 2D, the apical spine of one partner was shown to be anchored in the other cell. We can suppose that the phenomenon is symmetrical but not visible on the opposite face. It is probable that each cell uses its apical spine to anchor itself in the apical notch of its partner during pairing and before the formation of the fertilization tube.

Fluorescence light microscopy: precise steps of mating

New data concern also the *in vivo* observation of the nucleus and chromatin in the 4q DNA zygote (Fig 2H, Fig 3H'). For the first time we were able to observe chromatin rotation in *Prorocentrum micans*. This nuclear cyclosis movement was observed just before the first meiotic division. For the first time, Commandon & Fonbrune (1935) (*in* Biecheler 1952), filmed rotation of nuclear chromatin in the hematoblasts of *Salamandra* immediately before their division. Biecheler (1952), observed this chromatin movement in six species of dinoflagellates from three different genera, both autotrophic (*Peridinium, Goniodoma*) and one heterotrophic (*Gyrodinium*). In *Peridinium sociale* she observed a slow rotation (about 2 µm/sec, estimated from the nucleolus position) accompanying the migration of the nucleus from the hypo- to epicone and preceding the vegetative division. In several cases, Biecheler (1952) also observed faster nuclear rotation, always in "bigger nuclei with punctuated chromatin" and asked the question what could be the relation between this phenomenon and sexuality. After our observations, we are able to say that these nuclei are those of zygotes (4q DNA), just before the first meiotic division. And that, of course, chromatic cyclosis is linked to sexual stages. Moreover, we recently described the presence of several motility proteins in the nucleus such as actin (Soyer-Gobillard *et al.* 1996), P80 and myosin (Ausseil *et al.* 1999, 2000). These proteins probably play an important role in the chromatin movement during cyclosis. However, the function of this chromatin rotation is yet uncertain; it clearly has no connection with crossing over, which was already completed, after the fusion of the nuclei of the isogametes (see Fig. 3D, E) but just before the first meiotic mitosis (see Fig. 3G, G', H).

Chromosome transformations during meiosis

Previous work in which we described the morphology and behaviour of *Crypthecodinium cohnii* chromosomes during the cell cycle revealed their high decondensation during the S-phase (Bhaud *et al.* 2000).

Very rare electron microscopic observations were realized during fertilization of dinoflagellates: at our knowledge, only Spector *et al.* 1981, described the state of chromatin during the injection of nucleus of the donor cell into the receiver, in *Peridinium cinctum*. They showed in their work that numerous extensions project from the gamete chromosomes and that chromosomes of the injected nucleus were stretched.

In the different phases of sexuality in *P. micans*, we observed the transformation of chromosome architecture: stretching during the injection of the nucleus, unwinding, loosening of the nucleofilaments, parcelling of chromatin. During the crossing over steps, chromosomes are reduced to a skeleton, and in the zygote (4q DNA), this axial skeleton is surrounded by numerous DNA loops different in structure from the chromosome extensions described by Spector *et al.* (1981) in *Peridinium cinctum*. During the first meiotic division, nucleofilaments are loose and not twisted. The difference of organization of nucleofilaments between the G1-phase cell (Fig. 4A) and all other meiotic nuclei is evident although the preparation of the cells (very fast cryopreparation in 2 nanoseconds) was identical. This technique allowed a good chromatin preservation. It is known that the dinoflagellate chromatin is devoid of histones and nucleosomes (Herzog & Soyer 1981) and that chromosomal architecture is maintained by divalent cations (Ca^{2+} and Mg^{2+}) (Herzog & Soyer 1983) and structural RNAs (Soyer & Herzog 1985). It is highly probable that divalent cations are chelated during meiosis and that specific RNAses intervene to unwind chromosomes, a necessary step allowing either the crossing over and/or the DNA replication in the zygote, before the first meiotic division.

E. CONCLUSION

In spite of blocking of development after the first meiotic division of *Prorocentrum micans*, we were able to observe the course of meiotic stages induced by cold temperature (4°C) and darkness, except the second meiotic division.

Darkness could be also an ultimate additional cofactor partially responsible of the blocking after the first meiotic division, the day-night cycle of this autotrophic cell having been interrupted during the several days of its stay at 4°C. So both these cofactors could represent a useful tool to block the dinoflagellate *P. micans* meiosis at the precise stage of end of first meiotic division (nuclei with 2q DNA), this could provide additional data to our knowledge of the dinoflagellate proliferation mechanism.

ACKNOWLEDGEMENTS. – We are very grateful to M J Bodiou for preparation of Fig. 3, to Dr T Jalanti who made SEM photographs of Fig. 1, and to D Boissé and S Boletzky for critical reading of the manuscript. We warmly acknowledge MA Faust (Smithsonian Institution, Washington) and M Sleigh (University of Southampton) for suggestions and positive review of the text. This work was supported by CNRS, UMR 7628 "Modèles en Biologie Cellulaire et Evolutive".

REFERENCES

Anderson DM, Coats DW, Tyler MA 1985. Encystement of the dinoflagellate *Gyrodinium uncatenum*; temperature and nutrient effects. *J Phycol* 21: 200-206.

Ausseil J, Soyer-Gobillard MO, Géraud ML, Bhaud Y, Baines I, Preston T, Moreau H 1999. Characterization of p80, a novel nuclear and cytoplasmic protein in Dinoflagellates. *Protist* 150: 197-211.

Ausseil J, Soyer-Gobillard MO, Géraud ML, Bhaud Y, Perret E, Barbier M, Albert M, Plaisance L, Moreau H 2000. Dinoflagellate centrosome: associated proteins old and new. *Europ J Protistol*: 36: 1-19.

Bhaud Y, Soyer-Gobillard MO 1986. DNA synthesis and cell cycle of a primitive Dinoflagellate *Prorocentrum micans* Ehr. *Protistologica* 22: 23-30.

Bhaud Y, Soyer-Gobillard MO, Salmon JM 1988. Transmission of gametic nuclei through a fertilization tube during mating in a primitive dinoflagellate, *Prorocentrum micans* Ehr. *J Cell Sci* 89: 197-206.

Bhaud Y, D Guillebault, JF Lennon, H Defacque, MO Soyer-Gobillard, Moreau H 2000. Morphology and behaviour of dinoflagellate chromosomes during the cell cycle and mitosis. *J Cell Sci* 113: 1231-1239.

Biecheler B 1935. Existence d'une cyclose chromatique chez les Péridiniens. *C R Acad Sci* Paris 201: 503-505.

Biecheler B 1938. La cyclose chromatique des Péridiniens est un stade (prophase-métaphase) de leur division. *C R Acad Sci* Paris 207: 1067-1070.

Biecheler B 1952. Recherches sur les Péridiniens. *Bull Biol Fr et Belg* Suppl 36: 1-149.

Chatton E, Biecheler B 1934. Les Coccidinidae, Dinoflagellés, coccidiomorphes parasites de Dinoflagellés et le phylum des Phytodinozoa. *C R Acad Sci* Paris 199: 252-255.

Faust MA 1992. Observations on the morphology and sexual reproduction of *Coolia monotis* (Dinophyceae). *J Phycol* 28: 94-104.

Faust MA 1993. Sexuality in a toxic Dinoflagellate, *Prorocentrum lima*. *In* Toxic Phytoplankton Blooms in the Sea. TJ Smayda & Y Shimizu editors, Elsevier Science Publi B.V.: 121-126.

Haapala OK, Soyer MO 1973. Structure of Dinoflagellate chromosomes. *Nature* 244: 195-197.

Haapala OK, Soyer MO 1974. Absence of longitudinal differenciation of dinoflagellate (*Prorocentrum micans*) chromosomes. *Hereditas* 78: 141-145.

Herzog M, Soyer MO 1981. Distinctive features of dinoflagellate chromatin. Absence of nucleosomes in a primitive species *Prorocentrum micans* E. *Eur J Cell Biol* 23: 295-302.

Herzog M, Soyer MO 1983. The native structure of dinoflagellate chromosomes and their stabilization by Ca^{2+} and Mg^{2+} cations. *Eur J Cell Biol* 30: 33-41.

Nicolas G 1991. Advantage of fast-freeze fixation followed by freeze-substitution for preservation of cell integrity. *J Electron Microsc Techn* 8: 395-405.

Pfiester LA 1989. Dinoflagellate sexuality. *Internat Rev Cytol* 114: 249-272.

Pouchet G 1883. Contribution à l'étude des Cilio-flagellés. *Jour Anat Physiol* 19: 399-455.

Pouchet G 1885. Nouvelle contribution à l'histoire des Péridiniens marins. *Journ Anat Physiol* 21: 28-88.

Reynolds ES 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J Cell Biol* 17: 208.

Soyer-Gobillard MO, Ausseil J, Géraud ML 1996. Presence of nuclear and cytoplasmic actin in Dinoflagellate protists: immunocytochemical and biochemical study. *Biol Cell* 87: 17-35.

Soyer MO, Herzog M 1985. The native structure of dinoflagellate chromosomes. Involvement of structural RNA. *Eur J Cell Biol* 36 (2): 334-342.

Soyer MO, Prevot P, De Billy F, Jalanti T, Flach F, Gautier A 1982. *Prorocentrum micans* E., one of the most primitive dinoflagellates: I. The complex flagellar apparatus as seen in scanning and transmission electron microscopy. *Protistologica* 18: 289-298.

Soyer-Gobillard MO, Moreau H 2000. Dinoflagellates. Encyclopedia of Microbiology, vol 2, 2nd ed, Academic Press, San Diego: 42-54.

Spector DL, Pfiester LA, Triemer RE 1981. Ultrastructure of the dinoflagellate *Peridinium cinctum* F. *ovoplanum*. II. Light and electron microscopic observations on fertilization. *Amer J Bot* 68 (1): 34-43.

Von Stosch HA 1972. La signification cytologique de la "cyclose nucléaire" dans le cycle de vie des Dinoflagellés. *Soc Bot Fr Mém*: 201-212.

Von Stosch HA 1973. Observations on vegetative reproduction and sexual life cycles of two freshwater dinoflagellates, *Gymnodinium pseudopalustre* Schiller and *Woloszynskia apiculata* sp. nov. *Br phycol J* 8: 105-134.

Reçu le 18 avril 2002; received April 18, 2002
Accepté le 16 juillet 2002; accepted July 16, 2002

VIE MILIEU, 2002, 52 (4) : 177-187

From Marine Ecology to Developmental Biology
In Honour of Pierre Tardent (1927-1997)

TRICHOPLAX ADHAERENS: DISCOVERED AS A MISSING LINK, FORGOTTEN AS A HYDROZOAN, RE-DISCOVERED AS A KEY TO METAZOAN EVOLUTION

T. SYED[1,3], *B. SCHIERWATER*[1,2]

[1]*ITZ, Ecology and Evolution, Tierärztliche Hochschule Hannover, 30559 Hannover, Germany*
[2]*Dept. of Molecular Cellular and Developmental Biology, Yale University, 165 Prospect St., New Haven, CT 06520 U.S.A.*
[3]*Present address: Forschungs-Institut und Naturmuseum Senckenberg (Sektion vergleichende Anatomie und Histologie), Senckenberganlage 25, 60325 Frankfurt, Germany*

Corresponding author: bernd.schierwater@ecolevol.de

TRICHOPLAX ADHAERENS
PLACOZOA
METAZOAN EVOLUTION
DIPLOBLASTS
PLACULA HYPOTHESIS
BILATEROGASTRAEA HYPOTHESIS
GALLERTOID HYPOTHESIS

ABSTRACT. – *Trichoplax adhaerens* is more simply organized than any living metazoan. After its original description by FE Schulze 1883, it attracted particular attention because it possibly possessed the basic and ancestral state of metazoan organization. The interest of zoologists and evolutionary biologists suddenly vanished for more than half a century when *Trichoplax* was claimed to be an aberrant hydrozoan planula larva. Recently, *Trichoplax* has been rediscovered as a key species for unraveling early metazoan evolution. Hox genes and whole genome sequencing promise insights into the genetics underlying the origin and development of basal metazoan phyla. We here review the history of research on *Trichoplax*, and provide a modern interpretation of special *Trichoplax* features in an evolutionary context.

TRICHOPLAX ADHAERENS
PLACOZOAIRES
ÉVOLUTION DES MÉTAZOAIRES
DIPLOBLASTES
HYPOTHÈSE DE LA PLACULA
HYPOTHÈSE DE LA BILATEROGASTRAEA
HYPOTHÈSE DES GALLERTOIDES

RÉSUMÉ. – L'organisation de *Trichoplax adhaerens* est plus simple que celle des autres métazoaires vivants. Décrit par F E Schulze en 1883, cet organisme attirait l'attention des scientifiques qui pensaient d'abord que son organisation pourrait représenter un état basal et ancestral pour les métazoaires. Mais par la suite les zoologistes et les biologistes de l'évolution délaissèrent ce modèle, et durant plus d'un demi-siècle, *Trichoplax* passa pour une larve planula aberrante d'un Hydrozoaire. Récemment cet organisme a été redécouvert en tant qu'espèce clé pour la compréhension de l'évolution précoce des métazoaires. Les gènes Hox et le séquençage complet du génome devraient permettre d'aborder la génétique sous-jacente qui pourrait élucider l'origine et le développement des grands embranchements de métazoaires. Nous passons en revue l'histoire des recherches consacrées à *Trichoplax* et proposons une interprétation moderne des caractères particuliers de *Trichoplax* placés dans un contexte évolutif.

I. The discovery of Trichoplax adhaerens – *Schulze's original description and Bütschli's "Placula-Hypothesis"*

In 1883, the German zoologist Franz Eilhard Schulze published as a short communication the description of a new species, *Trichoplax adhaerens* (the "sticky hairy plate"; Greek *trich*=hair, *plax*=plate): a flattened, crawling marine animal of up to a few millimeters in size (Fig. 1). Schulze found these organisms settling on the glass sides of seawater aquaria at the University of Graz (Austria), recognizing their amoeba-like movements and continual shape changes. These were new features for metazoan animals.

Schulze's histological analysis of *Trichoplax,* based on microtome sections and various staining procedures, revealed a three-layered sandwich organization of the animal, with morphologically different upper and lower epithelia. The epithelia enclose an inner, connective-tissue-like union of cells. A more detailed description was published by Schulze in 1891, and most of his results are still valid today (Fig. 2). The upper epithelium consists of a thin squamous layer while the lower epithelium consists of relatively large columnar cells and smaller gland cells. The lower epithelium is spe-

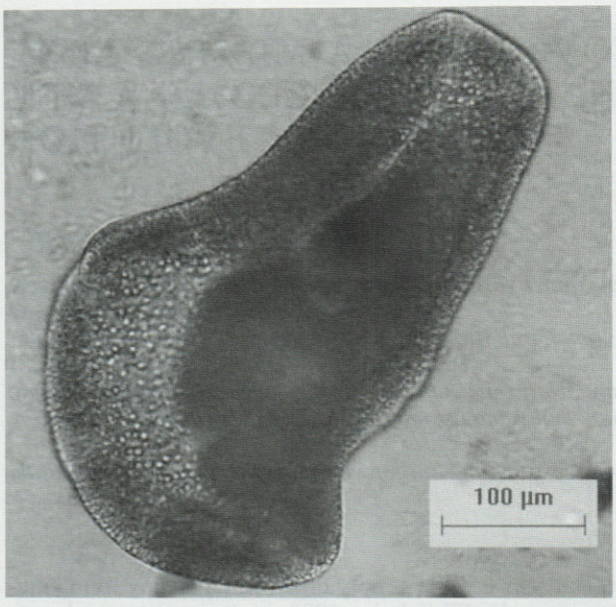


Fig. 1. – Photograph of living specimen of *Trichoplax adhaerens*. The animal shown is changing from an infolded (upper and right side) to its preferred flattened form (left side). The "shiny spheres" of the upper epithelium (cf. Fig. 2) are mainly visible on the left, where the body is already flattened.

cialized for extracellular digestion (the animal creeps over small food particles). The only obvious specialized structures of the upper epithelium are large lipid droplets, which were named "Glanzkugeln" ("shiny spheres") by Schulze (1891). Probably, the latter are degenerate cells that serve as a nutritive reservoir (but see also below, IV). Apparently degenerate cells are regularly found also in the lower epithelium. Cells of both epithelia are monociliated; the animal moves by ciliary walking. The interior cells are connected by cytoplasmatic extensions and form a three-dimensional meshwork. Schulze (1883, 1891) already noted that contractions of these cells cause the shape-changes of the animal. He named these contractile cells "Faserzellen" ("fibre cells").

Since only four somatic cell types are found in *Trichoplax*, and since noncellular structures such as a basal lamina and extracellular matrix (=ECM) are lacking, the animal is the most simply organized metazoan animal known. Moreover, because of the non-fixed outer shape, no axes of symmetry are present, and only a top-bottom polarity is seen (Fig. 2). Summarizing his first results, Schulze (1883) concluded that *Trichoplax* does not fit into any of the bauplan patterns of sponges, coelenterates (ctenophores and cnidarians), or the

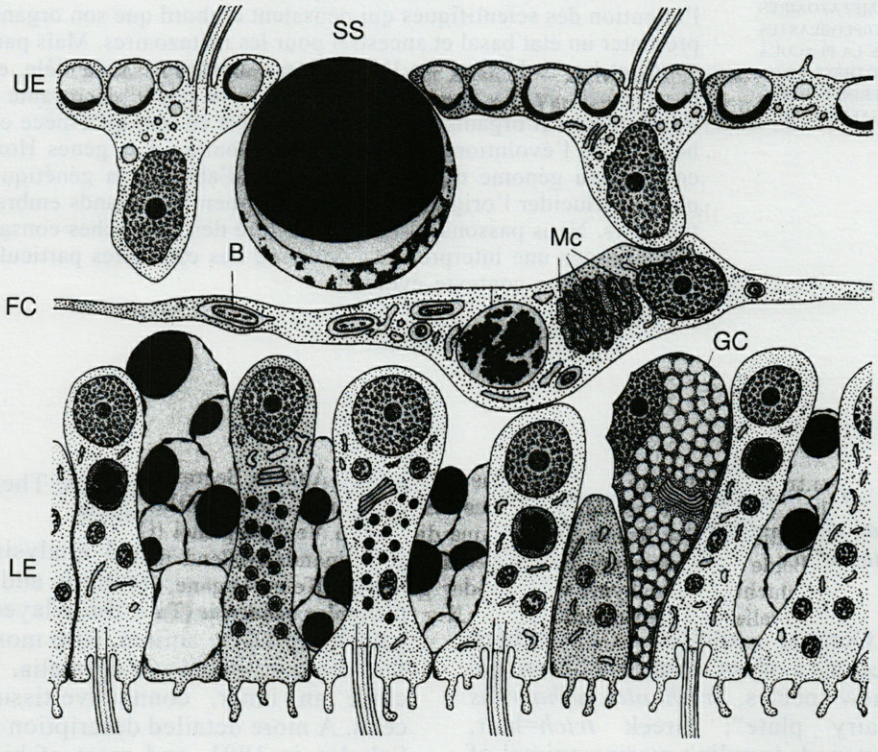


Fig. 2. – Schematic cross section of *Trichoplax adhaerens* (modified after Grell & Ruthmann 1991). UE = upper epithelium, LE = lower epithelium, FC = contractile fibre cell, GC = gland cell, SS = shiny sphere, Mc = mitochondrial complex, B = (endosymbiotic?) bacterium in endoplasmic reticulum. Note that the interspace between fibre cells and epithelia is free of ECM and that a basal lamina is missing.

vermiform phyla. Consequently, he assumed that *Trichoplax* was an isolated, basal offshoot close to the root of the metazoan phylogenetic tree.

Schulze's original description of *Trichoplax* soon sparked debate on the hypothetical first metazoan ("urmetazoa" or "archimetazoa") between Haeckel, Lankester, Metschnikoff, and other zoologists (for overview see: Gruner 1993). Only one year after Schulze's original description of *Trichoplax adhaerens*, O. Bütschli (University of Heidelberg) published an improved version of Haeckel's "gastraea-hypothesis". While Haeckel's "gastraea", a hypothetical spherical, pelagic organism, invaginates from a pelagic "blastaea" at its posterior pole, Bütschli (1884) tried to derive the gastraea from a flat, benthic-vagile ancestor, the hypothetical "placula" (cf. Fig. 3). According to Bütschli, the first metazoans emerged after colonial flagellates (Protozoa) fused into a benthic, single layered organism with ciliary locomotion. From this stage, the two-layered "placula" developed with an upper "ectoderm" and a lower "entoderm". Gradual invagination of the "entodermal" layer led to a benthic gastraea-like animal. The entodermal invagination finally led to closed gastric cavities or through-guts, as was already described in Haeckel's model.

Bütschli argued that the three-layered *Trichoplax* is a comparatively derived organism, still mirroring the two-layered placula's mode of life. It is important to note that both Schulze and Bütschli agreed in interpreting the upper epithelium of *Trichoplax* as an ectoderm and the lower epithelium as an entoderm homolog. The question then arises whether the interior fibre cell complex of *Trichoplax* is a mesoderm homolog or not. Both authors hesitated to interpret the fibre cells as a mesoderm homolog because this would have implied a close affinity of *Trichoplax* to the triploblastic phyla. Bütschli (1884, p. 425) therefore saw in the fibre cell layer an analogy to mesodermal structures of triploblasts. Schulze (1883) pointed out that observations on the ontogeny of *Trichoplax* would be required to solve this question. Both researchers were aware of the principal counter-hypothesis that *Trichoplax* might be a secondarily simplified organism, as this was already proposed for some parasitic mesozoans. The latter alternative was regarded as relatively unlikely as there was no evidence for parasitism by *Trichoplax*. After the first morphological descriptions and resulting phylogenetic interpretations it was expected that elucidating the ontogeny and the life cycle of *Trichoplax adhaerens* would be the next crucial step in resolving the phylogenetic position of the Placozoa.

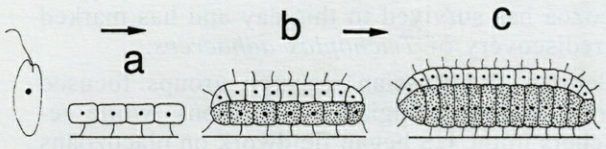


Fig. 3. – Placula-hypothesis of metazoan evolution according to O Bütschli 1884. Flagellated protozoans unite to form a benthic-vagile, plate-like metazoan organism. The one-layered form (a) evolves to the two-layered "placula" (b). Upper cells of the placula are presumed ectoderm, while the lower cells adapt to nutritive function and thus are presumed entoderm (c). Note that there is no gradual transformation from stage a to b (modified from Gruner 1993).

II. The hydrozoan-interpretation – Trichoplax *is forgotten*

In 1890, one year before Schulze's detailed *Trichoplax* description, FC Noll from the Senckenberg Museum in Frankfurt reported observations on the animals' normal mode of vegetative reproduction, which is binary fission of the entire body into two new individuals. Noll wrongly suggested the presence of otoliths in large *Trichoplax* specimens and thus a close relationship to the acoel turbellarians. This idea was supported by L von Graff (1891), an expert on Acoela. Schulze (1891) remained skeptical about this interpretation, mainly because of the arrangement of the inner contractile cells of the animal (which do not resemble a myoepithelium), and also because of the lack of any fixed axes of symmetry. At this time, most zoologists agreed with Schulze (1883) that the functional layer of contractile cells rejects a close relationship to either coelenterates or sponges. Through the end of the 19th century a close relationship of *Trichoplax* to acoel turbellarians or mesozoans was discussed. After FS Monticelli (1893, 1896) described another *Trichoplax*-like animal, *Treptoplax reptans*, both forms were united as Mesenchyma, in reference to the fibre cells, and grouped within the Mesozoa (Delage & Herouard 1899), a phylum that had already become "a dumping ground for a host of multicellular but presumed nonmetazoan organisms" (Brusca & Brusca 1990). True metazoan phyla were seen as showing an invaginating gastrula stage during embryogenesis (e.g., Neresheimer 1912), although this definition had been intensely debated from the very beginning.

The question of the complete life cycle of *Trichoplax* initially yielded a most surprising -- and completely wrong -- answer when the German zoologist Thilo Krumbach observed these animals in a seawater aquarium that was settled by sexual medusae of the hydrozoan *Eleutheria krohni* (Krumbach 1907). As in medusae of other *Eleutheria* species, for example *E. dichotoma*, the eggs of *E. krohni* develop in a brood pouch, which eventually opens to release well-developed planula larvae (Hauenschild 1956, Schierwater 1989,

Hadrys *et al.* 1990). Krumbach (1907) reported that he found *Trichoplax* individuals in exactly those positions where *Eleutheria* planulae had settled before. Krumbach was convinced that *Trichoplax adhaerens* was a deformed larva of *Eleutheria krohni*, although he never observed the supposed metamorphosis. Surely Krumbach's interpretations were influenced by speculations of other authors, who thought of *Trichoplax* as a "paranormal" organism that was unable to complete its life cycle under culture conditions (e.g., Ehlers 1887: 497). Although refused by Schulze (1891), this interpretation persisted in zoological textbooks of that time (e.g., Lankester 1901: 158). Krumbach's 1907 publication led to a corresponding statement on *Trichoplax* in the first installment of Bütschli's "Vorlesungen über vergleichende Anatomie" (1910) and was cited as fact in a reference book (Neresheimer 1912: 827). However, the hydrozoan interpretation was soon criticized by Schubotz (1912) and Schulze (1914). Schubotz compared the histological organization of *Eleutheria krohni* planulae and *Trichoplax*. He noted that the ectoderm of the planula already contains nematocysts, which would have to vanish during any transformation into a *Trichoplax*. Schulze completed Schubotz's argumentation by mentioning some special features of the inner fibre cell layer of *Trichoplax*. Note that Schulze's article of 1914 was the last publication on *Trichoplax* in a zoological journal for more than half a century.

How can an exciting animal like *Trichoplax* be pushed out of scientific research by a shaky larva-hypothesis ? It is an amazing fact that the completely unsupported larva-hypothesis remained in German, French, and Anglo-American textbooks for decades. After World War One, the first German encyclopedia on animal phyla was prepared by the meritorious zoologist W. Kuekenthal, who died one year before the first volumes were published in 1923 (also, F E Schulze died in 1921). The editor who finished the volumes was Thilo Krumbach, a supporter of the larva-hypothesis. Through his hands, volume one of the "Handbuch der Zoologie" contained the Protozoa, Porifera, Coelenterata, and Mesozoa. *Trichoplax* is briefly mentioned in the chapter "Hydroida" (H Broch, Oslo) and the chapter "Mesozoa" (M Hartmann, Berlin-Dahlem). Both authors interpreted *Trichoplax* as a transformed planula of *Eleutheria krohni*. Hartmann (p. 1014) cites Krumbach's paper from 1907 but ignores the replies of Schubotz (1912) and Schulze (1914). It was through this single, pivotal circumstance that Krumbach's larva-hypothesis became widely accepted. In her influential "Invertebrates", LH Hyman (1940) also cites only Krumbach's paper (p. 247) and ignores the other data: "... *Trichoplax* and *Treptoplax*, which have the construction of planulae, were found actually to be modified planulae of Hydroidea" (p. 243). PP Grassé's "Traité de zoologie IV" (1961: 694) mentions *Trichoplax* in a similar way.

III. The rediscovery of Trichoplax adhaerens *and birth of the phylum "Placozoa"*

It is often said that *Trichoplax adhaerens* was rediscovered when the German protozoologist KG Grell (University of Tübingen) found this animal in an algal sample from the Red Sea in 1969. Although it is true that the first electron-microscopical examinations by Grell were decisive for the final falsification of Krumbach's larva-hypothesis, at this time the animal had already found its way back into science.

In July 1961, the cell biologist W Kuhl (University of Frankfurt) found *Trichoplax* in a seawater aquarium containing organisms from the Mediterranean Sea. Although Kuhl's research on *Trichoplax* concentrated on locomotion and regeneration, he and co-workers clearly stated that they had never observed any connection between *Trichoplax* and hydrozoans in the aquarium (Kuhl & Kuhl 1966: 433). At about the same time, *Trichoplax* was also cultured in Moscow, where it inspired Russian researchers to reinforce E Metschnikoff's phagocytella-hypothesis of metazoan evolution (Ivanov 1968, cited in Ivanov 1973, 1988).

When Grell (1971b, 1972, Grell & Benwitz 1974) discovered oogenesis and cleavage processes (after mixing *Trichoplax* clones from different locations) it became clear that *Trichoplax* specimens in culture represent an adult stage. Unfortunately, the embryos regularly died after reaching the 64-cell-stage (cf. Ruthmann *et al.* 1981, Grell 1984), and the further development of *Trichoplax* remains unknown. However, Grell's meticulous research provided sufficient support for placing *Trichoplax adhaerens* in a new phylum, the "Placozoa" (Grell 1971a). The new phylum was named after Bütschli's placula hypothesis, and consequently the Russian researchers around AV Ivanov responded by proposing a phylum "Phagocytellozoa" for *Trichoplax* (Ivanov 1973). Grell's phylum Placozoa has survived to this day and has marked the rediscovery of *Trichoplax adhaerens*.

German and Russian research groups focused mainly on morphological descriptions, while researchers in the US began fieldwork on placozoans (close to nothing was known about the biology of *Trichoplax* in its natural habitat). It quickly became clear that *Trichoplax adhaerens* could be found worldwide in the littoral of subtropical and tropical regions (e.g. Pearse 1989). *Treptoplax reptans* Monticelli 1893 (see II.) has never been found again, and its existence can be doubted.

Detailed electron-microscopical studies by the groups around KG Grell in Tübingen and

A Ruthmann in Bochum confirmed and extended Schulze's (1883, 1891) classical descriptions of *Trichoplax*. No basal lamina could be found, and the interspace between the fibre cells and epithelia was found to be free of any collagenous ECM (e.g. Grell & Benwitz 1971, 1981). For the fibre cells, a syncytial (Buchholz & Ruthmann 1995) and wide-meshed organization (instead of a compact mass; Stiasny 1903) was described. Interestingly, von Graff (1891) and Stiasny (1903) had described unicellular algae in the cell bodies of the fibre cells and interpreted them as symbiotic or commensal zooxanthelles. Wenderoth (1986) found that algae and other food particles adhere to the slime layer of the upper epithelium and are subsequently phagocytized by the inner fibre cells. Food particles must be pulled through gaps of the upper epithelium, and Wenderoth (1986) called this unique mode of feeding "transepithelial cytophagy". Thus, the incorporated algae are prey. However, there may also be endosymbionts present in *Trichoplax*, as bacteria were regularly found in the endoplasmic reticulum of the fibre cells (Grell & Benwitz 1971).

The ability for transepithelial cytophagy indicates a relatively loose arrangement of the epithelia.

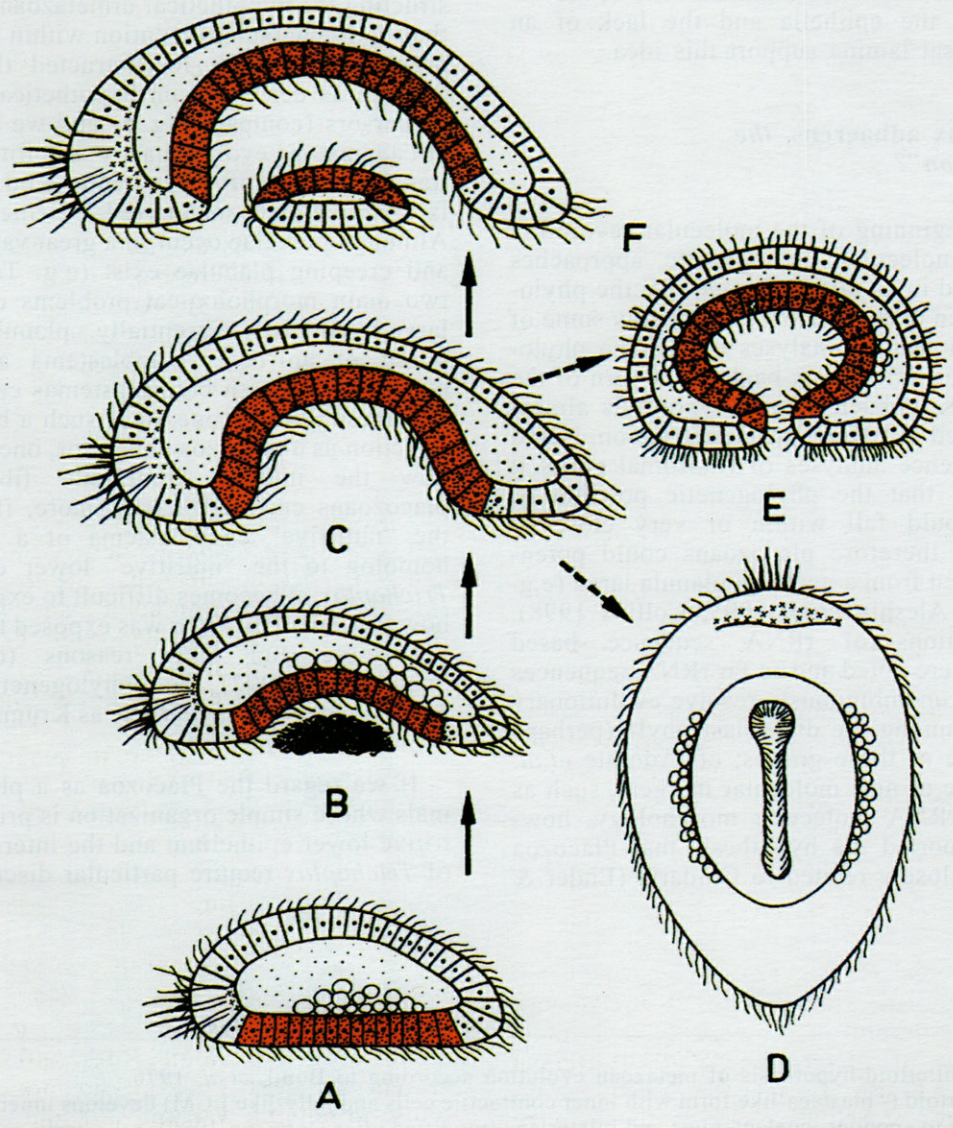


Fig. 4. – Bilaterogastraea-hypothesis of metazoan evolution according to G Jägersten 1955/59: A "benthoblastaea"-stage gives rise to the "bilaterogastraea" with a through-gut. A-benthoblastaea showing polarity, specialized lower epithelium and gonads, B- digesting benthoblastaea, C, E -benthoblastea changes to bilaterogastraea by developing an inner gastric cavity (D: transverse section showing "oral slit"), F: a through-gut develops by partial closing of the "oral slit". Note that the vagile benthoblastaea had already developed an A/P-axis and bilateral symmetry, inferring that a placozoan-construction without such axes of symmetry would have to be derived from an earlier stage. In the given scenario, the presumed entoderm of the benthoblastaea (orange color) would be homologous to the nutritive lower epithelium of *Trichoplax*.

Only two types of epithelial cell-cell connections are present in *Trichoplax*, belt and septate desmosomes (Ruthmann *et al.* 1986, Ruthmann 2000). Connections between the epithelia and the fibre cells remain unknown. It seems likely that these connections get continually rearranged. Studies on isolated fibre cells (Thiemann & Ruthmann 1989) revealed their ability to build up cytoplasmatic extensions by microtubuli-assembly. Those extensions are probably mediated by an actinomyosin system (Ruthmann 2000). Since isolated fibre cells live for hours in seawater, Grell & Ruthmann (1991) suggest that the interspace between epithelia and fibre cells may not be very different from the seawater medium. The loose arrangement of the epithelia and the lack of an underlying basal lamina support this idea.

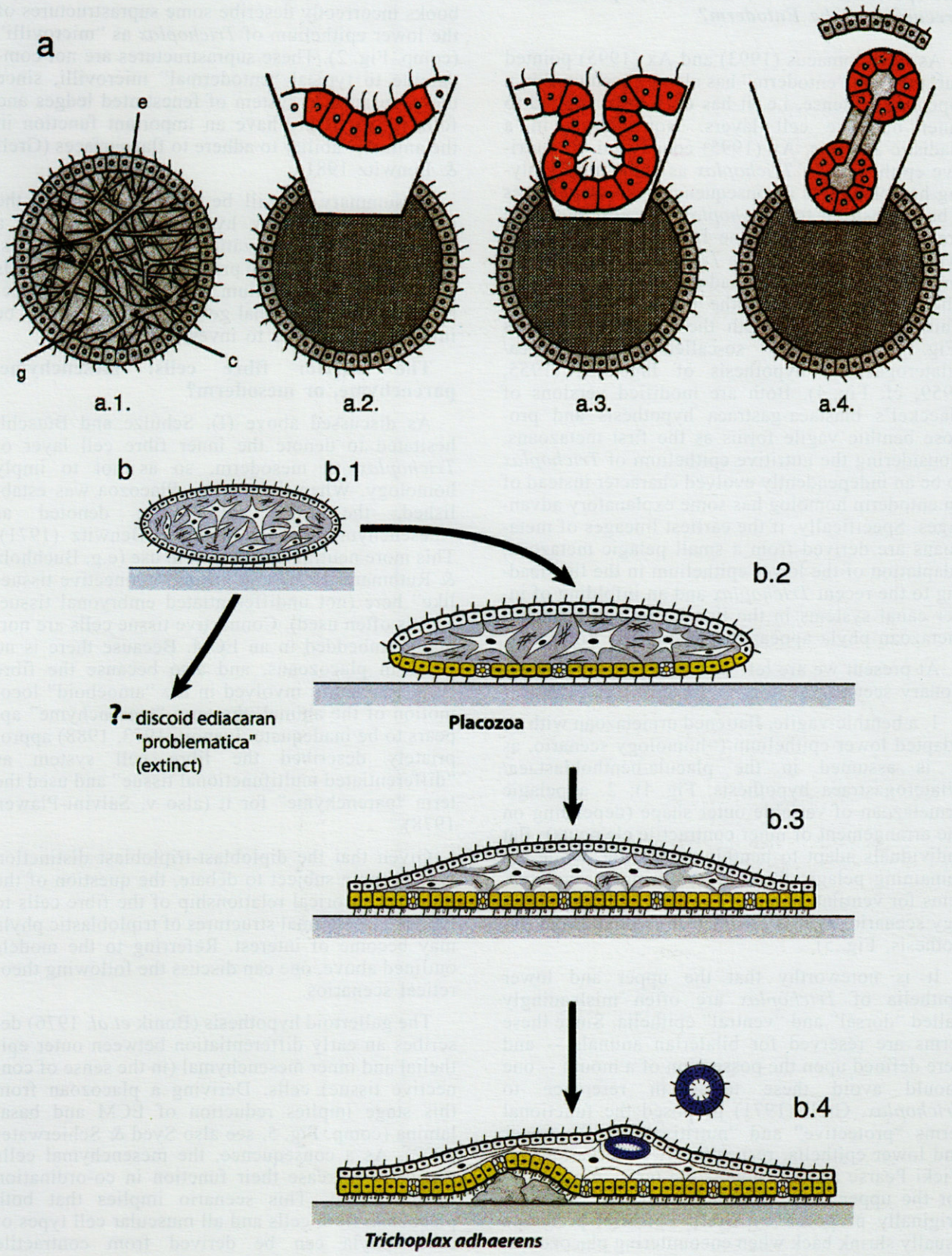
IV. Trichoplax adhaerens, *the "Archimetazoon"?*

With the beginning of the molecular revolution in biology, molecular phylogenetic approaches have been used in an attempt to unravel the phylogenetic position of the Placozoa. Ironically some of the modern molecular analyses moved the phylogenetic view on *Trichoplax* back to the turn of the century, and Krumbach's larva hypothesis almost was rejuvenated in a "phylogenetic" version. Based on DNA sequence analyses of ribosomal genes it was proposed that the phylogenetic position of *Trichoplax* could fall within or very close to Cnidaria, and therefore placozoans could potentially be derived from a neotenic planula larva (e.g. Bridge 1994, Aleshin *et al.* 1995, Collins 1998). Later, limitations of rRNA sequence based phylogenies were noted and so far rRNA sequences have failed to unambiguously resolve evolutionary relationships among the diploblast phyla (perhaps due to the age of these groups; cf. Adoutte *et al.* 2000). The use of new molecular markers, such as mtDNA and rRNA molecular morphology, however, has supported the hypothesis that Placozoa are not very closely related to Cnidaria (Ender & Schierwater 2002). For example, the secondary structure of the 16S rRNA molecule is substantially more complex in *Trichoplax* than what is known in any cnidarian. At present, molecular systematics has not resolved the issue of the phylogenetic position of the Placozoa. The sum of evidence available to date suggests a basal position for *Trichoplax* within Metazoa (e.g. Schierwater & DeSalle 2001, Syed & Schierwater 2002, Ender & Schierwater 2002). Resolving this issue is clearly of key importance to our understanding of the origin of the Metazoa.

Identification of the earliest diverging lineage of metazoans will be of decisive value for reconstructing the hypothetical urmetazoan bauplan, and thus early character evolution within Metazoa. In a previous paper we reconstructed the placozoan bauplan as derived from hypothetical "gallertoid" precursors (compare Fig. 5) and we argued in detail against the evolutionary possibility of a gradual transformation of a neotenic cnidarian planula larva into a placozoan (Syed & Schierwater 2002). Although planulae occur in a great variety of forms and creeping planulae exist (e.g. Tardent 1978), two main morphological problems contradict the larva-hypothesis. Essentially, planulae are composed of an outer ectoblastema and an inner endoblastema with both blastemas connected by a thin mesogloea. If one takes such a bilayered construction as a placozoan ancestor, one must explain how the interior contractile fibre cells of placozoans emerged. Furthermore, if one regards the "nutritive" endoblastema of a planula as a homolog to the "nutritive" lower epithelium of *Trichoplax*, it becomes difficult to explain why and how the inner cell layer was exposed to the outside. For these and other reasons (cf. Syed & Schierwater 2002) the "phylogenetic" larva-hypothesis seems as unrealistic as Krumbach's (1907) "ontogenetic" precursor.

If we regard the Placozoa as a phylum of animals whose simple organization is primary, the nutritive lower epithelium and the interior fibre cells of *Trichoplax* require particular discussion.

Fig. 5a, b. – Gallertoid-hypothesis of metazoan evolution according to Bonik *et al.* 1976.
a) pelagic gallertoid (=blastaea-like form with inner contractile cells and jelly-like ECM) develops inner canal systems, later giving rise to sponges, coelenterates and bilaterians (modified after Gutmann 1989). a.1.- early gallertoid (e=epithelium, c=contractile cell, g=gelatinous ECM), a.2., a.3.- infolding of grooves and canals, a.4.- superficial canals can be displaced into the interior of the body.
b) flattened, benthic gallertoids give rise to placozoan constructions, and probably some of the Ediacaran "problematica" (modified from Syed & Schierwater 2002). b.1. - flattened, benthic gallertoid b.2., b.3.- specialization of the lower epithelium and reduction of ECM. b.4. - recent *Trichoplax adhaerens* showing different (optimized) modes of locomotion, nutrition and reproduction. Note that the nutritive epithelium of *Trichoplax* (yellow color) evolves independently from the presumed entoderm of the other phyla (red color). Swarmers are shown in blue. Details can be found in Syed & Schierwater 2002.


a
e
g
c
a.1.
a.2.
a.3.
a.4.
b
b.1
b.2
?- discoid ediacaran
"problematica"
(extinct)
Placozoa
b.3
b.4
Trichoplax adhaerens

The specialized lower ("nutritive") epithelium: Precursor of the Entoderm?

As Bartolomaeus (1993) and Ax (1995) pointed out, the term "entoderm" has always been used in a topographic sense, i.e. it has only been applied to inner nutritive cell layers. Nonetheless, in a cladistic analysis, Ax (1995) considered the nutritive epithelium of *Trichoplax* as entoderm, implying homology. As a consequence – if one assumes a basal position for *Trichoplax*, the possibility we are addressing here – one faces the hypothetical evolutionary scenario of a *Trichoplax*-like, benthic animal, which gradually had its lower, nutritive epithelium displaced into the interior of the body. This is the case in both the placula hypothesis (Fig. 3) and the so-called benthoblastea/ bilaterogastraea hypothesis of Jägersten (1955, 1959; cf. Fig. 4). Both are modified versions of Haeckel's blastaea-gastraea hypothesis and propose benthic vagile forms as the first metazoans. Considering the nutritive epithelium of *Trichoplax* to be an independently evolved character instead of an entoderm homolog has some explanatory advantages. Specifically, if the earliest lineages of metazoans are derived from a small pelagic metazoan, adaptation of the lower epithelium in the line leading to the recent *Trichoplax* and an infolding of inner canal systems in the line leading to the other metazoan phyla appear reasonable (cf. Fig. 5).

At present we are left with two plausible evolutionary scenarios:

1. a benthic-vagile, flattened urmetazoan with an adapted lower epithelium (=homology scenario, as it is assumed in the placula/benthoblastaea/ bilaterogastraea hypothesis, Fig. 4); 2. a pelagic urmetazoan of variable outer shape (depending on the arrangement of inner contractile elements): flat individuals adapt to benthic vagile life, while the remaining pelagic forms develop inner canal systems for ventilation and filtration/digesting (=analogy scenario, as it is assumed in the gallertoid hypothesis, Fig. 5).

It is noteworthy that the upper and lower epithelia of *Trichoplax* are often misleadingly called "dorsal" and "ventral" epithelia. Since these terms are reserved for bilaterian animals -- and here defined upon the possession of a mouth -- one should avoid these terms in reference to *Trichoplax*. Grell (1971) proposed the functional terms "protective" and "nutritive" for the upper and lower epithelia, respectively. Observations by Vicki Pearse and us support the term "protective" for the upper layer in a more concrete way than originally proposed by Grell. Potential predators usually shrink back when encountering placozoans, perhaps because the shiny spheres (cf. Figs 1, 2) serve as reservoirs of chemical defense substances (V Pearse, pers comm). A biochemical analysis of the shiny spheres would be desirable.

It should further be mentioned that some textbooks incorrectly describe some suprastructures of the lower epithelium of *Trichoplax* as "microvilli" (comp. Fig. 2). These suprastructures are not comparable to typical "entodermal" microvilli, since they consist of a system of fenestrated ledges and folds which likely have an important function in the animals' ability to adhere to flat surfaces (Grell & Benwitz 1981).

In summary, it will be difficult to verify the homology or analogy hypothesis. Potentially, a biochemical characterization of digestive enzymes, which are thought to be produced by the gland cells of the nutritive epithelium, and a molecular characterization of entodermal gene expression would be informative features to investigate.

The interior fibre cells: mesenchyme, parenchyme, or mesoderm?

As discussed above (I), Schulze and Bütschli hesitated to denote the inner fibre cell layer of *Trichoplax* as mesoderm, so as not to imply homology. When the phylum Placozoa was established, the fibre cells were denoted as "mesenchyme-like" by Grell & Benwitz (1971). This more neutral term is still in use (e.g. Buchholz & Ruthmann 1995) and means "connective tissue-like" here (not undifferentiated embryonal tissue, as it is often used). Connective tissue cells are normally embedded in an ECM. Because there is no ECM in placozoans, and also because the fibre cells are clearly involved in the "amoeboid" locomotion of the animal, the term "mesenchyme" appears to be inadequate. Ivanov (1973, 1988) appropriately described the fibre cell system as "differentiated multifunctional tissue" and used the term "parenchyme" for it (also v. Salvini-Plawen 1978).

Given that the diploblast-triploblast distinction may become subject to debate, the question of the putative historical relationship of the fibre cells to typical mesodermal structures of triploblastic phyla may become of interest. Referring to the models outlined above, one can discuss the following theoretical scenarios.

The gallertoid hypothesis (Bonik *et al.* 1976) describes an early differentiation between outer epithelial and inner mesenchymal (in the sense of connective tissue) cells. Deriving a placozoan from this stage implies reduction of ECM and basal lamina (comp. Fig. 5, see also Syed & Schierwater 2002). As a consequence, the mesenchymal cells gradually increase their function in co-ordination of movement. This scenario implies that both placozoan fibre cells and all muscular cell types of other phyla can be derived from contractile mesenchymal cells of early pelagic gallertoids. According to this model both lines diverge very early, which – together with biomechanical reasons – could explain why placozoan fibre cells are diffi-

cult to compare to other muscular cell types or mesodermal tissues, as they are known from more derived phyla.

Because 19th century models of early metazoan evolution mainly focused on the ectoderm-entoderm specialization, they offer less precise statements about connective tissue-like elements. Therefore, Haeckel's classical blastaea-gastraea model was often criticized from a biomechanical point of view, as it is hard to imagine how gradual invagination and forming of inner canals could be stabilized in a hollow sphere (e.g., Bonik *et al.* 1978). Jägersten (1959: 99), whose bilaterogastraea-hypothesis was based on Haeckel's view, addressed this problem by modifying the early benthoblastaea-stages: "It is quite conceivable, and nothing prevents it, that the entire blastocoel was filled with a mesogloea-like substance, containing also somatic cells that had immigrated from the blastoderm. In other words, it is possible that a kind of mesoderm existed even prior to the evolution of the entoderm and the intestine." Thus, Jägersten (1959: 100) describes the term "mesoderm" as follows: "It is now obvious that the mesoderm is not a uniform germ layer in the same sense as the ectoderm and the entoderm. The very fact that it is formed exclusively mesenchymatically in several recent groups, but mainly the circumstance that this way of formation ought to be considered as the original in the metazoans, prevents its interpretation as a strict germ layer." To some extent, this view fits modern definitions of the mesoderm, as they are given by Bartolomaeus (1993) and Ax (1995).

Moreover, it seems clear that the gallertoid-hypothesis and the bilaterogastraea-hypothesis describe nearly the same type of earliest metazoan, from which the Placozoa would have to be derived. The assumption of Jägersten (1955) and Bonik *et al.* (1978) that a mesogloea-like connective tissue served as precursor of the later mesoderm may find support in current molecular studies. Homologs of triploblast mesodermal transcription factors were found to be expressed in the entocodon (an interconnecting structure) of a hydrozoan (Spring *et al.* 2000).

V. Two models of metazoan evolution

At the time when Jägersten (1955, 1959) outlined his bilaterogastraea-model of early metazoan evolution, *Trichoplax* was still assumed to be an aberrant hydrozoan planula and therefore not considered as a distinct phylum. Thus, after Grell established the phylum Placozoa in 1971, the bilaterogastraea-hypothesis was modified by some authors. While Jägersten (1955) derived his bilaterogastraea from a "benthoblastaea" (comp. Fig. 4), Grell (1971) proposed a placula sensu Bütschli (Fig. 3) as precursor of the bilaterogastraea. Today such modified versions of Jägersten's bilaterogastraea-hypothesis occur in several textbooks (e.g. Siewing 1987, Erben 1990, Ax 1995, Ruthmann 2000). However, there are some critical points in this scenario to note. First, Bütschli's (1884) original model of a one-layered organism that develops a second layer – resulting in precursors of the ecto- and entoderm – looks dubious, because this scenario hides a phylogenetic saltation. The two-layered stage cannot emerge in a gradual way (comp. Fig. 3). Bütschli did not mention this problem. He notes, however, that there is no plausible selective advantage for the newly developed placula.

Bütschli regarded the three-layered *Trichoplax* as derived from the two-layered placula. This is another critical point of the placula-hypothesis, because it is not straightforward how the interior fibre cells could have gradually emerged from a two-layered construction. It might be helpful in this regard to examine the hollow amphiblastula of some sponges, which flatten out after settlement (Gruner 1993). Also, the behavior of *Trichoplax adhaerens* may provide insight on this question. Starving placozoans change from their normal flattened shape to a spherical form, as the interior fibre cells degenerate and lose contact with the epithelia (personal observations). These starving forms are not able to regenerate and soon die (see also Thiemann & Ruthmann 1990). Thus, Jägersten's (1959) improved description of the benthoblastaea (as cited in IV) and the "gallertoid" as proposed by Bonik *et al.* (1976) are among the preferable models of early metazoans.

When comparing the gallertoid-hypothesis with Jägersten's benthoblastaea-bilaterogastraea scenario, there is one important difference regarding the evolution of placozoans. In Jägersten's model, the early benthic metazoans develop an anterior-posterior polarity before transforming to the bilaterogastraea-stage (cf. Fig. 4). Since *Trichoplax* does not exhibit any indication of an A/P polarity, we prefer the alternative shown in Fig. 5. According to this view, Placozoa would be the oldest extant metazoan group, probably a sister group of some enigmatic discoid "Vendobionta" as they are known from about 600 million year old strata (comp. Fig. 5 and Syed & Schierwater 2002).

VI. Current Research and Conclusions

Trichoplax attracts the attention of modern multi-disciplinary research for at least three good reasons. First, it is the most simply organized metazoan animal; second, it possesses the smallest genome of all known metazoans (Ruthmann & Wenderoth 1975, Ruthmann 1977, Ruthmann *et al.* 1981); and third, it might be relatively basal to all recent metazoan phyla (see above). Thus understanding the genetic control of its development will

redefine the basic, and possibly also the ancestral genetic programming of metazoan organization. With excitement we await the answers to how many genes control the development of a basic metazoan bauplan, how big these genes are, how different they are compared to protists, how regulatory genes in *Trichoplax* have switched or gained new functions in derived animals, and how these genes interact. For example, at present it seems that *Trichoplax* possesses a single Hox gene only (Schierwater & Kuhn 1998), which is substantially smaller and more simply structured than other Hox genes (Schierwater & DeSalle 2001), and which in sharp contrast to those of higher animals has no function with respect to the formation of body polarity (Jakob *et al.* in prep; see also Schierwater & DeSalle 2001). Furthermore, the deduced amino acid sequence of the *Trichoplax* Hox gene, *Trox-2*, looks like a genetic chimera that harbors diagnostic domains from several families of Hox genes (Kuhn *et al.* 1999, Schierwater & DeSalle 2002). At the same time we study the genetics, and particularly the developmental genetics, we need to unravel the complete life cycle, describe the morphological changes during development and reproduction in more detail, and finally resolve the phylogenetic position of *Trichoplax* and the relationships among the diploblastic animals. The answer to the latter seems close, the complete mtDNA genome of *Trichoplax* has been sequenced (Stephen Dellaporta, Bernd Schierwater, and co-workers), and comparative mtDNA genomes from sponges, hydrozoans, scyphozoans, cubozoans, and ctenophores are being sequenced now. At present it is probable that *Trichoplax* is not very closely related to the cnidarians (Ender & Schierwater 2002) and that only the Placozoa or Porifera qualify as candidates for the basal position within recent diploblasts.

Some key answers to the above questions are expected soon, since expression and functional studies on regulatory genes and whole genome sequencing of the *Trichoplax* genome are in progress (*Trichoplax* Consortium 2002). Soon the no-longer hydrozoan *Trichoplax adhaerens* might become what the hydrozoan *Hydra* has been, one of the most promising diploblastic model organisms for development and evolution (Tardent 1988).

ACKNOWLEDGEMENTS. – We are grateful for the very helpful reviews from Vicki Pearse and an anonymous referee, and to Allen Collins for comments and assistance with revision. We also acknowledge support from the German Science Foundation (DFG Schi 277/10-2) and the Human Frontier Science Program (HFSP RGP0221/2001-M).

LITERATURE

Adoutte A, Balavoine G, Lartillot N *et al.* 2000. The new animal phylogeny. Reliability and implications. *Proc Nat Am Sc* 97 (9): 4453-4456.

Aleshin VV, Vladychenskaya NS, Kedrova OS *et al.* 1995. Phylogeny of invertebrates deduced from 18S rRNA comparisons. *Molecular Biology* 29 (6): 843-855.

Ax P 1995. Das System der Metazoa I. Gustav Fischer, Jena-New York.

Bartolomaeus T 1993. Die Leibeshöhlenverhältnisse und Nephridialorgane der Bilateria-Ultrastruktur, Entwicklung und Evolution. Habilitationsschr Univ Göttingen.

Behrendt G, Ruthmann A 1986. The cytoskeleton of the fiber cells of *Trichoplax adhaerens* (Placozoa). *Zoomorphology* 106: 123-130.

Bonik K, Grasshoff M, Gutmann WF 1976. Die Evolution der Tierkonstruktionen I. *Natur und Museum* 106: 129-143.

Bonik K, Grasshoff M, Gutmann WF 1978. Warum die Gastraea-Theorie Haeckels abgelöst werden muß. *Natur und Museum* 108 (4): 106-117.

Bridge DM 1994. Phylogeny and life cycle evolution in the phylum Cnidaria. PHD-Thesis, Yale University.

Brusca RC, Brusca GJ 1990. Invertebrates. Sinauer Associates, Sunderland, Massachusetts.

Buchholz K, Ruthmann A 1995. The mesenchyme-like layer of the fibre cells of *Trichoplax adhaerens*: A syncytium. *Z Naturforsch* 50c: 282-285.

Bütschli O 1884. Bemerkungen zur Gastraea-Theorie. *Morph Jahrb* 9: 415-427.

Bütschli O 1910. Vorlesungen über vergleichende Anatomie. 1. Lieferung: Einleitung; vergleichende Anatomie der Protozoen, Integument und Skelet der Metazoen. W Engelmann, Leipzig.

Collins AG 1998. Evaluating multiple alternative hypotheses for the origin of Bilateria: An analysis of 18SrRNA molecular evidence. *Proc Nat Acad Sci USA* 95: 15458-15463.

Delage Y, Herouard E 1899. Traité de Zoologie Concrète II: Classe Mesenchymiens-Mesenchymia. Masson, Paris, 1: 9-12.

Ehlers E 1887. Zur Auffassung des *Polyparium ambulans* (Korotneff). *Zeitschr f wiss Zool* 45: 491-498.

Ender A, Schierwater B 2002. Placozoa are not derived cnidarians: Evidence from molecular morphology. *Mol Biol Evol* (in press).

Erben HK 1990. Evolution. Enke, Stuttgart.

Graff L v 1891. Die Organisation der Turbellaria acoela. W Engelmann, Leipzig.

Grassé PP 1961. Traité de Zoologie IV: Plathelminthes, Mésozoaires, Acantocéphales, Némertiens. Masson, Paris.

Grell KG 1971a. *Trichoplax adhaerens* und die Entstehung der Metazoen. *Naturw Rundsch* 24 (4): 160-161.

Grell KG 1971b. Embryonalentwicklung bei *Trichoplax adhaerens* F.E. Schulze. *Naturwiss* 58: 570.

Grell KG 1972. Eibildung und Furchung von *Trichoplax adhaerens* F.E. Schulze (Placozoa). *Z Morph Tiere* 73: 297-314.

Grell KG 1984. Reproduction of Placozoa. *Adv Invertebr Reprod* 3: 541-546.

Grell KG, Benwitz G 1971. Die Ultrastruktur von *Trichoplax adhaerens* F.E. Schulze. *Cytobiologie* 4: 216-240.

Grell KG, Benwitz G 1974. Elektronenmikroskopische Beobachtungen über das Wachstum der Eizelle und die Bildung der "Befruchtungsmembran" von *Trichoplax adhaerens* F.E. Schulze (Placozoa). *Z Morph Tiere* 79: 295-310.

Grell KG, Benwitz G 1981. Ergänzende Untersuchungen zur Ultrastruktur von *Trichoplax adhaerens* F.E. Schulze (Placozoa). *Zoomorphology* 98: 47-67.

Grell KG, Ruthmann A 1991. Placozoa. *In* Microscopic Anatomy of Invertebrates. Edited by FW Harrison & JA Westfall, Wiley-Liss, New York: 13-28.

Gruner HE 1993. Einführung, Protozoa, Placozoa, Porifera. *In* Lehrbuch der speziellen Zoologie Band I. Edited by A Kaestner, Fischer, Jena, 1.

Gutmann WF 1989. Die Evolution hydraulischer Konstruktionen. Waldemar Kramer, Frankfurt.

Hadrys H, Schierwater B, Mrowka W 1990. The feeding behaviour of a semi-sessile hydromedusa and how it is affected by the mode of reproduction. *Anim Behav* 40: 935-944.

Hauenschild C 1956. Experimentelle Untersuchungen über die Entstehung asexueller Klone bei der Hydromeduse *Eleutheria dichotoma*. *Z Naturforsch* 11b: 394-402.

Hyman LH 1940. The invertebrates. Protozoa through Ctenophora. Mc Graw Hill, New York.

Ivanov AV 1973. *Trichoplax adhaerens*, a phagocytella-like animal. *Zoologiceskij Zurnal 52*: 1117-1131 (Russian with English abstract).

Ivanov AV 1988. On the early evolution of the Bilateria. *Fortschr Zool* 36: 349-352.

Jägersten G 1955. On the early evolution of the metazoa. The Bilaterogastraea theory. *Zoologiska Bidrag* 30: 321-354.

Jägersten G 1959. Further remarks on the early phylogeny of metazoa. *Zoologiska Bidrag* 33: 79-108.

Krumbach T 1907. *Trichoplax*, die umgewandelte Planula einer Hydromeduse. *Zool Anz* 31: 450-454.

Kuhl W, Kuhl G 1966. Untersuchungen über das Bewegungsverhalten von *Trichoplax adhaerens* F.E. Schulze. *Zeitschr Ökolog Morph Tiere* 56: 417-435.

Kuhn K, Streit B, Schierwater B 1999. Isolation of Hox genes from the Scyphozoan *Cassiopeia xamachana*: Implications for the early evolution of Hox genes. *J Exp Zool (Mol Dev Evol)* 285: 63-75.

Lankester ER 1901. A treatise on Zoology Part IV: Platyhelmia, Mesozoa and Nemertini. A & C Black, London.

Monticelli FS 1893. *Treptoplax reptans* n.g., n.sp. *Atti dell' Academia dei Lincei, Rendiconti* (5)II: 39-40.

Monticelli FS 1896. Adelotacta Zoologica. *Mittheil zoolog Station Neapel* 12: 432-462.

Neresheimer E 1912. Mesozoen. *In* Handwörterbuch der Naturwissenschaften. Edited by E Korschelt *et al.* Gustav Fischer, Jena, Band VI: 817-829.

Noll FC 1890. Über das Leben niederer Seetiere. *Ber Senckenb Naturforsch Gesellsch (Abt. Berichte)*: 85-87.

Pearse V 1989. Growth and Behaviour of *Trichoplax adhaerens*: First Record of the Phylum Placozoa in Hawaii. *Pacific Science* 43 (2): 117-121.

Ruthmann A 1977. Cell differentiation, DNA content and chromosomes of *Trichoplax adhaerens* F.E. Schulze. *Cytobiologie* 10: 58-64.

Ruthmann A 2000. Evolution und die Vielfalt des Lebens. Shaker, Aachen.

Ruthmann A, Behrendt G, Wahl R 1986. The ventral epithelium of *Trichoplax adhaerens* (Placozoa). *Zoomorphology* 106: 115-122.

Ruthmann A, Grell KG, Benwitz G 1981. DNA-content and fragmentation of the egg-nucleus of *Trichoplax adhaerens*. *Z Naturforsch* 60: 564-567.

Ruthmann A, Wenderoth H 1975. Der DNA-Gehalt des primitiven Metazoons *Trichoplax adhaerens* F.E. Schulze. *Cytobiologie* 10: 421-431.

Salvini-Plawen L v 1978. On the origin and evolution of the lower Metazoa. *Z zool Syst Evolut Forsch* 16: 40-88.

Schierwater B 1989. Allometric changes during growth and reproduction in *Eleutheria dichotoma* (Hydrozoa, Athecata) and the problem of estimating body size in a microscopic animal. *J Morphol* 200: 255-267.

Schierwater B, Kuhn K 1998. Homology of Hox genes and the zootype concept of early metazoan evolution. *Mol Phyl Evol* 9 (3): 375-381.

Schierwater B, Dellaporta S, DeSalle R 2002. Is the evolution of *Cnox-2* Hox/ParaHox genes multicolored and polygenealogical? *Mol Phyl Evol* 24 (3): 374-378.

Schierwater B, DeSalle R 2001. Current Problems with the Invention of the Zootype. *J Exp Zool (Mol Dev Evol)* 291: 169-174.

Schubotz H 1912. Ist *Trichoplax* die umgewandelte Planula einer Hydromeduse? *Zool Anz* 39: 582-585.

Schulze FE 1883. *Trichoplax adhaerens* nov. gen. nov. spec. *Zool Anz* 6: 92-97.

Schulze FE 1891. Über *Trichoplax adhaerens*. *Physik Abh Kgl Akad Wiss Berlin:* 1-23.

Schulze FE 1914. Einige kritische Bemerkungen zu neueren Mitteilungen über *Trichoplax*. *Zool Anz* 64 (1): 33-35.

Siewing R 1987. Evolution. G Fischer, Stuttgart.

Spring J, Yanze N, Middel AM *et al.* 2000. The mesoderm specification factor Twist in the life cycle of jellyfish. *Developmental Biology* 228: 363-375.

Stiasny G 1903. Einige histologische Details über *Trichoplax adhaerens*. *Zeitschr wiss Zool* 75: 430-436.

Syed T, Schierwater B 2002. The Evolution of the Placozoa: A new morphological model. *Senckenbergiana lethaea* 82 (1): 315-324.

Tardent P 1978. Coelenterata, Cnidaria. *In* Morphogenese der Tiere, I Reihe, Edited by F Seidel, Gustav Fischer, Stuttgart: 83-289.

Tardent P 1988. Hydra. Neujahrblatt der Naturforschenden Gesellschaft in Zürich. NGZH, Zürich.

Thiemann M, Ruthmann A 1989. Microfilaments and microtubules in isolated fibre cells of *Trichoplax adhaerens* (Placozoa). *Zoomorphology* 109: 89-96.

Thiemann M, Ruthmann A 1990. Spherical forms of *Trichoplax adhaerens*. *Zoomorphology* 110: 37-45.

Wenderoth H 1986. Transepithelial cytophagy by *Trichoplax adhaerens* F.E.Schulze (Placozoa) feeding on yeast. *Z Naturforsch* 41c: 343-347.

Reçu le 9 juillet 2002; received July 9, 2002
Accepté le 27 août 2002; accepted August 27, 2002

VIE MILIEU, 2002, 52 (4) : 189-199

From Marine Ecology to Developmental Biology
In Honour of Pierre Tardent (1927-1997)

STASIS, CHANGE, AND FUNCTIONAL CONSTRAINT IN THE EVOLUTION OF ANIMAL BODY PLANS, WHATEVER THEY MAY BE

G.A. WRAY[1], *R.R. STRATHMANN*[2]

[1]*Department of Biology, Box 90338, Duke University, Durham, NC 27708-033, USA*
[2]*Friday Harbor Laboratories and Department of Zoology, University of Washington, 620 University Road, Friday Harbor, WA 98250, USA*

gwray@duke.edu rrstrath@u.washington.edu

BODY PLAN
BAUPLAN
DEVELOPMENT
FUNCTION
CONSTRAINT
EVOLUTION

ABSTRACT. – The phrase "body plan" or "bauplan" has been used to mean (1) the characteristic features of a phylum or other taxon of high rank, (2) architectural features of animals (such as symmetry; modular units; types of body walls, body cavities, body openings, and body subdivisions; types of supporting structures; position and structure of organ systems), (3) traits characteristic of an especially invariant stage in a life history (phylotypic stage), or (4) patterns of gene expression that first indicate the development of regions of the body. Multiple meanings of bodyplan within one argument can be misleading, but under all four meanings, body plans of animals have changed after stasis for long periods and after stasis during divergence of other traits. Change in body plans is often associated with an identifiable change in a functional constraint. Examples include decreases in body size and changes in requirements for feeding or locomotion. These observations support the hypothesis that functional constraints contribute to stasis in body plans. There is evidence that ancestral developmental processes constrain directions of evolutionary changes in body plans. There is little evidence that developmental processes prevent changes in body plans, but evidence for developmental constraint is more difficult to obtain than evidence for functional constraint.

PLAN DU CORPS
"BAUPLAN"
DÉVELOPPEMENT
FONCTION
CONTRAINTE
ÉVOLUTION

RÉSUMÉ. – L'expression "plan du corps" ou "plan de construction [bauplan]" a été employée dans le passé pour désigner (1) les caractéristiques d'un phylum ou d'un taxon de rang élevé, (2) les caractères architecturaux des animaux (exemples: la symétrie; les unités modulaires; les types de tégument, de cavités du corps, d'ouvertures du corps, et de subdivisions du corps; les types de structures de renforcement; la position et la structure des systèmes d'organes), (3) les propriétés d'un stade ontogénétique particulièrement stable (stade phylotypique), ou encore (4) des motifs d'expression de gènes qui indiquent déjà le développement des différentes régions du corps. Des significations multiples de « plan du corps » à l'intérieur d'une même argumentation peuvent générer des erreurs, mais dans toutes les conditions évoquées de (1) à (4), les plans du corps des animaux ont été modifiés après de longues périodes de stase et après des stases accompagnées d'une divergence au niveau d'autres traits. Une modification du plan du corps est souvent liée à un changement identifiable dans le contexte d'une contrainte fonctionnelle. Parmi les exemples, on peut citer la réduction de taille du corps et les modifications des conditions requises pour l'alimentation et la locomotion. Ces observations confortent l'hypothèse selon laquelle les contraintes fonctionnelles contribuent à la stase d'un plan du corps. Il y a de très bons indices montrant que les processus ancestraux du développement sont contraignants quant aux directions possibles des changements évolutifs de plans du corps. Il y a très peu d'indices pour que les processus du développement empêchent tout changement de plan du corps, mais les preuves d'une contrainte du développement sont plus difficiles à trouver que les indices de contraintes fonctionnelles.

INTRODUCTION

The potential for confusion from multiple meanings of "body plan"

The term body plan (often used as a synonym of ground plan and *bauplan*) has been used with quite different meanings. The use of "body plan" in studies of evolutionary stasis and change can produce mistaken inferences when disparate meanings are mixed within an argument. As an example, stasis in body plans of animals has been inferred from the fossil record because first appearances of taxa of high rank are early in the Phanerozoic fossil record rather than scattered through the Phanerozoic. Animal phyla with fossilization potential appear as fossils in the Cambrian and not subsequently. Similarly, many of the animal classes are known from the Cambrian or Ordovician. If all members of a phylum or class share the same body plan, then about 500 million years of stasis in body plans is implied. This apparent stasis has prompted the question: Why have no new body plans originated since the Cambrian ? The answer depends in part on the definitions of body plan that are used and how they are combined. Not all members of a phylum or class have the traits that are used to characterize that group. They are classified with it because of evidence that they belong to the same clade. If the traits of animals rather than the traits that are used to characterize clades as taxa are considered, then the answer is that new body plans have originated since the Cambrian.

The straw-man premise for the question "Why have there been no new body plans ?" was flawed, but it must be admitted that many ancestral traits characterizing animal phyla and classes have been retained for more than 500 million years. Thus some traits do exhibit remarkable stasis. These observations prompt questions that are not so easily dismissed. Why do body plans remain unchanged over such long periods ? Given such extended stasis, what enables them to change ? Such questions are being answered by functional and developmental biologists, but to interpret the answers one must consider the differing uses of the term "body plan."

Diverse meanings of "body plan"

1. The body plan often means the distinguishing morphological characteristics of a phylum or class. Under this definition, it would not be surprising that the traits that comprise a body plan are ancient and conservative. In so far as the hierarchical arrangement of taxa represents a sequence of evolutionary divergences, the traits that characterize higher taxa must have originated early. To be useful as diagnostic characters for descendants within the clade, the traits must change rarely. For more than two centuries zoologists have been searching for these ancient and conservative traits and redefining the animal phyla, and they are not done yet (Nielsen *et al.* 1996). Higher level systematics of animals has been an enduring source of controversy, and many zoologists are hoping that molecular evidence will help resolve questions about homology and homoplasy of traits that have been used to characterize taxa of high rank. Thus, even under this definition of body plan, one can find aspects of body plans that have changed in descendant lineages. Some of the traits characterizing a phylum vary within the phylum. We recognize the animals as members of a phylum or class because some indication of relationship remains. The observation that there are no new phyla since the Cambrian does not imply that there are no new body plans. It only implies that the animals with good potential for fossilization that originated later can be assigned to one of the clades known from the Cambrian. Similar observations apply to animal classes, with few new ones since the Ordovician, and to other ancient animal clades given high taxonomic rank. Nevertheless, there does appear to be a phenomenon to be explained. Many traits have remained unchanged for the majority of animals in a phylum or class. Groups characterized by these traits, if possessing readily fossilized parts, can now be traced as separate clades back to the Ordovician or Cambrian. For this meaning of body plan, observations of stasis and change in body plans are observations on traits that are considered to be persistent synapomorphies (shared derived characters) of clades of ancient origin.

These traits gained the attention of biologists because they are ancient and persistent. The bias in the biologists' choice is for prolonged stasis. With choice from a large number of traits, what is the probability that some would be so persistent by chance alone ? An estimate of that probability might be based on a null hypothesis of random change in traits during speciation and extinction, but we shall not attempt such an estimate here.

2. Body plan can also refer to architectural features of animals, such as types of body cavities, body walls, types of epithelia (Rieger 1994), organ systems, and skeletal support. Also included are arrangement of parts, as in segmentation, symmetry, position of mouth relative to nerve cords, or colonial versus solitary habit. Sometimes cell lineages (as in spiralian development) or cell movements (as in schizocoely and enterocoely), or cell types (such as choanocytes) are included as features of body plans. When body plans are defined as features of body organization rather than traits characterizing higher taxa, biases in selection of traits are eliminated (Fitch & Sudhaus 2002). The traits do not depend on relationships among clades, and other changes qualify as changes in body plans.

3. The traits of a phylotypic stage (Sander 1983, Raff 1996) have been suggested as the definition of the body plan, at least for those phyla or classes for which a phylotypic stage can be identified. Attention to phylotypic stages arose from the observation that some intermediate stage of development has diverged less than both earlier and later stages. For vertebrates the pharyngula has been considered to be a phylotypic stage, despite its variation among taxa (Ballard 1976, Richardson 1995). Most generally, a life-history stage has been considered to be phylotypic if it is the least varying stage of development in the most inclusive clade. To our knowledge there have been no attempts at a quantitative application of these dual criteria. Should greater weight be given to inclusiveness or to constancy in the identification of phylotypic stages ? Phylotypic stages are a special case of persistent synapomorphies. Like other persistent synapomorphies, they are selected by biologists for their stasis out of a large and uncounted set of traits. One explanatory hypothesis suggested for such especially conserved stages is that necessary interactions among developing body parts constrain evolutionary changes in this stage of development (Raff 1996).

4. A new use of the phrase "body plan" is its application to patterns of gene expression in embryos. Of particular interest are genes that establish the organization of developing embryos, including those that pattern general architectural features of body plans (body axes, germ layers, etc.) and distinguishing characteristics of particular body plans (notochord, segmentation, etc.). Most of the genes that pattern these features in embryos encode regulatory proteins such as transcription factors and intercellular signaling systems (Gerhart & Kirschner 1997, Carroll *et al.* 2001). The phrase "body plan" is increasingly used in association with the embryonic expression patterns of these genes (Fig. 1). Although there is clearly a relationship between regulatory genes and body plan features, gene expression profiles are not the same set of traits that have been used to distinguish phyla or classes, and they do not include all the features that distinguish types of body organization. As discussed in a later section, the phylogenetic distribution of most embryonic gene expression patterns does not match specific phyla and classes particularly well.

The term "body plan" seems to gain special significance for biologists when disparate meanings are combined in one argument. Confounding several meanings in one argument can produce a false impression of stasis. Not all traits that characterize higher taxa are architectural. Clearly not all architectural features of animals are useful in characterizing higher taxa. Homologous genes may be expressed in a similar pattern yet quite different structures result in subsequent development, and similar structures can develop via rather different patterns of gene expression. By using the term body plan (or ground plan or *bauplan*) ambiguously, one can attribute properties of one set of traits to another. When the attributes of the traits that characterize taxa of high rank are applied to the architectural features of multicellular animals or to the patterns of expression of homologous genes, or *vice versa*, confusion is likely. Use of "body plan" with double or triple meanings can bias assessment of the frequency or causes of stasis and change.

What one means by evolutionary stasis or change in "body plans" clearly depends on the traits considered to be part of a body plan. Nevertheless, for all of the meanings listed above, there are examples of changes in body plan following change in a functional constraint. In contrast, it is more difficult to demonstrate developmental constraint as a cause of stasis in body plans. In selecting examples, we examined correlates of change because they are easier to examine than correlates of stasis. We selected examples that met one of two criteria. For some cases, the inferred phylogeny, the distribution of traits, and the fossil record indicated that the change occurred after prolonged stasis. In other cases there was little evidence on the duration of stasis or time of the change, but differences in body plan within taxa of low rank indicated that a trait remained unchanged during the evolution of marked morphological disparity but nevertheless changed subsequently (the alternative and unparsimonious hypothesis for examples in this second category is that the trait represents the ancestral condition and traits in all other members of the clade were convergently derived from it).

CHANGES IN FUNCTIONAL REQUIREMENTS

One explanation for stasis is stabilizing selection, with the explanation of change being a change in functional requirements. The functional requirements that may account for stasis and change in body plans are varied. Here we emphasize ancient synapomorphies and architectural features of animals.

Nutrition from symbionts

A variety of free-living animals with chemoautotrophic symbiotic bacteria have lost the gut lumen and in some cases the mouth and anus. As a change in the construction of the body, the loss of a gut may be considered a change in body plan. Indeed the absence of a recognizable gut in the adult contributed to the Pogonophora being considered a phylum, although morphological and molecular ev-

idence now nest them within the annelids (George & Southward 1973, McHugh 2000).

A gut has been lost within families or genera within a number of clades (Gustafson & Lutz 1992, Krueger *et al.* 1992). The distribution of gutlessness within clades indicates extended stasis in which a gut was present followed by loss of a gut. Such distributions of gutlessness are found among species of protobranch bivalves, oligochaetes, and nematodes (Giere & Langheld 1987, Fisher 1990, Ott *et al.* 1982). As an example, the bivalve *Solemya reidi* has a stomach rudiment as a larva but lacks a functional gut. At metamorphosis, the larva ingests its own test cells, but the material enters the lumen of the perivisceral cavity (Gustafson & Reid 1988).

Nutrition by parasitism

The evolutionary transition from a free living habit to parasitism can involve extensive changes in body plans as ancestral structures for feeding and locomotion are lost and other structures elaborated. An extreme example from the crustacean arthropods illustrates the magnitude of possible changes. Rhizocephalan barnacles are allied to other cirripede crustaceans (Høeg 1995), but as adults the rhizocephalans lack such arthropod traits as segmentation and jointed appendages and in some cases are colonial. The female develops from a vermiform slug of cells injected into the host (Glenner *et al.* 2000). It becomes an interna, a set of branching roots with an epithelium and a cellular core. The externa, the reproductive part of the female, develops from the system of roots and extends outside the host. In one group of rhizocephalans, multiple externas develop from a set of rootlets (Høeg & Lützen 1993), thus achieving a colonial construction, with tissue connections and nutrient exchange maintained among morphological individuals. Thus the arthropod body plan has been so modified that it has been possible for coloniality to evolve as a novel feature of the body plan. The male rhizocephalan develops into a gametogenic structure hyperparasitic on the female. The multicellular structures of postlarval rhizocephalans give no morphological evidence of relationship to arthropods.

Nutrient content of eggs

Loss of a feeding larval stage has occurred numerous times within diverse phyla (Thorson 1950, Strathmann 1978, Hanken 1992). The transition from feeding to non-feeding development is often accompanied by loss or reduction of larval structures and accelerated development of postlarval structures. Loss of the larval mouth is common and loss of the entire gut has occurred in bryozoans, with development of the gut delayed until metamorphosis. These are substantial changes in the larval body plan. In some cases the loss has occurred after a demonstrably long period of stasis (Wray 1996). Cases of facultatively feeding larvae demonstrate that nutrient rich eggs and independence of exogenous food have evolved prior to evolutionary changes in the larval body (Hart 1996). Such changes are in part the result of loss of a functional requirement for obtaining food during the larval stage and as such could result from release from stabilizing selection (Strathmann 1975), but some of the changes may result from selection for improved performance of the non-feeding larva in swimming (Emlet 1994) and more rapid development to competence for metamorphosis (Wray & Bely 1994) and therefore could result from new directional selection. The changes in size of eggs and larval morphology and accelerated development of postlarval structures are associated with changes in cell fates that are evident early in development (Wray & Raff 1990).

Among the most dramatic developmental changes are the transitions from holoblastic cleavage to incomplete (meroblastic) early cleavages. Such changes have occurred in both directions with increases and decreases in size of eggs, as in arthropods. The processes establishing developmental fates of portions of the embryo can differ between syncytial embryos, in which nuclei share cytoplasm without diffusion barriers, and embryos composed of separate cells.

Other nutritional change

A radula is characteristic of most classes of molluscs and is a plesiomorphic trait for gastropods, but it has been lost independently in several lineages that diverged since the Paleozoic (Oliverio 1995). These animals have been assigned to families or genera in which other species have a radula and they are easily identified as gastropods, but they have lost part of the gastropod body plan. Losses may be concentrated in a clade in which the radula had become specialized for delivery of toxin and the losses may be associated with a shift in diet (Kantor & Sysoev 1989).

Changes from microphagous suspension feeding to macrophagy have occurred in several clades, with the derived macrophagous animals often occupying habitats that are poorer in suspended food. Sponges in the Cladorhizidae have lost the aquiferous system and choanocytes that are used in suspension-feeding and that are characteristic of sponges. These sponges trap larger prey, such as crustaceans. The condition is inferred to be derived rather than ancestral. "Such a unique body plan would deserve recognition as a distinct phylum, if these animals were not so evidently close relatives of Porifera.

Their siliceous spicules resemble those in several families of poecilosclerid Demospongiae" (Vacelet & Boury-Esnault 1995).

The small ctenophore *Ctenella aurantia* lacks the colloblasts characteristic of Ctenophora and also lacks the peripheral canals (Carré & Carré 1993). It appears to be a highly modified cydippid, with functional changes associated with exogenic cnidocysts and small size.

Motility and habitat selection

Notochord, dorsal nerve cord, and postanal tail are chordate features that have become restricted to a non-feeding larval stage in ascidians. Their functional role is thus restricted to habitat selection and perhaps other kinds of dispersal. This restricted role has preceded loss of the larval tail and thus most of the chordate body independently in several lineages. A restricted functional role preceded their loss. Taillessness appears to have evolved more times within molgulids than in other ascidians, which raises the possibility of a developmental predisposition for tail loss in molgulids and by implication a greater developmental constraint on tail loss in other ascidians (Huber *et al.* 2000). Nevertheless, mutations resulting in loss of most of the body axis are known for other chordates, as in the floating head and no tail mutants of zebra fish (Halpern 1997). Stasis in the vertebrate body axis has not been from an absence of mutations. Most of the body posterior to the head can be removed by a mutation, but whereas some ascidians thrive without axial or appendicular locomotion, no vertebrates have adopted a mode of life that permits such a loss (Strathmann 2000).

Adult size

Evolution of smaller adults is often associated with changes in body plans. The annelids offer examples of changes in several components of the annelid body plan. In marine annelids, smaller size is associated with an interstitial habit and can be associated with loss of parapodia, loss of setae, and acquisition of rings of cilia in the adult (Westheide 1985). Reduction in size can also be associated with an acoelomate condition (Smith *et al.* 1986, Fransen 1988). Such divergences in body plan occur within families, as in the Hesionidae and in the Dorvilleidae together with the allied Dinophilidae.

Loss of a blood vascular system and changes in excretory systems are also associated with reduction in size. In the hesionid polychaetes, small species that are apparently derived from larger ones have lost the blood vascular system and reacquired protonephridium-like excretory organs (Westheide 1986). This trend conforms to a broad correlation among multicellular animals (Ruppert & Smith 1988). In animals with a blood-vascular system, muscle-mediated filtration from blood vessels through podocytes into the coelomic cavity produces the filtrate. The filtrate is then modified as fluid passes through an open duct from the coelom to the outside. In contrast, in animals without a blood vascular system, cilia-mediated filtration of fluids occurs from the coelom into the excretory duct from coelom to the outside. The cilia extend into the duct, and fluid is filtered through a weir formed by extensions of cells at the inner ends of the ducts. Ruppert and Smith note that development of these protonephridia-like and metanephridia-like systems does not conform to germ lines, and they question homology among protonephridia. An example of consequences of divergent size of adults within a species is provided by *Bonellia*, an echiuran with large females and minute parasitic dwarf males (Schuchert 1990). The females have a blood vascular system and open ducts between coelom and the outside. The males lack a blood vascular system, and have protonephridia-like excretory organs. The excretory organs of the male do not develop from a larval excretory system but rather as a new structure. Body plans can diverge between sexes within a species in association with different functional constraints.

Skeletons and requirements for support, defense, and density

Accretionary growth of a shell, as opposed to molting, has evolved in several arthropods. A bivalved carapace (as in conchostracans) or sessile habit (as in barnacles) appear to have been the preconditions for this change. Presumably the zoologists who initially classified barnacles as molluscs considered a permanent shell and mantle cavity to be important parts of a body plan.

A particular form of calcite skeleton is the most consistent distinguishing feature of living and fossil echinoderms. Each skeletal element is composed of a latticework of anastomosing rods, the whole element optically like a single crystal of calcite. Skeletal elements are absent in the Pelagothuriidae, a family of elasipod sea cucumbers, although they belong to a clade of undoubted echinoderms and their inferred sister group within the suborder has skeletal ossicles (Hansen 1975). This condition may be associated with their pelagic life at great depths. Reduced skeletons, of which this is an extreme, carry consequences for buoyancy, structural support, flexibility, and defense.

Protection of embryos

Protection of gastropod embryos is associated with especially long cell cycles for the cells that will form trochoblasts and apical ectoderm, and the specification of the mesentoblast and dorso-ventral body axis occurs when the embryo has fewer cells

(van den Biggelaar *et al.* 1997). As a consequence, trochoblasts and other ectodermal cells can depart from a radial arrangement of cell fates and diverge in differentiation when the embryo has fewer cells. For example, fewer cells form prototrochal cilia and some instead form the head vesicle in pulmonate gastropods (van den Biggelaar 1993). Protection has led to modified cell interactions and cell fates to modify the trochophore. In addition to changes in ancient synapomorphies and architectural features, these are changes in what could be considered to be a phylotypic stage and presumably also in gene expression in the cells that do or do not form trochoblasts in the divergent clades.

Conclusion on functional constraints on ancient synapomorphies and body structures

The foregoing examples suggest that stasis in body plans (as architectural features of animals) results from stabilizing selection for performance of certain activities. Change results from changes in selection on performance. The foregoing examples include change and stasis in body plans under other definitions as well, with different subsets concerning traits characterizing major groups, body architecture, and changes at nearly all stages, including some that could be candidates for phylotypic stages.

Functional requirements are, by themselves, sufficient to account for many instances of stasis and change in traits that are considered parts of body plans, but support for a hypothesis of functional constraint does not in itself exclude developmental constraints in these or other instances. A hypothesis of functional constraint emphasizes that high performance of some function is maintained by stabilizing selection. Developmental processes may influence the effect of mutations on performance and thus affect selection for or against the change. Our examples simply indicate that changes in body plans are associated with changes in the need to perform certain identifiable tasks. We now turn to meanings of "body plan" that are explicitly developmental.

CHANGES IN DEVELOPMENT

The two definitions of body plans that are based on development produce a somewhat different perspective on stasis and why it might occur. Here too, however, changes in body plans are clearly possible in post-Cambrian time and are commonly associated with shifts in functional constraints.

Phylotypic stages and body plans

Only three clades within phyla have been proposed to possess phylotypic stages: vertebrates, insects, and sea urchins (Ballard 1976, Sander 1983, Raff 1996). Within these clades, the phylotypic stage is generally well conserved (although exceptions exist, as described in the next paragraph). Most phyla, however, seem to lack a phylotypic stage. There are few obvious phylum-wide similarities in the embryonic or larval development of (for instance) cnidarians, bryozoans, nemerteans, and platyhelminthes. Either these and most other phyla lack phylotypic stages, or those stages are evolutionarily labile. In either case, a body plan definition based on phylotypic stages does not match the perception that body plans have remained static since the Cambrian.

Modifications in phylotypic stages

Some modifications to the phylotypic stage have evolved within each of the three groups proposed to possess one. Waddington (1956) and Elinson (1987) pointed out that the phylotypic stage of vertebrates is not the beginning of development, but rather a point of convergence that is preceded by more disparate earlier stages and followed by more disparate later ones. Duboule (1994) and Raff (1996) called this the "developmental hourglass" to emphasize the many changes in early (i.e., prephylotypic) development, most of which are associated with changes in functional constraints on embryonic nutrition (Elinson 1987, Raff 1996). The famous phylotypic "pharyngula" of vertebrates is itself not as stereotypic as implied by Haeckel's famous figures, with component features often appearing in different sequences (Richardson 1995). The phylotypic stages of insects (germ band) and sea urchins (adult rudiment and juvenile) (Emlet 2000) show similar differences in the order of events and relative size of component structures. Thus, simple modifications to the phylotypic stage are possible. In a few cases, the phylotypic stage has been more extensively modified. The best examples come from sea urchins and other echinoderms with essentially direct development, where formation of the adult body plan shows almost no similarities to that of other species with overall rather similar adult anatomy (e.g., McEdward 1992, Schatt & Feral 1996, Emlet 2000).

Gene expression and body plans

Anatomical and gene expression indices of body plans show rather different patterns of stasis and change. Indeed, if all we had to go on were com-

parisons of gene expression, it seems unlikely that we would recognize the same clades of animals as most distinctive. Many regulatory genes and features of their expression are very widely shared among animal phyla (Slack *et al.* 1993, Gerhart & Kirschner 1997, Carroll *et al.* 2001). Enthusiastic assertions that all animal embryos are patterned in essentially the same manner with the same set of regulatory genes (DeRobertis & Sasai 1996, Holland 1999, Carroll *et al.* 2001) probably overstate the case (Davidson 2001, Wilkins 2002). Nonetheless, some striking similarities exist. Genes of the *Hox* complex pattern position along the anteroposterior axis in all phyla for which the relevant functional information exists (Gerhart & Kirschner 1997). This applies to phyla with body plans on anatomical grounds that are about as different as they come, including cnidarians, arthropods, echinoderms, and chordates (Davidson 2001). Another good example is homologous regulatory proteins that control the differentiation of muscle and neuronal cell types in the same broad set of phyla (Davidson 2001). Most published comparisons of regulatory gene expression among phyla have emphasized similarities rather than differences.

Some features of embryonic gene expression are apparently restricted to particular body plans, however. One is the expression of functionally antagonistic domains of *engrailed* and *wingless* at future segment boundaries in a wide variety of arthropods (Patel *et al.* 1989, Brown *et al.* 1994b). Unfortunately, no data are yet available from other ecdysozoan phyla with metameric or segmented body organization. Superficial similarities in *engrailed* expression have been reported from other phyla (Wedeen & Wiseblat 1991, Holland *et al.* 1997), but these cases do not appear to involve segment patterning, since expression is restricted to only a few taxa and occurs too late to play a role in segmentation (Bely & Wray 2001, Davidson 2001). Another example of body plan-specific regulatory gene expression appears to be the expression of *brachyury* in the notochord of urochordates and chordates. Although this gene has rather different developmental roles in other phyla, its function in notochord differentiation is apomorphic for chordates and appears to be widespread within the phylum.

Additional examples of regulatory gene expression that closely match anatomical body plan features are scarce. This may be due partly to insufficient information. The depth of phylogenetic sampling of embryonic gene expression does not approach that for anatomical data , and for most animal phyla, the expression of not even a single gene has been examined. Four phyla have been extensively studied (arthropods, nematodes, chordates, and echinoderms) and scattered information is available for several others (cnidarians, annelids, mollusks, platyhelminths, and hemichordates). With data from more phyla and from more taxa within each phylum, a closer match between gene expression and anatomical body plans may emerge. For now, however, regulatory genes are primarily remarkable for how many similarities they reveal among phenotypically disparate phyla and for how many changes have evolved within phyla (see below).

Changes in body-patterning gene expression

Losses and modifications in the expression of body-patterning genes have occurred within phyla, and some are associated with life history transformations. A well-studied example involves the wasp *Copidosoma*, which is a parasitoid with polyembryonic development. Unlike other wasps and holometabolous insects in general, the earliest body-patterning genes are no longer expressed in this species, including the "gap" genes and those that establish the anteroposterior axis. Later in embryogenesis, however, the conserved network of segmentation and homeotic gene expression is instituted (Grbic & Strand 1998). Another clear example involves the urochordate genus *Molgula*, where reduced larval dispersal has evolved several times independently by loss of the tail (Huber *et al.* 2000). Genes that pattern the notochord, a characteristic chordate feature, are expressed in embryos of species with tailed larvae but not in those whose larvae lack tails (Swalla & Jefferey 1996).

Although these losses of characteristic aspects of embryonic gene expression in *Copidosoma* and *Molgula* both evolved in conjunction with shifts in life history, this correlation is not always evident. Some other dramatic changes in the expression of body-patterning genes have evolved without any obvious connection to functional changes in development or body plan organization. Examples include the role of *bicoid* in anteroposterior patterning of embryos of Diptera but not other holometabolous insects (Stauber *et al.* 2000), the role of even-skipped in pair-rule patterning of holometabolous but not hemimetabolous insects (Patel 1994), and the loss of homeotic patterning functions of *zen* and *fushi-tarazu* in holometabolous insects (Brown *et al.* 1994a, Dawes *et al.* 1994, Falciani *et al.* 1996).

DISCUSSION

Evolutionary changes in body plans abound, under four different meanings of the term body plan. The changes support the hypothesis that stasis in body plans depends on functional requirements. When functional requirements change, widely conserved features with long periods of stasis can be

changed. Extensive modifications in development often accompany these changes. Some of our examples are post-Paleozoic changes in body plans. For others, the variation is found within extant taxa of sufficiently low rank that one can infer stasis for the body plan through repeated evolutionary divergences in many other features prior to the change in the body plan.

Multiple parallel cases are known for changes in body plans. These replicated evolutionary events are associated with parallel changes in functional requirements. Examples include changes in nutrient content of eggs, changes in requirements for motility, changes in nutrition of adults associated with parasitism, and changes in size of adults. Thus functional constraints on body plans are well documented.

We did not, however, tally instances in which there was a change in functional requirements for a body plan but little change in the body plan. The concentration of evolutionary loss of tails in molgulids is consistent with differing constraints on mutational "knock outs" in different ascidians. The comparative evidence on developmental processes is suggestive. Molgulids lack an ankyrin-like protein that is present in the cortex of eggs in other sampled ascidian families, and this trait might be a developmental precondition that results in loss of tails in molgulids more readily than in other families of ascidians (Huber *et al.* 2000). Some sea urchins with large eggs and no apparent requirement for larval feeding, like *Brisaster*, have retained the pluteus form whereas other echinoids with large eggs and no requirement for feeding have lost the pluteus form (Hart 1996, Wray 1996). Also, some sea urchins with a feeding pluteus larva require thyroxine from their diet for development of the echinus rudiment, but at least one sea urchin with non-feeding larval development produces thyroxine (Saito *et al.* 1998). Endogenous production of thyroxine could be a developmental precondition for the evolutionary loss of a pluteus larval form. If so, dependence on diet for thyroxine could be considered a developmental constraint on the evolutionary transition to a non-feeding larva. It therefore remains possible that developmental processes differ in clades in which there have been apparently similar changes in functional requirements but difference in whether there has been a change or no change in body plan. Although hypotheses of developmental constraint have not fared well thus far, differences among clades in incidence of stasis and change raise the possibility of differing developmental constraints in different clades. Such differences offer the possibility of testing hypotheses of constraint by the comparative method.

Despite intense interest, body plan-constraining features of development have yet to be demonstrated. Instead, studies of the *cis*-regulatory elements involved in body patterning have indicated

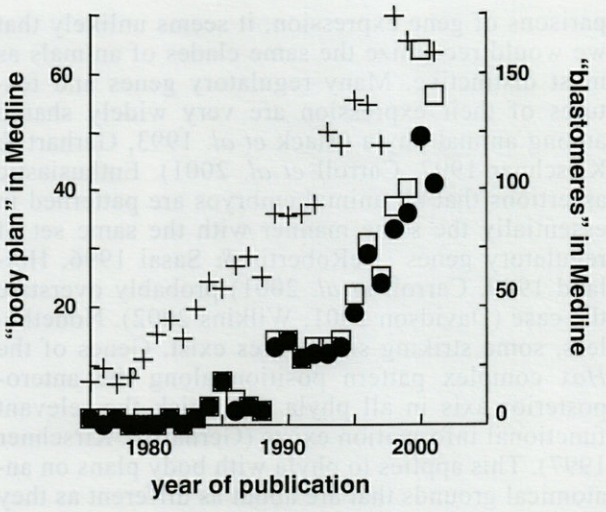


Fig. 1. – Number of articles published between 1975 and 2001 that were identified with the key words "body plan" and "blastomeres" from the data base Medline/PubMed. Open squares are total number each year that were obtained with "body plan" and filled circles are for those articles whose titles indicated that gene expression or pattern specification were part of the study. The latter account for most of the accelerating increase in use of "body plan." The + symbols are numbers of articles obtained with "blastomeres,"which increased during the same period, but only linearly.

remarkably great scope for evolutionary change (Carroll *et al.* 2001). This does not, however, rule out body plan-constraining features of development. It seems unlikely from current evidence that tissue interactions constrain body plan features. It remains possible that gene interactions do so. Technical advances that would allow this possibility to be tested have come into widespread use in model systems and are now being applied in a comparative context.

An extreme hypothesis of developmental constraint on body plans would be that variation in the traits comprising a body plan is impossible. This would mean that there are no mutations that change the body plan. An alternative hypothesis is that stabilizing selection accounts for stasis in body plans. Although changes are possible, they result in poorer performance of some key activity and are therefore selected against. As a functional arrangement of body parts is improved, there may be a decreasing number of routes to further improvements that do not pass through a functionally inferior transitional state. In addition, as ecological "space" is filled by a variety of animals, the minimal functional requirements may become greater and possible transitions between body plans thereby restricted (Frazzetta 1970, Valentine 1973, Strathmann 1978). New features arise very rarely, not because preexisting ones are "optimal" solu-

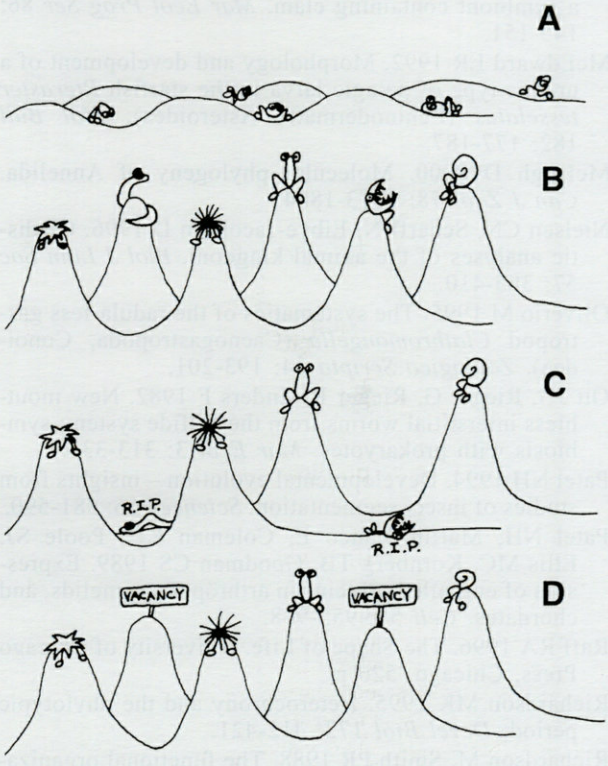


Fig. 2. – A model, based on Sewall Wright's metaphor of an adaptive landscape, that illustrates constraints on evolutionary change in body plans. A, When a new way of life is first entered, clumsy transitional stages can nevertheless be improvements over coexisting forms. Many adaptive peaks may occupied, or at least closely approached. B, In an occupied way of life, reaching new adaptive peaks is less likely because occupation of adaptive peaks has produced deeper maladaptive valleys and perhaps because the adaptive changes involved in reaching the peaks removed traits that provided the initial evolutionary flexibility. C, In a mass extinction, adaptive peaks temporarily disappear, but the valleys between peaks may nevertheless remain steep because of survivors' traits. D, Even when adaptive peaks reappear, they may remain unoccupied. Adaptive body plans may not appear as before because survivors continue to produce deep valleys in the adaptive landscape and because some ancestral traits are no longer represented in the descendents (After Strathmann 1978).

tions (pan-adaptationist hypothesis) but because new features are very unlikely to be better than preexisting ones. Moreover, local optima that were once attained may not be attained a second time. One such situation is modeled in Fig. 2).

ACKNOWLEDGEMENTS. – This study was aided by the Friday Harbor Laboratories, NSF grant IBN 0113603 to RRS, NSF grant IBN 96346 to GAW, a meeting on evolution of body plans organized by R A Raff at the University of Indiana, discussions with numerous colleagues, and an anonymous reviewer.

REFERENCES

Ballard WW 1976. Problems of gastrulation: real and verbal. *Bioscience* 26: 36-39.

Bely AE, Wray GA 2001. Evolution of regeneration and fission in annelids: insights from *engrailed-* and *orthodenticle*-class gene expression. *Development* 128: 2781-2791.

Brown SJ, Hilgenfeld RB, Denell RE 1994a. The beetle *Tribolium castaneum* has a *fushi tarazu* homolog expressed in stripes during segmentation. *Proc Natl Acad Sci (USA)* 91: 12922-12926.

Brown SJ, Patel NH, Denell RE 1994b. Embryonic expression of the single *Tribolium engrailed* homolog. *Dev Genet* 15: 7-18.

Carré C, Carré D 1993. *Ctenella aurantia*, genre et espèce nouveaux de Cténophore tentaculé (Ctenellidae fam. nov.) méditerranéen sans colloblastes et avec ventouses labiales. *Canadian J Zool* 71: 1804-1810.

Carroll SB, Grenier JK, Weatherbee SD 2001. From DNA to Diversity: Molecular Genetics and the Evolution of Animal Design. Blackwell Scientific, Malden, Massachusetts.

Davidson EH 2001. Genomic Regulatory Systems: Evolution and Development. Academic Press, San Diego.

Dawes R, Dawson I, Falciani F, Tear G, Akam M 1994. *Dax*, a locust *Hox* gene related to *fushi-tarazu* but showing no pair-rule expression. *Development* 120: 1561-1672.

DeRobertis EM, Sasai Y 1996. A common plan for dorsoventral patterning in Bilateria. *Nature* 380: 37-40.

Duboule D 1994. Temporal colinearity and the phylotypic progression: a basis for the stability of a vertebrate Bauplan and the evolution of morphologies through heterochrony. *Development* 1994 supplement: 135-142.

Elinson R 1987. Changes in developmental patterns: embryos of amphibians with large eggs. *In* Development as an Evolutionary Process, RA Raff & EC Raff, editors. Liss, New York: 1-21

Emlet RB 1994. Body form and patterns of ciliation in nonfeeding larvae of echinoderms: functional solutions to swimming in the plankton? *Amer Zool* 34: 570-585.

Emlet RB 2000. What is a juvenile sea urchin? A comparative and phylogenetic survey of post-metamorphic juveniles. *Zygote* 8: S40-S41.

Falciani F, Hausdorf B, Schröder R, Akam M, Tautz D, Denell R, Brown S 1996. Class 3 *Hox* genes in insects and the origin of *zen*. *Proc Natl Acad Sci (USA)* 93: 8479-8484.

Fisher CR 1990. Chemoautotrophic and methanotrophic symbioses in marine invertebrates. *Rev Aquat Sci* 2: 399-436.

Fitch DHA, Sudhaus W 2002. One small step for worms, one giant leap for "Bauplan ?" *Evol & Devel* 4: 243-246.

Fransen M 1988. Coelomic and vascular systems. *Microfauna Marina* 4: 199-213.

Frazzetta TH 1970. From hopeful monsters to bolyerine snakes. *Amer Nat* 104: 55-72.

George JD, Southward EC 1973. A comparative study of the setae of Pogonophora and polychaetous Annelida. *J Mar Biol Assoc UK* 53: 403-424.

Gerhart J, Kirschner M 1997. Cells, embryos, and evolution. Blackwell Science, Malden, Massachusetts. 642 p.

Giere O, Lanheld C 1987. Structural organization, transfer and biological fate of endosymbiotic bacteria in gutless oligochaetes. *Mar Biol* 93: 641-650.

Glenner H, Høeg JT, O'Brien JJ, Sherman TD 2000. Invasive vermigon stage in the parasitic barnacles *Loxothylacus texanus* and *L. panopaei* (Sacculinidae): closing of the rhizocephalan life-cycle. *Mar Biol* 136: 249-257.

Grbic M, Strand MR 1998. Shifts in the life history of parasitic wasps correlate with pronounced alterations in early development. *Proc Natl Acad Sci (USA)* 95: 1097-1101.

Gustafson RG, Lutz RA 1992. Larval and early post-larval development of the protobranch bivalve *Solemya velum* (Mollusca: Bivalvia). *J Mar Biol Assoc UK* 72: 383-402.

Gustafson RG, Reid RGB 1988. Larval and post-larval morphogenesis in the gutless protobranch bivalve *Solemya reidi* (Cryptodonta: Solemyidae). *Mar Biol* 97: 373-387.

Halpern ME 1997. Axial mesoderm and patterning of the zebrafish embryo. *Amer Zool* 37: 311-322

Hanken J 1992. Life history and morphological evolution. *J Evol Biol* 5: 549-557.

Hansen B 1975. Systematics and biology of the deep-sea holothurians. *Galathea-Report* 13: 1-262.

Hart MW 1996. Evolutionary loss of larval feeding: development, form and function in a facultatively feeding larva, *Brisaster latifrons*. *Evolution* 50: 174-187.

Hodin J, Hoffman JR, Miner BG, Davidson BJ 2001. Thyroxine and the evolution of lecithotrophic development in echinoids. *In* Echinoderms 2000. Edited by M Barker. A A Balkema, Lisse: 447-452.

Høeg JT 1995. The biology and life cycle of the Rhizocephala (Cirripedia). *J Mar Biol Assoc UK* 75: 517-550.

Høeg JT, Lützen J 1993. Comparative morphology and phylogeny of the family Thompsoniidae (Cirripedia, Rhizocephala, Akentrogonida), with descriptions of three new genera and seven new species. *Zoologica Scripta* 22: 363-386.

Holland LZ, Kene M, Williams NA, Holland ND 1997. Sequence and embryonic expression of the amphioxus *engrailed* gene (*AmphiEn*): the metameric pattern of transcription resembles that of its segment-polarity homolog in *Drosophila*. *Development* 124: 1723-1732.

Holland ND, Holland LZ 1999. Amphioxus and the utility of molecular genetic data for hypothesizing body part homologies between distantly related animals. *Amer Zool 39:* 630-640.

Huber JL, da Silva KB, Bates WR, Swalla BJ 2000. The evolution of anural larvae in molgulid ascidians. *Seminars Cell Devel Biol* 11: 419-426.

Kantor YI, Sysoev AV 1989. The morphology of toxoglossan gastropods lacking a radula, with a description of new species and genus of Turridae. *J Molluscan Studie* 55: 537-549.

Krueger DM, Gallager SM, Cavanaugh CM 1992. Suspension feeding on phytoplankton by *Solemya velum*, a symbiont containing clam. *Mar Ecol Prog Ser* 86: 145-151.

McEdward LR 1992. Morphology and development of a unique type of pelagic larva in the starfish *Pteraster tesselatus* (Echinodermata: Asteroidea). *Biol Bull* 182: 177-187.

McHugh D 2000. Molecular phylogeny of Annelida. *Can J Zool* 78: 1873-1884.

Nielsen CN, Scharff N, Eibye-Jacobsen D 1996. Cladistic analyses of the animal kingdom. *Biol J Linn Soc* 57: 385-410.

Oliverio M 1995. The systematics of the radula-less gastropod *Clathromangelia* (Caenogastropoda, Conoidea). *Zoologica Scripta* 24: 193-201.

Ott JG, Rieger G, Rieger R, Enders F 1982. New mouthless interstitial worms from the sulfide system: symbiosis with prokaryotes. *Mar Ecol* 3: 313-333.

Patel NH 1994. Developmental evolution – insights from studies of insect segmentation. *Science* 266: 581-590.

Patel NH, Martin-Blanco E, Coleman KG, Poole SJ, Ellis MC, Kornberg TB, Goodman CS 1989. Expression of engrailed proteins in arthropods, annelids, and chordates. *Cell* 58: 955-968.

Raff RA 1996. The Shape of Life. University of Chicago Press, Chicago. 520 p.

Richardson MK 1995. Heterochrony and the phylotypic period. *Devel Biol* 172: 412-421.

Richardson M, Smith PR 1988. The functional organization of filtration nephridia. *Biol Rev* 63: 231-258.

Rieger RM 1994. Evolution of the "lower" Metazoa. *In* Early Life on Earth, Edited by S Bengston, Columbia Univ Press, New York: 475-488.

Ruppert EE, Smith PR 1988. The functional organization of filtration nephridia. *Biol Rev* 63: 231-258.

Saito M, Seki M, Amemiya S, Yamasu K, Suyemitsu T, Ishihara K 1998. Induction of metamorphosis in the sand dollar *Peronella japonica* by thyroid hormones. *Develop Growth Differ* 40: 307-312.

Sander K 1983. The evolution of patterning mechanisms: gleanings from insect embryogenesis and spermatogenesis. *In* Development and Evolution. Edited by BC Goodwin, N Holder, CC Wylie. Cambridge University Press, Cambridge: 137-159.

Schatt P, Féral J-P 1996. Completely direct development of *Abatus cordatus*, a brooding schizasterid (Echinodermata: Echinoidea) from Kerguelen, with description of perigastrulation, a hypothetical new mode of gastrulation. *Biol Bull* 190: 24-44.

Schuchert P 1990. The nephridium of the *Bonellia viridis* male (Echiura). *Acta Zoologica* 71: 1-4.

Slack JMW, Holland PWH, Graham CH 1993. The zootype and the phylotypic stage. *Nature* 361: 490-492.

Smith PR, Lombardi J, Rieger RM 1986. Ultrastructure of the body cavity lining in a secondary acoelomate, *Microphthalmus* cf. *listensis* Westheide (Polychaeta: Hesionidae). *J Morph* 188: 257-271.

Stauber M, Taubert H, Schmidt-Ott U 2000. Function of *bicoid* and *hunchback* homologous in the basal cyclorrhaphan fly *Megaselia* (Phoride). *Proc Natl Acad Sci (USA)* 97: 10844-10849.

Strathmann RR 1975. Larval feeding in echinoderms. *Amer Zool* 15: 717-730.

Strathmann RR 1978. The evolution and loss of feeding larval stages of marine invertebrates. *Evolution* 32: 894-906.

Strathmann RR 1978. Progressive vacating of adaptive types during the Phanerozoic. *Evolution* 32: 907-914.

Strathmann RR 2000. Functional design in the evolution of embryos and larvae. *Seminars Cell Devel Biol* 11: 395-402.

Swalla BJ, Jeffery WR 1996. Requirement of the *Manx* gene for expression of chordate features in a tailless ascidian larva. *Science* 274: 1205-1208.

Thorson G 1950. Reproductive and larval ecology of marine bottom invertebrates. *Biol Rev* 25:1-45.

Vacelet J, Boury-Esnault N 1995. Carnivorous sponges. *Nature* 373: 333-375.

Valentine JW 1973. Evolutionary Palaeoecology of the Marine Biosphere. Prentice-Hall, Englewood Cliffs, New Jersey. 511 p.

van den Biggelaar JAM 1993. Cleavage pattern in embryos of *Haliotis tuberculata* (Archaeogastropoda) and gastropod phylogeny. *J Morphol* 216: 121-139.

van den Biggelaar JAM, Dictus WJAG, van Loon AE 1997. Cleavage patterns, cell-lineages and cell specification are clues to phyletic lineages in Spiralia. *Cell Develop Biol* 8: 367-378.

Waddington CH 1956. Principles of Embryology. George Allen & Unwin, London, 510 p.

Wagner GP, Misof BY 1993. How can a character be developmentally constrained despite variation in developmental pathways ? *J Evol Biol* 6: 449-455.

Westheide W 1985. The systematic position of the Dinophilidae and the archiannelid problem. *In* The Origins and Relationships of Lower Invertebrates. Edited by S Conway Morris, R Gibson, HM Platt. Oxford Univ Press, Clarendon. 310-326.

Wedeen CJ, Weisblat DA 1991. Segmental expression of an *engrailed*-class gene during early development and neurogenesis in an annelid. *Development* 113: 805-814.

Westheide W 1986. The nephridia of the interstitial polychaete *Hesionides arenaria* and their phylogenetic significance (Polychaeta, Hesionidae). *Zoomorphology* 106: 35-43.

Wilkins AS 2002. The Evolution of Developmental Pathways. Sinauer Press, Sunderland Massachusetts.

Wray GA 1996. Parallel evolution of nonfeeding larvae in echinoids. *Syst Biol* 45: 308-322.

Wray GA, Bely AE 1994. The evolution of echinoderm development is driven by several distinct factors. *Development* 1994 supplement: 97-106.

Wray GA, Lowe CJ 2000. Developmental regulatory genes and echinoderm evolution. *Syst Biol* 49: 28-51.

Wray WG, Raff RA 1990. Novel origins of lineage founder cells in the direct-developing sea urchin *Heliocidaris erythrogramma*. *Devel Biol* 141: 41-54.

Reçu le 31 mai 2002; received May 31, 2002
Accepté le 19 septembre 2002; accepted September 19, 2002

VIE MILIEU, 2002, 52 (4) : 201-207

From Marine Ecology to Developmental Biology
In Honour of Pierre Tardent (1927-1997)

SEAHORSES – MASTERS OF ADAPTATION

M.S. SCHMID, D.G. SENN**

**Laboratory for Vertebrate Morphology, University of Basle, Claramattweg 8, 4057 Basle, Switzerland*

SEAHORSE
HIPPOCAMPUS
ADAPTATION
EVOLUTION
REPRODUCTION

ABSTRACT. – Seahorse habitats are hard substrates (rocks, gravel, corals, gorgonians, sponges). Special adaptations are needed for survival in such an environment. Seahorses show adaptations in body shape, appearance, locomotion and behaviour as well as (most outstanding) in reproductive biology. In contrast to the majority of marine teleosts, seahorses avoid a planktonic larval phase. Males develop a brood pouch in which, after mating, the eggs are fertilized and kept for cleavage and embryonic development. Like this, a maximum degree of protection is provided for the clutch. This paper gives an overview on seahorse biology, emphasizing most important features and discussing them with reference to the benthonic way of life of these extraordinary animals.

HIPPOCAMPE
HIPPOCAMPUS
ADAPTATION
ÉVOLUTION
REPRODUCTION

RÉSUMÉ. – Les Hippocampes vivent sur fonds durs (roches, graviers, Coraux, Gorgones, Eponges). Des adaptations spéciales sont nécessaires pour survivre dans un tel biotope. Elles concernent leur morphologie, leur locomotion et leur comportement aussi bien que leur reproduction. Contrairement à la plupart des Téléostéens marins, les Hippocampes évitent la phase larvaire planctonique. Les mâles possèdent une poche abdominale d'incubation dans laquelle (après l'accouplement) les œufs sont fécondés et maintenus pendant leur segmentation et leur développement embryonnaire, ce qui leur assure une protection maximale. Cette étude présente une vue d'ensemble de la biologie des Hippocampes et fait ressortir les points les plus importants liés à la vie benthique de ces Poissons extraordinaires.

INTRODUCTION

Teleosts represent the modern type of fish among the ray-finned fishes (Actinopterygii). Their body structure shows differentiations referring to a light construction, maneuverability and swift swimming. Even generalized teleosts (Greenwood *et al.* 1973, Arratia 1996), the type of herring-like fishes (Clupeomorpha), have thin and light scales; furthermore they get perfect buoyancy by an adjustable swim-bladder in order to move in the three-dimensional environment of the open ocean. The fusiform-shaped body (Hertel 1966, Senn 1997) permits an efficient and energy-saving locomotion. Some reproductive patterns emphasize that the modern fish differentiated for a high degree of mobility. Eggs and larvae develop as part of the drifting plankton. When clupeomorph (herring-like) teleosts radiated during Cretaceous and early Tertiary they primarily populated the pelagic environment.

However, teleosts as the richest group of vertebrates did not only adapt to open waters. Numerous independent lines secondarily led to bottom-dwelling life-styles. Such substrate-dwelling fishes developed different body shapes and structures. Not speed and swiftness became important but adaptations to the substrate and to slower swimming. Some fishes differentiated flattened bodies in order to get in intimate contact with the bottom. Others developed skin structures resembling the ground in order to obtain a perfect camouflage. Some forms show an astonishing ability to change coloration; they produce rapid adaptations while frequently changing the substrate. Flounders and plaices are perfectly invisible on sandy bottoms (Portmann 1956, Bürgin 1986).

On hard substrates adaptations are a bit special. Here on rocks, gravel, corals and gorgonians, which are frequently overgrown by calcifying red algae (Lithophyllum), sponges, tubeworms and Bryozoa, camouflage is more elaborate. A variety of structures contribute to make fishes (scorpion fish, stone fish, file fish etc.) invisible. In terms of camouflage on structured hard bottoms as well as in sea-grass the tubemouth fishes (Syngnathiformes) are unsurpassable (Kuiter 2001).

The pipe fishes of the genus *Syngnathus* resemble a sea-grass leaf in shape and coloration; thus they are perfectly camouflaged in their environment of Posidonia fields. Leafy pipe fishes

(*Phyllopteryx*) seamlessly imitate all irregular parts of thalli (stalks and phylloids) of brown algae in Australian waters. And the seahorses (genus *Hippocampus*), with the earliest representatives known from the Pliocene of Fiume Merecchia, Italy (Frickhinger 1991), show enormous adaptations for their living on hard substrate bottoms of Mediterranean and North Atlantic coastal waters.

Hippocampus shows various adaptations to the hard substrate. We observe adaptations in body shape, appearance and behaviour in order to move safely and get a perfect camouflage on the richly structured bottom. Body shape does not at all resemble a fish; there is no need for a hydrodynamic, fast swimming animal. Secondly locomotion is neither swift nor buoyant. The seahorse rather creeps on the substrate while bending its body and, if there is low current, uses the dorsal fin to hover slowly to the next structure. It then gets firmly fixed on a hard object by its seizing tail in order to harvest plankton by suction feeding. Among the most surprising adaptations to a substrate dwelling biology is the pattern of reproduction. In contrast to most marine teleosts seahorses (and other syngnathids e.g. pipe fish) avoid a planktonic larval phase in their life history. Males develop an incubating pouch in which, after mating, the eggs are fertilized and kept for cleavage and embryonic development under a maximum degree of protection.

MATERIAL AND METHODS

All observations (*H. hippocampus, H. bleekeri* and *H. reidi*) have been realised thanks to the Vivarium of the Zoological Gardens in Basle. Big thanks are expressed to the whole team of the Vivarium for their help in various respects.

The seahorses used for paraffin and alcohol preparation were taken from the clutch of *H. hippocampus* on January 2nd, 2001. On the first day after hatching few specimens were taken out of the salt-water aquarium and given into the fixation solution AFE (90 % alcohol, 10 % formaldehyde, 10% glacial acetic acid) for two days. After that all specimens were given into alcohol (80 %) for storage. Living animals were taken, though, individuals that already had serious problems with keeping themselves upright in the water column, helplessly drifting on the water surface.

RESULTS AND DISCUSSION

The entire biology of seahorses is harmonized with their benthonic habitat (Bellomy 1969). The aspect of adaptation can be seen as the key to the understanding of seahorse biology. Subsequently, the most important parts in seahorse biology will be emphasized and discussed in the context of the benthonic way of life of these animals.

A. Morphologic adaptations

One of the most outstanding changes with reference to the morphology of original teleosts is the transformed and now prehensile tail of seahorses. As a consequence, the tail fin was lost. Most of the time this prehensile tail works as anchorage in the highly structured sea-grass habitat. While swimming it fulfils balance and steering duties. Additionally the tail takes action during the greeting rite (performed daily), the mating dance of pairs (reproductive season) as well as in the competition of males. If not more or less stretched out during swimming, adult seahorses do generally keep their tail rolled up towards their belly. The morphology of the tail tells us more about the phenomena of grasping (according to Hale 1996) and the impossibility of lateral and s-ward bending in adults.

In the context of tail structure and function the existing exoskeleton is worth a glance. It is built by dermal bones (joint pieces of the skeleton, located right below the skin), evolving directly from embryonic connective tissue (desmic way of bone formation). The trunk of seahorses is surrounded by 7 longitudinal rows of these plates (forming 3 pairs of bony ridges to both sides and one ridge to the front). Four rows of bony plates do lie around the tail. This is how the striking appearance of seahorses and pipe fishes is created. Every segment of the tail consists of 4 bony plates. They overlap their anterior neighbors at any time, even at maximum bending (Fig. 1). Overlapping parts of succeeding plates are joined by connective tissue. The vertebrae attach to the plates with connective tissue at the lateral processes and the hemal spines (Hale 1996).

In comparison to our model of the 'classic' teleost the muscular apparatus in seahorses shows considerable modification. We find a redistribution and new orientation of muscular tissue within the myomeres. Fast and powerful contractions become possible by this special kind of muscle formation. Every single plate is connected to a myomere by a thick band of connective tissue (muscular tendon). During axial bending ventral tendons and plates project myomere-generated forces to the backbone. Ventral bending of the tail can therefore be achieved by simultaneous and evenly strong contraction of the left and right hypaxial muscle of a segment (Fig. 2a). Median ventral muscles are involved in axial bending, too. In this latter case the transformation of forces runs over the hemal spines of the vertebrae (Fig. 2b).

In the whole mechanism of bending we find a division of labor between myomeres and median ventral muscle groups. Myomeres do act in fast,

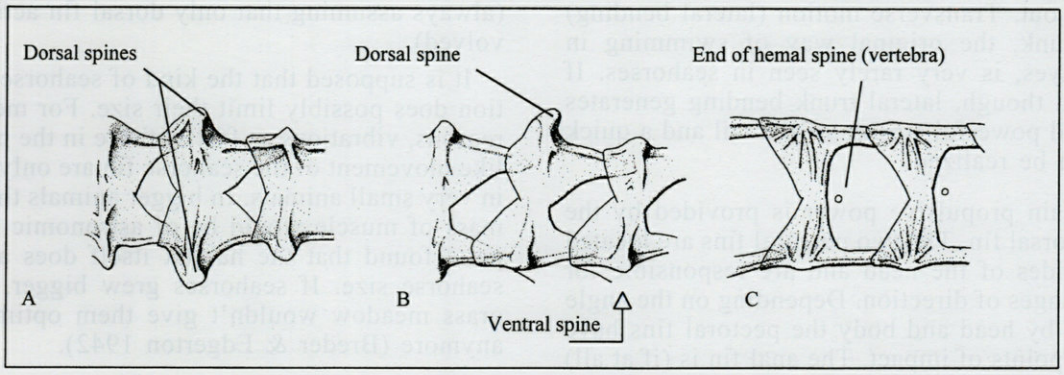


Fig 1. – Overlapping plates in the tail segments of *H. Kuka* (from Hale 1996): dorsolaterally and ventrolaterally the plates from edges, which dorsolaterally end in spiny projections. A, Dorsal view; B, Lateral view; C, Ventral view.

powerful tail movements. Median muscles (composed of tonic fibers) do generate rather small forces but seem to be responsible for maintaining a bent tail position for a longer period. The heavy plating of the seahorse tail plays a prominent role in the transmission of forces during axial bending. The plates provide rigid structures for the myomeres to pull upon. Therefore the plating can eventually even be seen as a necessary prerequisite for this extreme kind of force transmission and tail bending (Hale 1996).

According to Hale studies, the arrangement of plates and skin connective tissues prevents strictly lateral movement of the tail. Additionally, the overlapping of plates helps control the twisting of the tail in adults. In newborns the development of plating and connective tissues is still incomplete. This might be the/a reason for the extended lateral bending abilities in newborn seahorses (personal observation M Schmid 2001). In adults lateral grasping can only be achieved by controlled twisting of the tail. As in the normal grasping movement, the ventral side of the tail becomes the inside of the curl, when lateral grasping is necessary.

Most of the time seahorses keep themselves in an upright position. Like their relatives, the pipe fishes, they manage to maintain this position by the cooperation of air bladder and tail. For a change of position the gas mixture of the bladder can be moved to its anterior or posterior part. For animals depending on the ability of fast swimming (either for hunting of flight) only very few changes in the shape of their body are possible without suffering losses in velocity. In the habitat of seahorses, though, no advantage can be derived from fast swimming. For this reason a spindle-shaped body, as present in fast hunters like sharks, is no longer attractive for seahorses. Alternatively they count on optimized maneuverability which is much more advantageous in their complex habitat of sea-grass meadows.

Basically four modes of progression can be distinguished (Blake 1976 and personal observations).

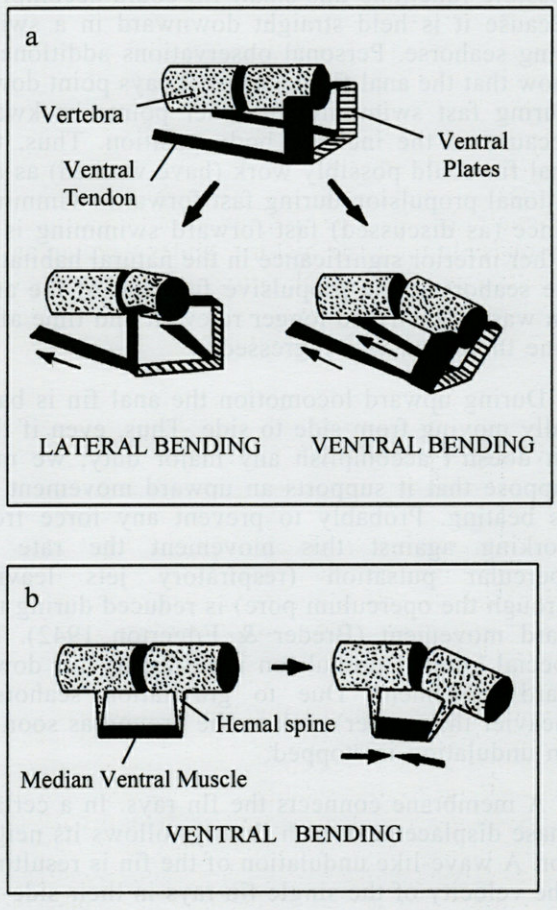


Fig. 2. – a, Model showing lateral and ventral bending in the seahorse tail. Ventral tendons project myomere generated forces on specific body segments. Evenly or unevenly (according to Hale 1996). b, Model showing ventral bending in the seahorse tail by contraction of median ventral muscles and transmission of forces over the hemal spines of the vertebrae (according to Hale 1996).

These are: slow forward, fast forward, turning and vertical movement. The faster the animal swims, the more its body will be inclined towards swimming direction and the more the tail will be

stretched out. Transverse motion (lateral bending) of the trunk, the original way of swimming in Osteichthyes, is very rarely seen in seahorses. If demanded though, lateral trunk bending generates strong and powerful strokes of the tail and a quick flight can be realized.

The main propulsive power is provided by the waving dorsal fin. The two pectoral fins are located at both sides of the head and are responsible for small changes of direction. Depending on the angle produced by head and body the pectoral fins have different points of impact. The anal fin is (if at all) only present in a reduced, tiny form and has almost no function with respect to locomotion (Vincent 1990). Breder & Edgerton (1942) consider a slightly lifting force or rudder-like action the only possible functions this small fin could accomplish because it is held straight downward in a swimming seahorse. Personal observations additionally show that the anal fin does not always point down. During fast swimming it rather points backward because of the inclined body position. Thus, the anal fin could possibly work (have worked) as additional propulsion during fast forward swimming. Since (as discussed) fast forward swimming is of rather inferior significance in the natural habitat of the seahorses, the propulsive function of the anal fin was probably no longer relevant and time after time the organ was regressed.

During upward locomotion the anal fin is basically moving from side to side. Thus, even if this fin doesn't accomplish any major duty, we may suppose that it supports an upward movement by its beating. Probably to prevent any force from working against this movement the rate of opercular pulsation (respiratory jets leaving through the operculum pore) is reduced during upward movement (Breder & Edgerton 1942). No special kind of propulsion is necessary for downward movement. Due to gravitation seahorses (heavier than water) sink to the ground as soon as fin undulation is stopped.

A membrane connects the fin rays. In a certain phase displacement each fin ray follows its neighbor. A wave-like undulation of the fin is resulting. The velocity of the single fin rays in their side-to-side movements is comparable to the wing-beating velocity of some flying animals of equivalent size. Outstanding though is the fact that the seahorse fin is able to maintain that same velocity in an atmosphere (water) that is far more dense than the one (air) in which the insect wing beats ! If there wasn't the connecting membrane, the force produced by the beating fin rays would carry the animal horizontally forward. The posterior moving waves do additionally create a force that works vertically downward. The cooperation of the two force vectors of waves and fin rays produces the actual propulsion that pushes the animals diagonally upward (always assuming that only dorsal fin action is involved).

It is supposed that the kind of seahorse locomotion does possibly limit their size. For mechanical reasons, vibrations as fast as those in the propeller-like movement of the seahorse fin are only possible in very small animals. In bigger animals the needed mass of muscle would be of astronomic size. Experts found that the habitat itself does also limit seahorse size. If seahorses grew bigger, the seagrass meadow wouldn't give them optimal cover anymore (Breder & Edgerton 1942).

B. Adaptations in reproductive biology

Syngnathidae are the only vertebrate group in which males literally get pregnant. The male seahorse carries the clutch either at the base of its belly or tail (male ghost fish), separated by thin walls at the bottom of its body, completely surrounded by folded skin or even in tightly closed brood pouches. This gives us an idea of how the brood pouch in seahorse males has evolved (Vincent 1990). Further development probably proceeded past the open pouch in pipe fish (at the back or tip of the tail) ending up with the pouch at the belly of seahorse males, which represents the version of highest development. Apart from a small pore this brood pouch is completely closed.

In an impressive way seahorse reproductive biology is tuned with habitat conditions. In the majority of species mating pairs are faithful. At least for the course of a breeding season, probably even longer. In natural habitats, seahorses don't live in big colonies. Mating pairs are spread in a patchy way and in low density. Therefore, finding a partner is a rather difficult attempt, requiring a great deal of energy. In contrast, living in pairs helps saving time and energy that would otherwise have to be spent when searching for new mates. Additionally, predation risk is reduced since the animals do have to leave their cryptic state less often.

When it comes to caring for the clutch we find an exchange of duties in seahorse pairs. The female deposits a set of eggs (several hundreds in most species!) into the pouch of the male who is responsible for all further care. The brood pouch with its ion-rich fluid is of optimal nutritional value and works as osmotic adaptation chamber for the developing embryos (Linton & Soloff 1964). The duration of egg and embryo incubation within the pouch does strongly depend on the species. The finally hatching newborns are miniatures of their parents and right from the start independent in every respect (Fig. 3).

A new clutch of eggs can already mature in the belly of the female while the male is carrying the previous breed in his pouch. This means that the male seahorse increases his own rate of reproduc-

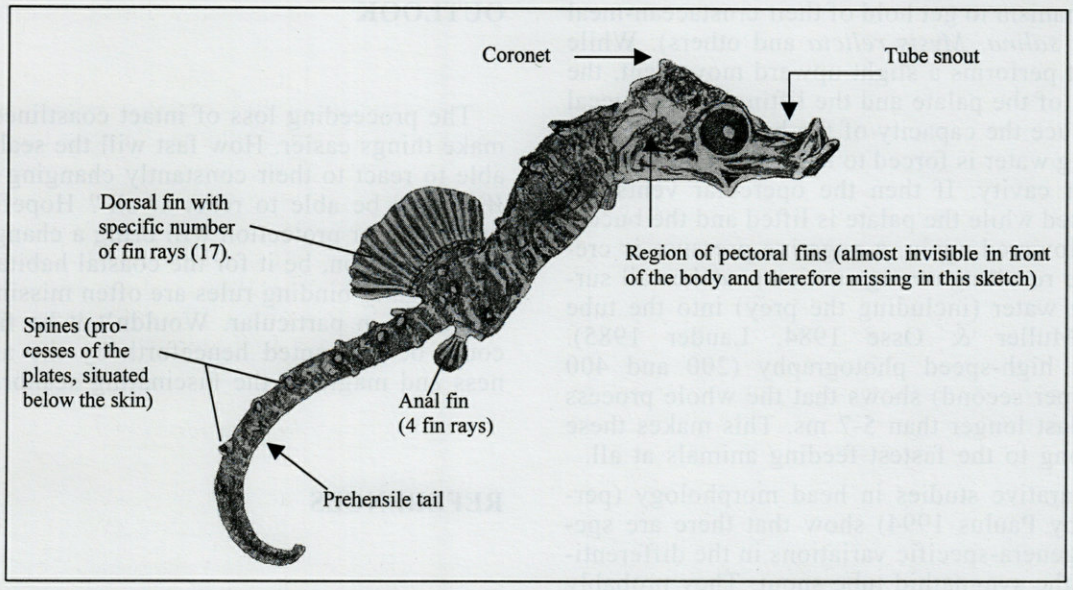


Fig. 3. – Newborn *H. hippocampus*, Zoo Basle (almost one day old, after fixation in AFE, kept in 70 % alcohol for storage). Body development comparable to an adult, though proportions are different. Total body length: 7 mm (Drawing by M Schmid 2001).

tion by breeding the young in his pouch (Woodruffe & Vincent 1994). Additionally a male seahorse can be sure of his paternity at 100 percent because the eggs of the female are only fertilized after they've reached his (and no other) brood pouch.

Literature tells us of daily-performed greeting rites at daybreak. The choice of this particular time of day might be connected to the attempt of keeping the rite unnoticed by enemies. Eventually we can talk of a so-called 'trade-off' effect. Minimal predation risk on one side and more or less good lighting conditions on the other side. As changes of body color are part of this morning rite some active role of the optic sense can be assumed. Considering this, it obviously makes sense to meet and 'dance' early in the morning before hiding away in the seagrass when the sun rises. Probably for the same reasons of protection the birth of the young is set in the early morning, too, or does even happen at night. In their natural habitat, the newborn seahorses are subject to a high mortality caused by predators and their hatching during darkness does certainly benefit their chances of survival.

C. Appearance – A factor for survival

The development of a brood pouch in the male seahorse provides optimal security for the clutch. Anyway, it would be useless if the fabulous camouflage wouldn't have been invented simultaneously. There is no exaggeration in calling the camouflage of seahorses 'close to perfection'. Seahorses are able to adapt their looks to their environment in a way that an unpracticed eye will have difficulties to spot a specimen in the wild. By their changing body color and by their ability to grow tufts, seahorses imitate sea-grass or gorgonians on which and in which they hide. The shield of bony plates below their skin is part of their subtle tactics of passive defense. The hard casing alone makes them relatively uneatable already and therefore less attractive to some potential predators.

Naturally the just hatched youngsters do face an elevated degree of danger. The risk of predation is reduced by the birth in darkness and by the transparency of the newborn. In contrast to many other adult features (which are already present at birth), pigmentation patterns are developed during the first weeks *after* birth.

D. 'Cool' hunters

Following the aim of optimum camouflage, seahorses are ambush-hunters. Remaining motionless they let their prey (small crustaceans) come to them. Their eyes can move chamaeleon-like and independently from each other, allowing the seahorse to observe a large part of the vicinity while remaining perfectly hidden. Problems arise when the water is troubled by a storm or by human water pollution along the coastline. Reduced visibility is problematic since the optic sense of seahorses is of great importance when it comes to localizing and catching the prey. Even newborns do already use their tube snout and their specialized pipet-feed-

ing-mechanism to get hold of their crustacean-meal (*Artemia salina, Mysis relicta* and others). While the snout performs a slight upward movement, the lowering of the palate and the lifting of the buccal floor reduce the capacity of the buccal cavity. The remaining water is forced to leave the mouth by the opercular cavity. If then the opercular vents are kept closed while the palate is lifted and the buccal floor is lowered again, a negative pressure is created. The resulting strong suction snatches all surrounding water (including the prey) into the tube snout (Muller & Osse 1984, Lauder 1985). Lauder's high-speed photography (200 and 400 pictures per second) shows that the whole process doesn't last longer than 5-7 ms. This makes these fish belong to the fastest feeding animals at all.

Comparative studies in head morphology (performed by Paulus 1994) show that there are species- or genera-specific variations in the differentiation of the syngnathid tube snout. They probably reflect a secondary specialization according to the adaptation to various food ressources. Sympatric living species (species, living in the same habitat) such as the syngnathids of the Jordanian Red Sea coastal waters are forced to use different niches when feeding. This is because of the enormous interspecific pressure by competition. Specialized differentiations in the tube snout with regards to a certain food item are favoured (Brauch 1966). These specializations are shown in the bones of the tube snout and, within the species, overall in lateral snout elements (Paulus 1994).

CONCLUSION

With regard to all the adaptations to the benthonic habitat, the biology of seahorses may without a doubt be of great fascination for evolution scientists. Unfortunately, some aspects of the seahorses specialized way of living are about to become their ruin. Several peculiarities in behaviour and ecology render seahorses extremely sensitive for disturbances. Their small density in natural populations would be an example. Additionally, seahorse territories are small as well. Males stay on a territory of only one square meter during breeding season. Seahorses are tied to their home territory and their mobility (particularly the mobility of males during pregnancy) is restricted. All this makes them an easy catch for fishermen. Their strong faithfulness means that widowed animals do have to face serious problems when looking for a new mate. From this point of view all the fascinating adaptations discussed above do unfortunately turn to a multitude of fatal facts with regards to seahorse survival.

OUTLOOK

The proceeding loss of intact coastlines doesn't make things easier. How fast will the seahorses be able to react to their constantly changing habitat ? *Will* they be able to react at all ? Hopefully new programs for protection will bring a change for today's situation, be it for the coastal habitat in general (legally binding rules are often missing) or for seahorses in particular. Wouldn't it be fine if we could be enchanted henceforth by the attractiveness and magic of the fascinating seahorses... ?

REFERENCES

Arratia G 1996. The Jurassic and early teleosts. *In* Mesozoic Fishes – Systematics and Paleontology. By G Arratia & G Viohl. Pfeil, München: 243-259.

Bellomy MD 1969. Encyclopedia of Seahorses. TFH. Publications. Jersey City, N.J.

Blake RW 1976. On seahorse locomotion. *J Mar Biol Ass U K* 56: 939-949.

Brauch GM 1966. The feeding mechanism of *Syngnathus acus* Linnaeus. *Zool Afr* 2(1): 69-89.

Breder CM, Edgerton HE 1942. An analysis of the locomotion of the seahorse, *H. hudsonius*, by means of high-speed cinematography. *Ann NY Ac Sci* 43: 145-172.

Burgin T 1986. Beiträge zur Kopfanatomie der Plattfische (Teleostei; Pleuronectiformes). Dissertation, Univ Basel.

Frickhinger KA 1991. Fossilien Atlas Fische. Mergus, Melle.

Greenwood PH, Miles RS, C Patterson 1773. Interrelationships of Fishes. Linn. Soc. Ac. Press.

Hale ME 1996. Functional morphology of ventral tail bending and prehensile abilities of the seahorse. *J Morph* 227(1): 51-65.

Hertel H 1966. Structure, Form and Movement. Reinhold Publishing Corporation, New York.

Kuiter RH 2001. Seepferdchen, Seenadeln, Fetzenfische und ihre Verwandten. Ulmer, Stuttgart.

Lauder GV 1985. Aquatic feeding in lower vertebrates. *In* Functional vertebrate morphology. Edited by M Hildebrand, DM Bramble, KF Liem & DB Wake. Belknap Press. Cambridge.

Linton JR, Soloff BL 1964. The physiology of the brood pouch of the male seahorse *H. erectus*. *Bull Mar Sci* 45-61.

Lourie SA, Vincent ACJ, Hall HJ 1999. Seahorses – An identification guide to the world's species and their conservation. Project Seahorse, London.

Muller ML, Osse JWM 1984. Hydrodynamics of suction feeding in fish. *Trans Zool Soc Lond* 37: 51-135.

Paulus Th 1994. Morphology of the head skull and the tube snout of the Syngnathidae from the Red Sea (Pisces: Teleostei). *Senckenberg Mar* 25(1/3): 53-62.

Portmann A 1956. Tarnung im Tierreich. Springer, Berlin.

Senn DG 1996. Environments and functional anatomy of certain Mesozoic fishes. *In* Mesozoic Fishes – Systematics and Paleontology. By G Arratia, G Viohl Pfeil, München: 551-554.

Senn DG 1997. Durch Wasser, Wind und Wellen. Eine Naturgeschichte der ozeanischen Wirbeltiere. R+R Verlag, Aarau.

Vincent ACJ 1990. A seahorse father makes a good Mother. *Nat Hist* 12: 33-42.

Woodruffe R, Vincent ACJ 1994. Mother's little helpers: pattern of male care in mammals. *TREE* 9: 294-297.

Reçu le 11 avril 2002; received April 11, 2002
Accepté le 30 mai 2002; accepted May 30, 2002

VIE MILIEU, 2002, 52 (4) : 209-215

From Marine Ecology to Developmental Biology
In Honour of Pierre Tardent (1927-1997)

DEVELOPMENTAL FEATURES OF *OCTOPUS MACROPUS* RISSO, 1826 (MOLLUSCA, CEPHALOPODA)

S.v. BOLETZKY, M. FUENTÈS, N. OFFNER

Observatoire Océanologique de Banyuls, UMR 7628, 66651 Banyuls-sur-Mer, France
boletzky@obs-banyuls.fr

CEPHALOPODA
OCTOPODA
DEVELOPMENT
LIFE-STYLE

SUMMARY – *Octopus macropus* Risso, 1826 is known from the Mediterranean Sea and may occur also in the Atlantic. Embryonic development is briefly described, the emphasis being on features related to the existence of a planktonic/micronektonic post-hatching phase. At the end of this phase, juveniles switch to the adult mode of bottom life, but the actual conditions of this change of life-style are not yet known for this species. The observations are discussed in relation to the difference, which is known only in the Octopodidae, between merobenthic species (characterized by a planktonic/micro-nektonic post-hatching phase) and holobenthic species (characterized by a benthic post-hatching phase).

CÉPHALOPODES
OCTOPODES
DÉVELOPPEMENT
MODE DE VIE

RÉSUMÉ – *Octopus macropus* Risso, 1826 est connu de la Méditerranée; l'espèce est également signalée dans l'Atlantique. Son développement embryonnaire est décrit brièvement, mettant en relief les caractères liés à l'existence d'une phase post-embryonnaire de type planctonique ou micronectonique. Celle-ci aboutit à l'adoption du mode de vie benthique, mais les conditions exactes de ce passage au mode adulte sont encore inconnues chez cette espèce. La discussion replace les observations dans le contexte d'une différence, connue uniquement chez les Octopodidae, entre espèces mérobenthiques (caractérisées par une phase post-embryonnaire planctonique) et espèces holobenthiques (caractérisées par une phase post-embryonnaire benthique).

INTRODUCTION

The white-spotted octopus, *Octopus macropus*, is a rather common species on both near-shore and deeper bottoms of the Mediterranean Sea. Originally described by Risso (1826) from the area of Nice, it was listed and figured by Férussac & d'Orbigny (1835-1848), Delle Chiaje (1841), Vérany (1851), Jatta (1896) and Naef (1923). Its occurrence outside the Mediterranean was stated by Férussac & d'Orbigny (1835-1848) who examined specimens from the eastern Atlantic, the Red Sea and the Indo-Pacific and considered them all as members of the same species as the specimens collected in the Mediterranean. [These authors claimed priority for the name *Octopus cuvierii* d'Orbigny, since an illustration of the animal under that designation was made available to the public as early as 1825; see Norman (1992) on synonymy with *O. lechenaultii* d'Orbigny]. *O. macropus* was considered a cosmopolitan species until recent decades (Voss & Williamson 1971). It now appears, however, that several species similar to the original *O. macropus* live in shallow tropical and temperate waters throughout the world (Norman 1992, Mangold 1998). Whether the Mediterranean species is the same as the one observed in the Atlantic Ocean remains to be established (Rees & Maul 1956, Norman 2000, Wirtz 2001). All these species are characterized by very long arms, those of the first arm pair being longer than others, a very shallow web, and an adult life-style marked by predominant or exclusive night activity (Norman 2000). When observed under day light in an aquarium, the bright red color and the brilliant white spots may convey the false impression of poor camouflage. In contrast, when animals active at night are observed in the field (Fuentès & Offner pers. obs.), the skin patterns of *O. macropus* offer particularly eloquent examples of cephalopodan background mimicry (Tardent 1993, Messenger 2001).

The biological cycle of *O. macropus* is poorly known, and until recently there were no reliable data on spawning, embryonic development, and post-hatching life-style (Hochberg *et al.* 1992). Although Naef (1923) identified very early juvenile stages of *O. macropus* from plankton samples obtained in the Bay of Naples and in the Straits of Messina, it was long before evidence was obtained that the smallest specimens described by Naef (1923: 705, Figs 417, 418) indeed represent an immediate post-hatching stage (Boletzky *et al.* 2001). In the Caribbean Sea, a closely related species was

observed to lay very similar eggs producing also similar hatchlings (see photographs by RT Hanlon & M Wolterding in CephBase, http://www.cephbase.utmb.edu/imgdb/imgsrch2.cfm).

This note considers some developmental features of *O. macropus*, with special emphasis on aspects relating to life-style switching during juvenile development. Although the eggs of this species are much larger than those observed for instance in *O. vulgaris*, they are still small relative to the adult body size. As in other octopus species producing such relatively small eggs, the short-armed hatchlings of *O. macropus* start out as active swimmers feeding on planktonic organisms (Boletzky 1974). These hatchlings are indeed planktonic as far as their limited mobility in greater water masses is concerned, but they are micro-nektonic in terms of their swimming and hunting behavior (Boletzky 1977). Their behavioral characteristics will be viewed here in relation to morphological features resulting from embryonic development, with special attention to the question whether arm development reflects specific size relationships between egg (or hatchling) and adult.

OBSERVATIONS

All observations reported here were made in autumn 2000 on eggs spawned in the aquarium by a female *O. macropus* that had been captured during early summer of the same year in Banyuls-sur-Mer, north-western Mediterranean (Boletzky *et al.* 2001).

Eggs and embryos

Eggs measured 4.0 × 1. 2 mm at laying, not including the chorion stalk that measured 4 mm in length (Boletzky *et al.* 2001). The female attached the chorion stalks individually or in small clusters to a hard surface, thus forming a sheet of eggs that covered the walls of the artificial den inhabited throughout brooding. Eggs were flask-shaped when freshly laid, the chorion membrane tapered into a poorly defined chorion stalk. The overall aspect of the eggs changed slowly during early embryonic development as a constriction formed at the stalk base (Fig. 1), but the lumen of the hollow stalk remained in open connection with the main chorion space (Fig. 8).

Embryonic development closely resembled the developmental patterns described in other octopodid species (Boletzky 1978-79). Similarities included the occurrence of a first reversal of the embryo, which results in the position shown in Figure 1 (Boletzky & Fioroni 1990). As in other octopus species, reversal can fail so the embryo continues to develop in the initial position (Fig. 2). Under normal conditions, an embryo having undergone the first reversal returns to the initial orientation at a later stage (Fig. 8).

The portion of the yolk remaining in the visceral complex of the embryo during organogenetic stages is strongly compacted (Boletzky 2002). It forms a distinct inner yolk sac, which decreases in size due to an overall contraction of the embryonic body (Fig. 3). The minimum size of the inner yolk sac is attained by stage XV of Naef (1928), i. e. at a time when the mantle begins to grow in length (Fig. 4). During the subsequent stages of embryonic development, yolk transfer from the outer yolk sac results in a strong size increase of the inner yolk sac (Fig. 5). During these stages, the arms grow in length, each developing a set of 7 suckers that are arranged in a zigzag pattern on the oral arm surface (Figs 6, 7). The rounded ends of the arms (Fig. 6) become more pointed, each forming a terminal bud (Fig. 7), which is the rudiment of a prospective whip-like arm end (cf. Fig. 9). The arms remain sub-equal in length till hatching. The surface structure is marked by the formation of a dense set of Kölliker's organs in the skin (Fig. 9).

During these advanced stages, the first chromatophores appear on the head surface (Fig. 5). At the final embryonic stages the integument shows about 145 red chromatophores (Boletzky *et al.* 2001). The arrangement of 22 chromatophores in a double row behind the mantle edge (Fig. 8) could be a species-specific feature. The single file of chromatophores on the aboral surface of each arm pleads against the tentative identification of post-hatching stages by Rees (1955).

From stage XIX of Naef (1928) to hatching, the outer yolk sac becomes very small due to the combined effects of yolk absorption in the outer sac and yolk transfer to the inner sac (Fig. 8). The young hatched (at 16°C), about 2 months after the onset of embryonic development (at 22°C). If the temperature had remained above 20°C, embryonic development would have been shorter.

Post-hatching mode of life

The newly hatched animals measured 4 mm in dorsal mantle length (ML) and were planktonic/micro-nektonic, in other words they actively swam or hovered in the water column. During hovering the body was in an oblique position, the funnel tube and the short arms pointing downward (Fig. 10). When placed in a very small volume of water, the young animals spread out their arms and attached the suckers to the substrate (Fig. 9). Unconstrained, swimming individuals occasionally approached a vertical hard surface (e. g. the window of the aquarium), spread their arms (Fig. 11), and pounced at the hard surface to attach their arms

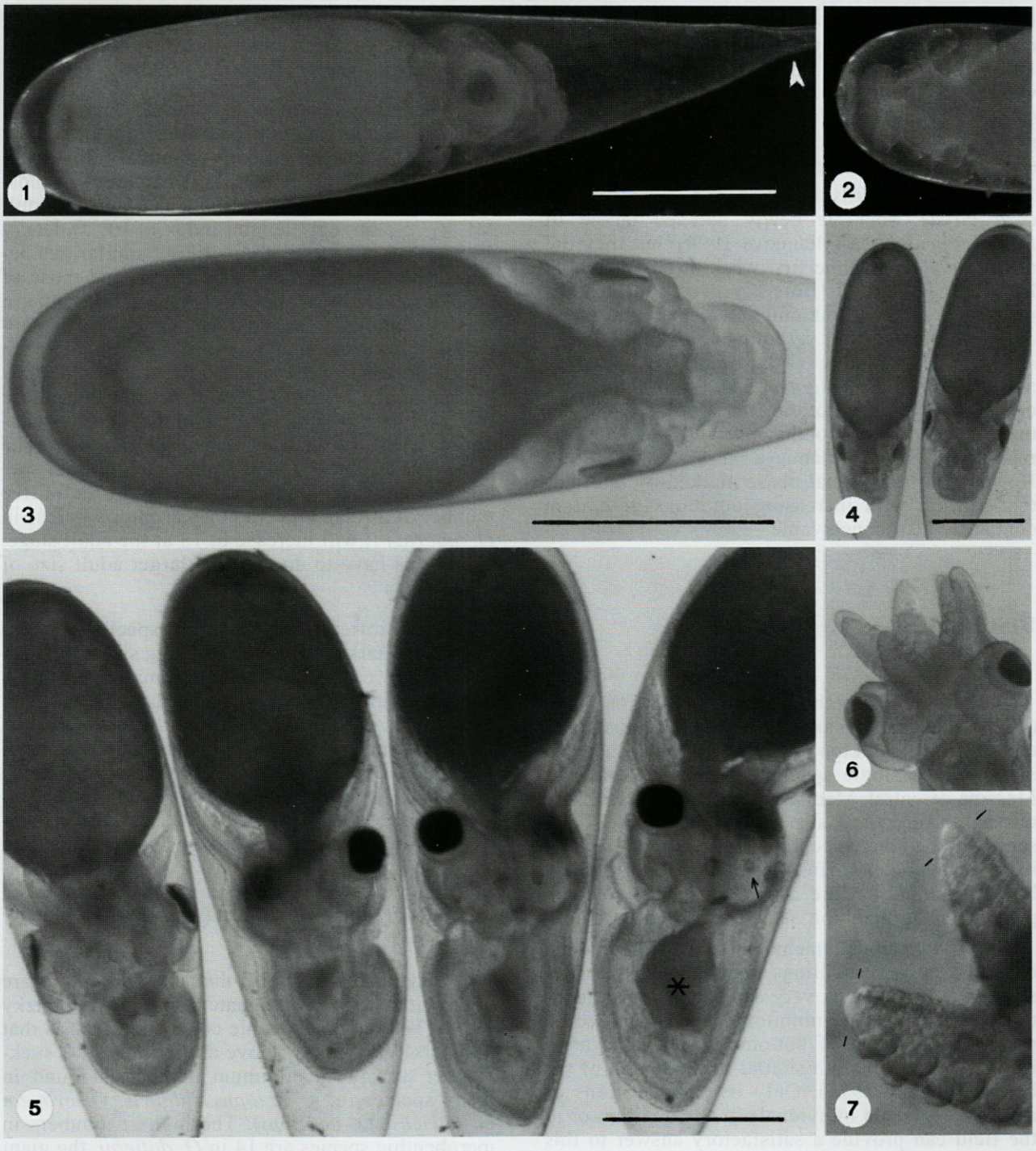


Plate I. – Fig. 1. Early organogenetic stage of *O. macropus* in lateral view (stage XI-XII of Naef); the orientation of the embryo in the chorion results from the first reversal. Note faint pigmentation of the retina. The arrow head points at the base of the chorion stalk. Scale bar = 1 mm. Fig. 2. Similar stage as in Fig. 1, but having missed the first reversal, so the embryo proper (seen from the oral side) lies close to the thick micropyle zone of the chorion. Fig. 3. Advanced organogenetic stage (stage XIII) in normal orientation, seen from the oral side. Scale bar = 1 mm. Fig. 4. Stages XIII (left) and XIV (right). Note size increase of head and mantle, and darkening of retinal pigmentation. Scale bar = 1 mm. Fig. 5. Stages XV (left) to XVI-XVII (right). Note the small size of the inner yolk sac (left) and its increase (at right*), and the differentiation of chromatophores (arrow). Scale bar = 1 mm. Fig. 6. Detail of head and arms at stage XIV after removal of the outer yolk sac. Note the rounded arm tips. Fig. 7. Some arms at stage XVI after removal of the outer yolk sac. Note the bare knobs (to the left of the bars) that are the rudiments of the future whip-like arm tips.

with the suckers (Fig. 12). It is significant that the mantle end was always slightly pointed (Figs 9, 10). This foreshadowed the tendency of benthic juveniles to stretch the mantle end into a conical shape (Naef 1923, p 707) and of benthic adults to give the whole mantle a slender cyrtoconic shape (Bergbauer & Humberg 1999).

Hatchlings readily attacked and seized crustacean larvae, such as newly hatched larvae of *Pagurus prideaux* (Villanueva 1994), but they did not survive beyond the first week of post-hatching life in the aquarium (Boletzky *et al.* 2001). Thus the length of the planktonic/micro-nektonic phase of *O. macropus* is not yet known. Given the larger size of the hatchling (ML 4 mm) compared to newly hatched *O. vulgaris* (ML 2 mm), the time before switching to bottom life is possibly shorter than the nearly two months observed in *O. vulgaris* reared at 21°C (Villanueva *et al.* 1995, 1996). However, a transitional phase marked by intermittent settling with increasing durations of bottom contact is also conceivable.

DISCUSSION

Based chiefly on chromatophore patterns, Naef (1923) recognized early juvenile individuals obtained from plankton samples to be young *O. macropus*. A single specimen from the Straits of Messina (Central Mediterranean) had 10 suckers on each arm, i. e. 3 suckers more than the 7 observed in the other specimens. This individual must have been older than the other, likely newly hatched animals. More advanced stages were not present in the plankton samples studied by Naef.

The spontaneous attachment to a hard substrate observed in our hatchlings may suggest that life-style switching occurs very early, but this assumption is unsafe. An inhibitory mechanism could postpone the onset of bottom life until the arms have grown to a length similar to the length of the body (Boletzky 1977). Only live observations of advanced juvenile individuals in an aquarium or in the field can provide a satisfactory answer to this question, as in the case of other octopodid species that have a planktonic/micro-nektonic phase but show very early settling responses (Boletzky 1987).

For all these species, two questions deserve particular attention. How far is life-style switching related to initial body proportions and behavioral maturity ? Are such relations linked to a specific magnitude of size difference between the hatchlings and their respective adults ? This is of interest in particular with regard to differences in the respective juvenile and adult morphometrics between merobenthic species (undergoing juvenile life-style switching) and holobenthic species (producing crawl-away young that skip the [supposedly primitive] pelagic phase) (Boletzky 1992).

Considering common features of merobenthic octopodids, the body proportions of the newly hatched animals are virtually identical (arms measuring about 40-50 % of ML at hatching) in most species producing eggs smaller than 10 % of adult ML (i. e., an egg length index < 10; Boletzky 1974). One may note other striking similarities between the hatchlings of *O. macropus* and those of *O. maorum*, a much larger species from southern Australia and New Zealand (Batham 1957) that appears to be a member of the *O. macropus* group (Norman 1992). The hatchlings of *O. macropus* (4.0 mm ML) are only slightly smaller than those of *O. maorum* (4.5 mm ML), and they have 7 suckers on each arm instead of 8 in *O. maorum*. The gills have 10 lamellae per demibranch in both species at hatching. However, this is the definitive number of gill lamellae in *O. macropus*, whereas in *O. maorum* it will increase to 13 at the adult stage. Could this have to do with the larger adult size of *O. maorum* ?

Indeed, different merobenthic species can exhibit very marked differences in egg size/adult size relations (i. e. egg length indices may range 2–8), which are reflected by corresponding differences in hatchling size/adult size relations. Thus in terms of the hatchling size *relative* to the adult size (90-100 mm ML in *O. macropus*, 200-300mm ML in *O. maorum*), hatchlings of *O. macropus* are much bigger than those of *O. maorum*. If a definitive number of gill lamellae may suggest something like 'morphological maturity' in *O. macropus* hatchlings, however the sucker numbers cannot be taken to reflect anything of the sort (as once suggested by Boletzky 1977, Table II).

It remains to recall also that sucker numbers are not related to *absolute* hatchling size (Boletzky 1977). The only reasonable conclusion then is that numbers vary widely above a minimum of 3 suckers per arm. This minimum number is found in many species (e. g. *Octopus vulgaris*, *O. cyanea*, *O. tetricus*, *O. defilippi*). The highest numbers in merobenthic species are 14 in *O. dofleini*, the giant octopus of the northeastern Pacific, and 16 in *O. fitchi*, a pygmy octopus of the eastern Pacific (Hochberg *et al.* 1992). These examples again emphasize that there is no positive relation with either absolute or relative hatchling size, as a high number of suckers indeed appears in small hatchlings (2-4 mm ML) that are either very small relative to the adult (< 2 % adult ML in *O. dofleini*) or very large relative to the adult (> 8% adult ML in *O. fitchi*). This large relative size of the hatchling results from an exceptionally high egg length index (ca 20) that would normally predict a holobenthic strategy, with long-armed, crawl-away young hav-

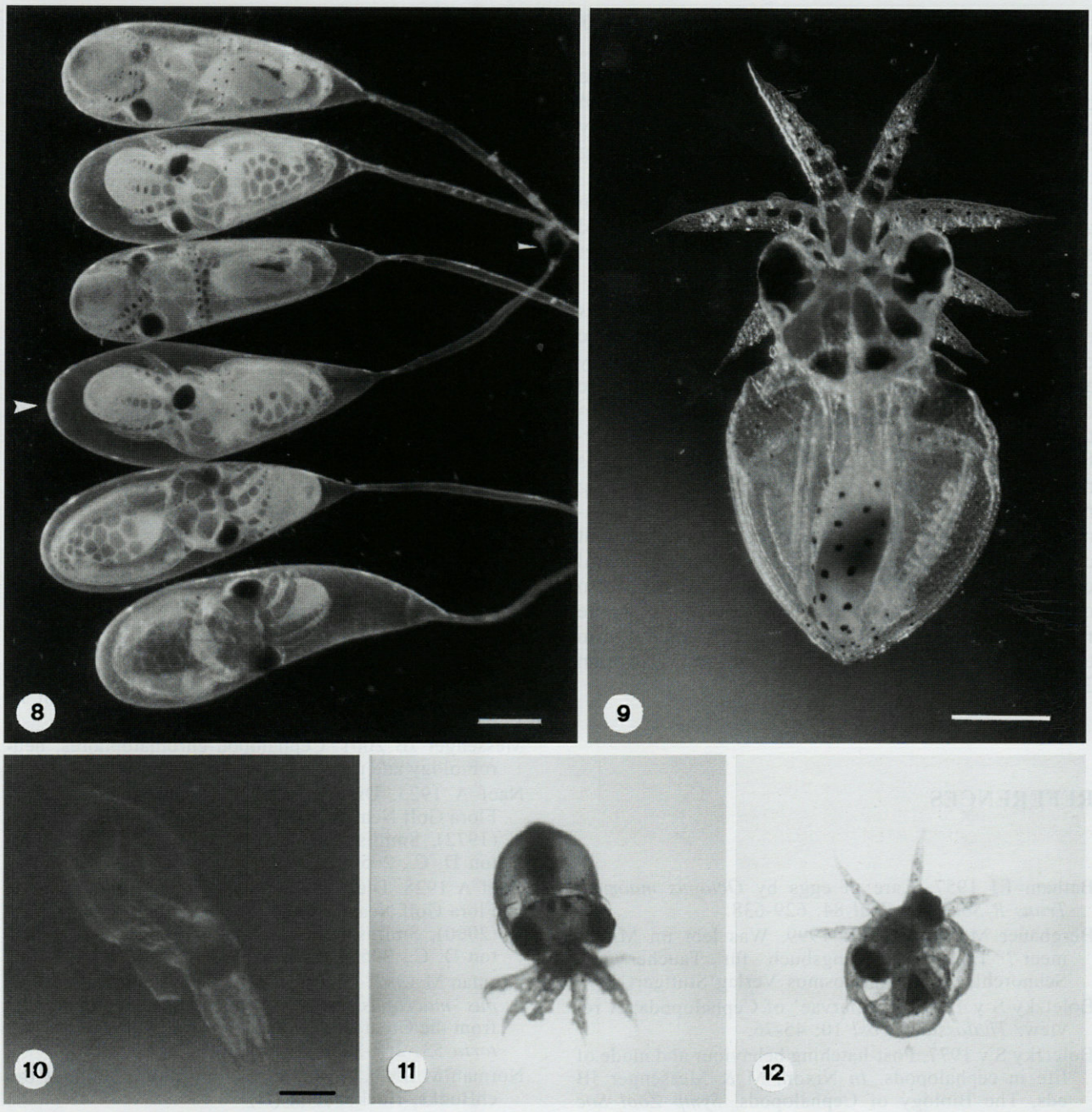


Plate II. – Fig. 8. Late embryonic stages (around stage XIX) before the second reversal (upper four) and after the second reversal (lower two, in normal hatching position). The large arrow head points at a strongly expanded chorion having an intact stalk anchored in a piece of fixation cement (hardened secretion) marked by the small arrow head. The third chorion from top has lost some perivitellin fluid due to rupture of the hollow chorion stalk. Note the dense set of chromatophores, especially the double row of chromatophores behind the mantle edge in the first and third embryo from top, and the very dark pigmentation of the retinae. Scale bar = 1 mm. Fig. 9. Newly hatched animal placed in a drop of sea water on a hollow microscope slide (photo taken through a dissecting microscope). Note the spread arms with their whip-like tips, the suckers being attached to the glass bottom. The granular appearance of the mantle is caused by Kölliker's organs in the skin. Scale bar = 1 mm. Fig. 10. Newly hatched animal hovering (lateral view in aquarium). Note the pointed mantle end at the top and the large funnel tube pointing downwards. Scale bar = 1 mm. Fig. 11. Very young individual (1-2 days after hatching) swimming, arms spread (frontal view in aquarium). Fig. 12. Same individual as in Fig. 11. attached to the aquarium window (oral view). Note the spread arms, the upper two being arm pair I, and the oblique position of the head and body (this twist is due to [perhaps incidental] muscular torsion of the arm crown attachment to the head).

ing at least 20 suckers on each arm (Boletzky 1997).

Among the species in which hatchlings have only 3 suckers per arm, *O. defilippi* differs from others in that the third arm pair is more strongly developed already at hatching, thus emphasizing the 'Macrotritopus' condition that seems to characterize an *O. defilippi* species complex (Hanlon *et al.* 1985). In contrast, the "first arm longest and stoutest" condition of adult *O. macropus* is not yet recognizable at hatching.

If most developmental features of *O. macropus* may appear rather commonplace for a merobenthic octopodid species, several details of morphology and behaviour deserve further attention. Meristic characters of juvenile and adult gills could be of interest. That the gills of the hatchlings have their definitive complement of lamellae in *O. macropus* is remarkable, although probably not unique among merobenthic species. There may be specific combinations of otherwise inconspicuous features that could provide some hints of a common ancestral condition, while some of these features may turn out instructive as discrete character states usable for the distinction of closely related species within the *Octopus macropus* species complex (Norman 2000).

REFERENCES

Batham EJ 1957. Care of eggs by *Octopus maorum*. *Trans R Soc New Zeal* 84: 629-638.

Bergbauer M, Humberg B 1999. Was lebt im Mittelmeer ? Ein Bestimmungsbuch für Taucher und Schnorchler. Franckh-Kosmos Verlag Stuttgart.

Boletzky S v 1974. The ‚larvae' of Cephalopoda: A review. *Thalassia Jugosl* 10: 45-76.

Boletzky S v 1977. Post-hatching behaviour and mode of life in cephalopods. *In* Nixon, M & Messenger JB eds, The Biology of Cephalopods. *Symp Zool Soc Lond* 38: 557-567.

Boletzky S v 1978-79. Nos connaissances actuelles sur le développement des Octopodes. *Vie Milieu* 28-29 (1AB): 85-120.

Boletzky S v 1987. Embryonic phase. *In* Boyle PR ed. Cephalopod Life Cycles vol. II. Academic Press, London: 5-31.

Boletzky S v 1992. Evolutionary aspects of development, life style, and reproductive mode in incirrate octopods (Mollusca, Cephalopoda). *Rev suisse Zool* 99: 755-770.

Boletzky S v 1997. Developmental constraints and heterochrony: a new look at offspring size in cephalopod molluscs. *Geobios* Mém Sp 21: 267-275.

Boletzky S v 2002. Yolk sac morphology in cephalopod embryos. *In* Summesberger H, Histon K & Daurer A eds, Cephalopods – Present and Past. *Abh Geol B-A* 57: 57-68.

Boletzky S v, Fioroni P 1990. Embryo inversions in incirrate octopods: the state of an enigma. *J Ceph Biol* 1(2): 37-57.

Boletzky S v, Fuentès M, Offner N 2001. First record of spawning and embryonic development in *Octopus macropus* (Mollusca: Cephalopoda). *J Mar Biol Ass UK* 81: 703-704.

Delle Chiaje S 1841. Descrizione e notomia degli animali invertebrati della Sicilia citeriore. I. Molluschi Cefalopodi e Pteropodi. Napoli.

Férussac AE, d'Orbigny A 1835-48. Histoire naturelle générale et particulière des Céphalopodes acétabulifères vivants et fossiles. Baillière, Paris.

Hanlon RT, Forsythe JW, Boletzky S v 1985. Field and laboratory behavior of «Macrotritopus larvae» reared to *Octopus deflippi* Verany, 1851 (Mollusca, Cephalopoda). *In* Mangold K & Boletzky S v eds, Biology and distribution of early juvenile cephalopods. *Vie Milieu* 35: 237-242.

Hochberg FG, Nixon, M, Toll, RB 1992. Order OCTOPODA Leach, 1818. *In* Sweeney MJ, Roper CFE, Mangold KM, Clarke MR & Boletzky S v, eds, "Larval" and Juvenile Cephalopods: A Manual for Their Identification. *Smiths Contr Zool* 513: 213-280.

Jatta G 1896. I Cefalopodi viventi nel Golfo di Napoli. *Fauna Flora Golf Neapel* 23: 1-264.

Mangold K 1998. The Octopodinae from the Eastern Atlantic Ocean and the Mediterranean Sea. *In* Voss NA, Vecchione M, Toll RB & Sweeney MJ eds, Systematics and Biogeography of Cephalopods, vol II. *Smiths Contr Zool* 586: 521-528.

Messenger JB 2001. Cephalopod chromatophores: neurobiology and natural history. *Biol Rev* 76: 473-528.

Naef A 1923. Die Cephalopoden (Systematik). Fauna Flora Golf Neapel 35(I-1): 1-836. [English translation (1972), Smithsonian Institution Libraries, Washington D. C., 20560, U.S.A.].

Naef A 1928. Die Cephalopoden (Embryologie). Fauna Flora Golf Neapel 35(I-2): 1-357 [English translation (2000), Smithsonian Institution Libraries, Washington D. C., 20560, U.S.A.].

Norman M 1992. Four new octopus species of the *Octopus macropus* group (Cephalopoda: Octopodidae) from the Great Barrier Reef, Australia. *Mem Mus Victoria* 53: 267-308.

Norman M 2000. Cephalopods – A World Guide. ConchBooks, Hackenheim (D).

Rees WJ 1955. The larvae and late-larval stages of *Octopus macropus* Risso. *Proc Malac Soc Lond* 31: 185-189.

Rees WJ, Maul GE 1956. The Cephalopoda of Madeira – records and distribution. *Bull Brit Mus Zool* 3: 259-281.

Risso A 1826. Aperçu sur l'histoire naturelle des Mollusques. *In* Histoire naturelle des principales productions de l'Europe méridionale et particulièrement de celles des environs de Nice et des Alpes maritimes, 4: 1-8.

Tardent P 1993. Meeresbiologie – Eine Einführung. Georg Thieme Verlag Stuttgart.

Villanueva R 1994. Decapod crab zoeae as food for rearing cephalopod paralarvae. *Aquaculture* 128: 143-152.

Villanueva R, Nozais C, Boletzky S v 1995. The planktonic life of octopuses. *Nature* 377: 107.

Villanueva R, Nozais C, Boletzky S v 1996. Swimming behaviour and food searching in planktonic *Octopus vulgaris* Cuvier from hatching to settlement. *J Exp Mar Biol Ecol* 208: 169-184.

Vérany JB 1851. Céphalopodes de la Méditerranée. *In* Mollusques Méditerranéens 1: 1-132.

Voss GL, Williamson G 1971. Cephalopods of Hong Kong. Hong Kong Government Press.

Wirtz P 2001. New records of invertebrates from the Cape Verde islands. *Arquipélago* 18A: 81-84.

Reçu le 29 août 2002; received August 29, 2002
Accepté le 16 octobre 2002; accepted October 16, 2002

VIE MILIEU, 2002, 52 (4) : 217-222

From Marine Ecology to Developmental Biology
In Honour of Pierre Tardent (1927-1997)

THE STORY OF *ALCYONIUM*: FROM HALCYON BIRDS TO ZOOPHYTES

E.A. ROBSON

School of Animal & Microbial Sciences, The University of Reading, P.O. Box 228, Reading RG6 6AJ, UK

ALCYONIUM
ALCYONARIA
HALCYONS
ZOOPHYTE
MYTHOLOGY
HISTORY

ABSTRACT. – The name of the soft coral *Alcyonium* is derived from the ancient Greek myth of Ceyx and Alcyone. They were transformed into halcyon birds whose floating nests, empty after the young hatched, were mistakenly identified with Mediterranean flotsam including the remains of sessile colonies or seaweeds detached by wave action and so variously referred to as alcyoniums. During the eighteenth century ground-breaking research on *Hydra* by Trembley (1744) was followed by precise descriptions of hydroids and of *Alcyonium digitatum* (L.) (de Jussieu 1742 and especially Ellis 1755) and the recognition that all "zoophytes" were animals. Subclass Alcyonaria and related taxonomic terms were derived later from the name *Alcyonium*.

ALCYONIUM
ALCYONARIA
ALCYONS
ZOOPHYTE
MYTHOLOGIE
HISTOIRE

RÉSUMÉ. – Le nom du polypier charnu *Alcyonium* provient de l'ancien mythe grec de Ceyx et Alcyone. Ils furent transformés en alcyons, dont les nids flottants, vides après l'éclosion des jeunes, furent identifiés par erreur parmi les débris flottants de Méditerranée où se trouvaient les déchets de colonies sessiles ou d'algues détachées par les vagues, et donc de soi-disant alcyoniums divers. Pendant le XVIII^{e} siècle les recherches révolutionnaires sur l'*Hydra* de Trembley (1744) furent suivies par les descriptions exactes d'Hydroïdes et d'*Alcyonium digitatum* (L.) (de Jussieu 1742 et surtout Ellis 1755), et la perception de tous "zoophytes" comme animaux véritables. La sous-classe Alcyonaria (= Octocorallia) et les noms de proches sous-groupements taxonomiques furent dérivés ensuite du nom *Alcyonium*.

The influence of Greek mythology upon biological nomenclature is considerable (Thompson 1895, 1947) though nowadays often forgotten. As the ancient origins of names recede into the mists of time the names become detached from them. Freed from their original context many names in use since the eighteenth century or earlier have survived far-reaching changes in the perspectives of science. The history of the name *Alcyonium*, briefly presented here, is an example which illustrates how nomenclature may evolve from myth and poetry as well as science. I hope Pierre Tardent would have liked this story, for he appreciated the resources of history and would never underestimate what earlier authors had discovered (Tardent 1987, 1993).

The story runs from antiquity to the eighteenth and nineteenth centuries and is set in scientific perspectives earlier than our own. Before telling it, an attempt is made to recapture these perspectives and to bring less familiar modes of thought into the picture. This account begins with thumbnail sketches which recede century by century from modern times to the Renaissance (Singer 1931, 1959, Singer & Underwood 1962, Bodenheimer 1958, Petit & Théodoridès 1962, and also Roger 1997, Jahn 1998).

Glancing back 50 years we see the post-war 1950's as trailblazing years for modern physiology, for medicine and for the future molecular biology and genetics. Powerful computers were being developed and chess players felt challenged. The theory of plate tectonics was still dormant and its implications were not generally recognized. On the other hand the centenary of "The Origin of Species" was about to be celebrated.

Stepping a hundred years thence to the 1850's one finds horsepower coexisting with steam as railways and factories developed. Darwin's seminal influence was as dawn still below the horizon. But new precepts of comparative anatomy and physiology were already being adopted, the cell theory had gained ground and generation and development were beginning to be understood. Scientific knowledge was increasingly enriched by travel and exploration. Scientists communicated through letters and meetings, and published thanks to a growing number of scientific societies and journals. Their early education at this time might include Latin if not Greek.

The century before this differed even more from our own. In the 1750's Linnaeus' work shone like a beacon. It may be recalled that at this time Aris-

totle's "Ladder of Nature" still spanned the living world (Singer 1931, 1959) and fossils remained puzzling. Many fine naturalists turned their attention to animals and plants, sometimes minerals; and they created or enriched museums and wrote books. By 1750 microscopy had become an important tool. Leeuwenhoek's sophisticated use of simple lenses for high magnification had been unique, but for other researchers even low magnifications were far better than none. The observant naturalists who transcribed what they saw were often pioneers (Trembley 1744). To an enthusiastic contemporary the century was remarkable for the discoveries of electricity and of *Hydra* spp. (Le Cat 1754). At the frontiers of science knowledge was communicated really fast through correspondence and meetings. It was an exhilarating period even if those interested in new ideas were sometimes held back by the influences of legend and history. Within medicine we would recognize the basis of anatomy and of some aspects of physiology and pharmacy, although in 1750 much still lay ahead: most of the basis of modern chemistry, the contributions to physiology of von Haller and J Müller, and advances in the understanding of disease due to Jenner, Pasteur and Lister. Latin served as a lingua franca for special texts, and was an accessible medium.

To step across another hundred years towards 1650 is to experience in a different era the full vigour of scientific discovery and discussion. Charters were granted to the Royal Society in 1662 and to the French Académie des Sciences in 1666. The giants on whose shoulders we stand include Newton, Boyle, Hooke, Malpighi, Hales and many others whose achievements still influence today's young scientist. By 1650 entrainment in Latin and Greek scholarship had become less germane to science and thus from the beginning, the Royal Society of London's Transactions, for example, were published in English.

The records of yet earlier authors in the sixteenth century are of significant interest here as will be seen. Works on natural history, whether erudite compilations (e.g. Gesner 1555, Aldrovandi 1603) or new (Belon 1555) provided detailed reviews of ancient literature, both Greek and Latin. Scholars of all persuasions used Latin themselves and were steeped in Homer and Ovid and so familiar with classical mythology.

The subject of this narrative are the soft corals represented by *Alcyonium digitatum* (L.), a British coastal species commonly known as dead man's fingers. It is in the family Alcyonaceae, order Alcyonacea, subclass Alcyonaria (Hickson 1930) otherwise Octocorallia (Manuel 1981, Fabricius & Alderslade 2001).

The source of the name *Alcyonium* is the Greek myth of Ceyx and Alcyone, which gave rise also to the metaphor "halcyon days". Ovid's well known version of the myth is the best introduction to other sources (e.g. Graves 1992, Forey 2002).

Alcyone, daughter of Aeolus god of the winds and of Anarete was the wife of Ceyx, son of Lucifer. They were king and queen of Trachis. Ceyx and Alcyone found perfect happiness together but tragedy befell them. Ceyx decided to consult an oracle and despite Alcyone's pleadings, he set sail, promising to return. His ship was wrecked in a storm and Ceyx was drowned. Alcyone, anxious with waiting, prayed for his safe return until Juno, weary of petitions, sent her messenger to the god of sleep, who dispatched Morpheus to appear in the form of Ceyx in Alcyone's dream. Alcyone sees her drowned husband only too clearly and awakes in anguish, crying out and too distraught to take comfort. She throws herself into the sea to join him and as she does so she is transformed. Jupiter takes pity on the bereft lovers and Alcyone and Ceyx are turned into halcyon birds.

Halcyons are kingfishers, but to relate the myth as transcribed to details of the birds known to Aristotle or other ancient observers is not straightforward. One might think of terns or gulls or else gannets but the halcyon had a tapered beak and was brightly coloured, with blue head and back and a reddish breast (Thompson 1895, 1910, Belon 1555: 218). None of the present species of kingfisher corresponds to halcyons in nesting habits or distribution.

There is more to the myth. Halcyon birds make a nest which floats unharmed upon a tranquil sea for seven days before and seven days after the winter solstice. The winds drop as Aeolus restrains them. The female lays her eggs and broods, the young birds are hatched and they fly away as the calm halcyon days end. Then Aeolus releases the unruly winds. The substance of the nest is uncertain, including sea foam and perhaps fishbones, but carefully fashioned with the beak to be seamless and unbreakable (e.g. Aldrovandi 1603: 497).

Sailors' legends follow: occasional sightings of "halcyon birds" or calm midwinter spells in the Gulf of Corinth confirm the myth. Halcyon birds in flight are propitious as they bring good weather. A female flying alone is heard to call repeatedly, as if in distress. Maritime legends about halcyons including the medicinal properties of sea foam persist well into the sixteenth century (e.g. Belon 1555: 219).

Ovid's transcriptions of earlier sources have stood the test of centuries and have influenced almost every Western writer (Hughes 1997: vii). The myth of Ceyx and Alcyone retains all its vitality. It has inspired poetry, opera and works of art (Reid 1993). The phrase "halcyon days" is still a literary or political metaphor indicating a fortunate period of untroubled calm (Simpson & Weiner 1989). Halcyon birds have featured in heraldry and also as

emblems (Giovio 1574, Scorza 1981). Fig. 1 is a device from Giovio (1562) which shows halcyon birds on their floating nest during the days of calm weather. The rubric and accompanying verse (Mr CG Wagstaff kindly furnished this translation) are innovative: "The halcyon birds know the elected time for the sea not to harm their nests or their eggs. Unhappy the man who knows not how to wait to give effect to his design". Giovio's device for the Fieschis refers to their strategy for taking up arms in revenge, which proved successful. It was an age when both myth and observation were significant. Paulo Giovio (1483-1552) and the naturalist Pierre Belon (1517-1564) were near contemporaries.

The term Alcyonium sprang from the coexistence of myth, legend and reality. Halcyon birds' nests of sea foam interwoven with fishbones might later reappear among the floating flotsam and jetsam of Mediterranean coasts. Poorly specified objects were called alcyonium or alcyoneum from ancient times (e.g. Pliny: see Thompson 1895). Light weight or of spongy texture, perhaps compound in origin, they might include soft corals, sponges, ascidians, bryozoans and encrusted algae detached by wave action (e.g. Ellis 1755, Ellis & Solander 1786, Johnston 1838). Some of the sponges referred to as alcyoniums had ancient medical uses, an aspect of sea foam which influenced later halcyon legends (e.g. Thompson 1895, 1947, Belon 1555: 219). By the end of the sixteenth century the misapprehension that the several kinds of alcyonium were halcyons' nests had faded away.

The soft coral *Alcyonium digitatum* appears in the 10th edition of Linnaeus' "Systema Naturae", the species described as *Alcyonium ramoso-digitatum molle* by John Ellis (1755) and shown in Fig 2. It was well known from flatfish grounds (Ray 1724). English fishermen called it dead man's hand, or dead man's toes, or cow-paps (Johnston 1838). The Mediterranean species *Alcyonium palmatum* (Pallas 1766) was called *manus marina* (the sea hand) (Gesner 1558:619, Aldrovandi 1606:593). Ancient Greek sponge divers would probably have seen it.

Early in the eighteenth century the boundaries between animals and plants were by no means clear (Robson 1975). In Aristotle's "Ladder of Nature" animals were higher than plants, and minerals were inanimate. The idea of a zoophyte, in the sense of an animal with vegetable attributes, began with Aristotle. Later naturalists observed that there were aquatic plants with stony parts (lithophytes). If the natural world showed every aspect of intergradation lithophytes and zoophytes offered categories which avoided taxonomic boundary problems and were consistent with contemporary outlook. The word "zoophyte" was widely used until the late nineteenth century, by then in a descriptive sense (Simpson & Weiner 1989).

Distinctions between animals and plants were sharpened by Abraham Trembley's seminal experiments on freshwater *Hydra* spp. (Trembley 1744). These were greeted with amazement if not disbelief. That animals which captured prey and walked about could bud, and even regenerate like plants, one type being green besides, caused a great stir, and an upheaval of previously held ideas (e.g. Réaumur 1742, Dawson 1987). Trembley's beautiful work is still as good as new and marks the rise of experimental biology (Baker 1952, Lenhoff & Lenhoff 1986, Tardent 1987). Trembley sent hydra to de Réaumur, who in 1741 confirmed his conclusions. It was he who suggested the term "polype" for hydra by analogy with the Polypi (i.e. cephalopods) of ancient authors, whose eight arms capture prey (Réaumur 1742: li-lxxx; Baker 1952).

The influence of Trembley's research was immediate and widespread. In 1755 Ellis refers to "the Freshwater Polype whose extraordinary properties have been so fully enquired by the ingenious Mr Trembley". Twenty years later it is common knowledge. "Every one knows, that the common *polype* sends out its young from its side, like buds..." (Ellis 1776).

Linnaeus (1758) placed *A. digitatum* in his Zoophyta nonetheless. These were composite animals with plant-like stems in which he included sea pens, gorgonians, hydroids and *Hydra*. He placed corals under Lithophyta. Ray (1724), however, had included *Alcyonium digitatum* and several lithophytes and zoophytes of Linnaeus (e.g. corals) under aquatic plants (Plantae Submarinae). Ellis (1755) used Ray's Latin description of *A. digitatum.* His English rendering for Fig. 2 is "*Alcyonium* of a soft fleshy Nature, with its Surface full of Stars".

Ellis used low magnification (a microscope by John Cuff with single lenses) to great advantage and he was besides an acute and thoughtful observer. He saw living polyps of hydroids and later those of *Alcyonium digitatum* (Ellis 1754, 1755, 1763). In the sponge *Halichondria panicea,* whose texture resembled that of *A. digitatum*, there were no polyps. Instead, oscula opened and closed and water passed through them (Ellis 1763, 1765). He was in no doubt that all of these specimens were animal in nature.

Comparable but less detailed observations were made by Bernard de Jussieu in 1741. Familiar with *Hydra* spp. and the earlier view of Peysonnel (1752) that corals were animals, he visited the north coast of France and upon studying *Tubularia indivisa* and *Alcyonium digitatum* he had reached the same conclusions (de Jussieu 1742, Allman 1871).

Although Linnaeus (1758) incorporated his discoveries Ellis did not think that zoophytes had affinities with plants. His careful observations re-

Fig. 1. – Device by Paolo Giovio (1562) for Sinibaldo and Ottobuono Fieschi of Genoa, showing halcyon birds on their nest under a clear sky. During seven days before and after the winter solstice (the "halcyon days") it floats unharmed upon a calm sea. Giovio (1574) had in mind Pliny's account of the myth. The birds were to be shown in bright colours (azure, red, white, green and yellow). The rubric in French "We know the right time" and the accompanying Italian verse (see text) refer to the Fieschi's strategy for revenging themselves upon their adversaries, which proved successful. Photo: Warburg Institute.

solved uncertainties about the status of those with branching skeletons such as gorgonians (Ellis 1776). As he was still called "the father of English Zoophytology" in 1883 (Simpson & Weiner 1989) his concluding views on myth and natural history may be quoted:

"... although they grow in a branched form, they are no more allied to vegetables than they are to the ramified configurations of *sal ammoniac*; to the elegant branched figures in the Mocha and other gems, called *dendrites;* to the *arbor Dianae*, or the arborescent figures of the Cornish native copper: consequently, that animal life does not depend on bodies growing according to a certain external form. Hence it appears, that this metamorphosis of a plant to an animal is a flowery expression, and in my opinion, better suited to the poetical fancy of an OVID, than to that precise method of describing which we so much admire in a natural historian." (Ellis 1776).

During the nineteenth century the range of soft corals and related Anthozoa (sea fans, sea pens, red coral and others) became better known: knowledge of local marine species increased as did the collection of specimens from much further afield. Several higher taxonomic terms derived from the name *Alcyonium* were introduced, such as the subclass Alcyonaria (Fabricius & Alderslade 2001: 8). These aspects may be traced in a splendid history of researches on Anthozoa by Carlgren (1903). For earliest use of the term Alcyonaria see Dana (1846), Milne-Edwards (1857) and Hickson (1930). For inspired illustrations of soft corals see Saville Kent (1893) and Fabricius & Alderslade (2001). Hickson's first impressions are still vivid (Hickson 1889): "This was my first introduction to

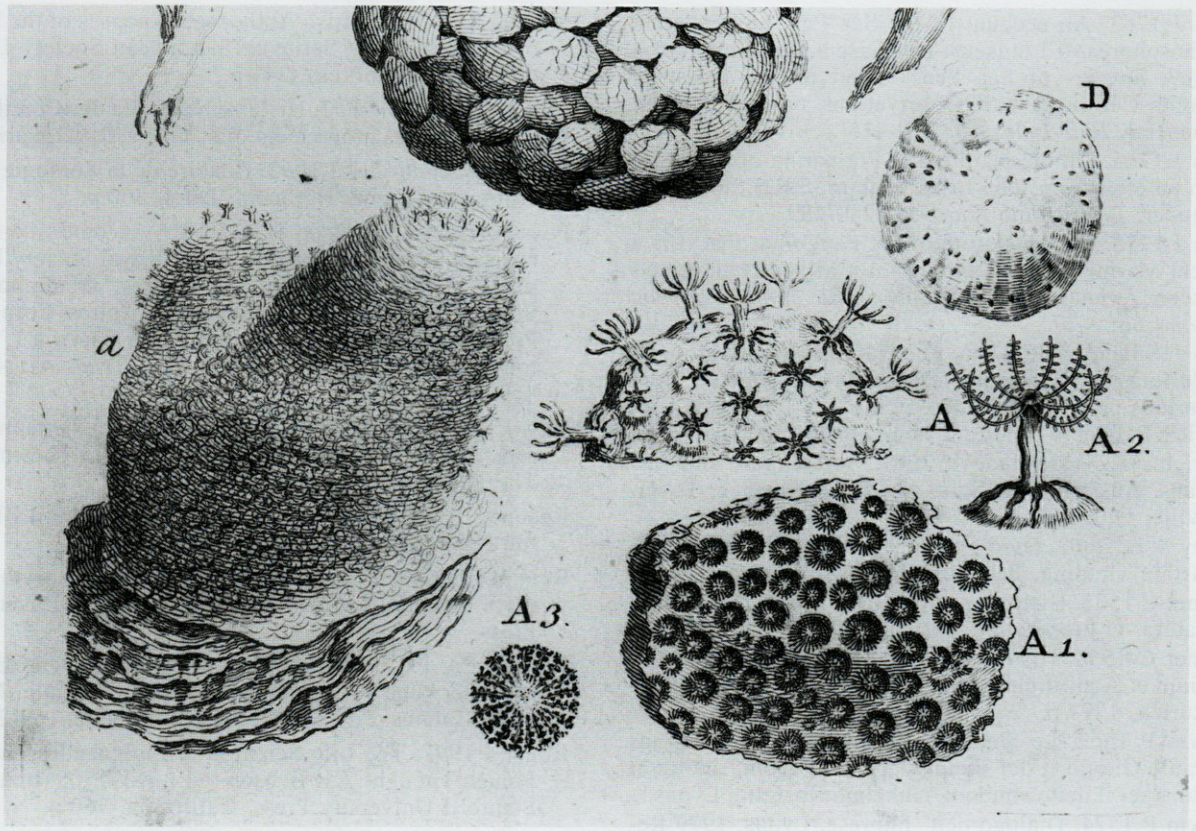


Fig. 2. – *Alcyonium digitatum* (L.), the "*Alcyonium* of a soft fleshy Nature with its Surface full of Stars", from John Ellis (1755). The specimen attached to an oyster shell shown at *a* was collected by fishermen near Whitstable, Kent. At *A* the surface papillae magnified by a lens are seen with 8-pointed stars from which polyps emerge. As shown in *A2*, their 8 tentacles are fringed. Photo: University Photographic Unit, Reading.

a coral reef... I was not prepared to find such brilliancy and variety of colour..." "... it was impossible to put one's foot down on anything save living zoophytes..." and while out on low spring tides "...watching... the slow and graceful waving movements of the Alcyonarian polypes...".

ACKNOWLEDGEMENTS. – I would like to thank Library Staff at the British Library, The Institute of Classical Studies, the Linnean Society, the Natural History Museum, the Royal College of Surgeons, the Royal Society, the University of Reading, the Wellcome Library, and the Librarians and Photocollection Staff at the Warburg Institute for their help, and Mrs M Barratt for typing the manuscript.

REFERENCES

Allman GJ 1871. A monograph of the Gymnoblastic or Tubularian Hydroids. The Ray Society, London, 450 p.

Aldrovandi U 1603. Ornithologiae Tomus Tertius acpostremus. Lib XIX – XX. Bologna, 560 p.

Aldrovandi U 1606. De Reliquis Animalibus exsanguibus libri quattuor... de Mollibus, Crustaceis, Testaceis et Zoophytis. Bellagamba, Bologna, 593 p.

Baker JR 1952. Abraham Trembley of Geneva scientist and philosopher 1710-1784. Edward Arnold, London, 259 p.

Belon P du M 1555. Histoire de la Nature des Oyseaux, avec leurs descriptions, et naifs portraicts retirez du naturel: escrite en sept livres, etc. Gilles Corrozet, Paris, 382 p.

Bodenheimer FS 1958. The History of Biology. William Dawson & Sons, London, 465 p.

Carlgren O 1903. Coelenterata (Hohlthiere). Anthozoa. HG Bronns Klassen und Ordnungen der Thierreichs. Bd II, Abt 2, Buch 3, Lief 1-6. CF Winter, Leipzig, 176 p.

Dana JD 1846. Structure and Classification of Zoophytes. Lea & Blanchard, Philadelphia, 132 p.

Dawson VP 1987. Nature's Enigma: the problem of the polyp in the letters of Bonnet, Trembley and Réaumur. *Mem Amer Phil Soc Philadelphia* 1 74: 266 p.

Ellis J 1754. A Letter from Mr John Ellis, F.R.S. to Mr Peter Collinson, F.R.S. concerning the animal Life of those Corallines that look like minute Trees, and grow upon Oysters and Fucus's all round the Seacoast of this Kingdom. *Phil Trans* 48: 627-633.

Ellis J 1755. An Essay towards a Natural History of the Corallines and other Marine Productions of the like Kind, Commonly found on the Coasts of Great Britain and Ireland, etc. London, 103 p.

Ellis J 1763. An account of the Sea Pen, or Pennatula Phosphorea of Linnaeus; Likewise a Description of a New Species of Sea Pen, Found on the Coast of South-Carolina, with Observations on Sea-Pens in General. *Phil Trans* 53: 419-435.

Ellis J 1765. On the Nature and Formation of Sponges: In a Letter from John Ellis, Esquire, F.R.S., to Dr Solander, F.R.S. *Phil Trans* 55: 280-289.

Ellis J 1776. On the Nature of the Gorgonia; that it is a real Marine Animal, and not of a Mixed Nature, between Animal and Vegetable . *Phil Trans Roy Soc Lond* 66: 1-17.

Ellis J, Solander DC 1786. The Natural History of many Curious and Uncommon Zoophytes, collected from various parts of the globe by the late John Ellis Esq, FRS Benjamin White & Son, London, 208 p.

Fabricius K, Alderslade P 2001. Soft Corals and Sea Fans. Australian Institute of Marine Science, Townsville, Qld, 264 p.

Forey M ed 2002. Ovid's Metamorphoses. Translated by Arthur Golding. Penguin Books, London, 535 p.

Gesner C 1555. Historiae Animalium. Lib III. De Avium natura. C Froschauer, Zürich, 779 p.

Gesner C 1558. Historiae Animalium. Lib IIII. De Piscium et Aquitilium animantium natura. C Froschauer, Zürich, 1297 p.

Giovio P 1562. Le Sententio se imprese di Monsignor Paulo Giovio et del signor Gabriel Simeoni, ridotte in rima per il detto simboni. Guhelmo Rosiglio, Lyons.

Giovio P 1574. Dialogo dell' Imprese. Lyons. 1979 Reprinted with translation. Garland Publishing Inc., New York & London.

Graves R 1992. The Greek Myths. Penguin Books, London, 782 p.

Hickson SJ 1889. A Naturalist in North Celebes. John Murray, London, 392 p.

Hickson SJ 1930. On the classification of the Alcyonaria. *Proc Zool Soc Lond* (1) 229-252.

Hughes Ted 1997. Tales from Ovid. Faber & Faber, London, 264 p.

Jahn I 1998. Geschichte der Biologie: Theorien, Methoden, Institutionen, Kurzbiographien. 3rd ed Gustav Fischer, Jena, 1088 p.

Johnston G 1838. A History of the British Zoophytes. WH Lizars, Edinburgh, 341 p.

Jussieu B de 1742. De quelques productions marines qui ont été mises au nombre des Plantes, et qui sont l'ouvrage d'une sorte d'Insectes de mer. *Hist Acad Roy Sci Paris:* 290-302.

Kent WS 1893. The Great Barrier Reef of Australia. London, 387 p.

Le Cat CN 1754. Abhandlung von den Polypen des süssen Wassers, welche in der Versammlung der königlichen Academie der Wissenschaften zu Rouen abgelesen ist. *Allg Mag Nat Kunst Wiss Leipzig* 3: 1-24.

Lenhoff SG, Lenhoff HM 1986. Hydra and the Birth of Experimental Biology – 1744. Abraham Trembley's *Mémoires* concerning the Polyps. Boxwood Press, California, 192 p.

Linnaeus C 1758. Systema Naturae. Vol 1. Regnum Animale. 10th ed. Stockholm. (1956 Facsimile. British Museum (Natural History), London, 823 p).

Manuel RL 1981. British Anthozoa. Synopses of the British Fauna: (New Series): 18. Linnean Society London. Academic Press, 241 p.

Milne-Edwards H 1857. Histoire Naturelle des Coraillaires ou Polypes proprement dits. Vol. 1. Roret, Paris.

Petit G, Théodoridès J 1962. Histoire de la Zoologie des Origines à Linné. Hermann, Paris, 360 p.

Peysonnel JA de, Watson W 1752. An Account of a Manuscript Treatise, Presented to the Royal Society Intituled, Traité du Corail, les Pores, Madrepores, Scharras, Litophitons, Eponges, et Autres Corps et Productions, que la Mer Fournit, pour Servir a L'histoire Naturelle de la Mer; etc. *Phil Trans* 47: 445-469.

Ray J 1724. Synopsis Methodica Stirpium Britannicarum. 3rd ed G & J Innys, London. (1973 Facsimile. The Ray Society, London).

Réaumur R-AF de 1742. Mémoires pour Servir à l'Histoire des Insectes. Tome 6. Paris.

Reid JD 1993. The Oxford Guide to Classical Mythology in the Arts, 1300-1990s. Vol 1. Oxford University Press.

Robson EA 1975. The nervous system in coelenterates. *In* Usherwood PNR & Newth DR eds, 'Simple' Nervous Systems. Edward Arnold Ltd, London: 169-209.

Roger J 1997. The Life Sciences in Eighteenth-Century French Thought. KR Benson (ed.), R Ellrich (transl.). Stanford University Press, California, 760 p.

Scorza RA 1981. Vincenzo Borghini and *Invenzione:* the Florentine *Apparato* of 1565. *J Warburg & Courtauld Insts* 44: 57-75.

Simpson JA, Weiner ESC eds 1989. The Oxford English Dictionary. 2nd ed. Clarendon, Oxford.

Singer C 1931. A Short History of Biology. Clarendon Press, Oxford, 572 p.

Singer C 1959. A Short History of Scientific Ideas to 1900. Clarendon Press, Oxford, 575 p.

Singer C, Underwood EA 1962. A Short History of Medicine. 2nd ed. Clarendon Press, Oxford, 854 p.

Tardent P 1987. *Hydra*. Neujahrsblatt/Naturforschende Gesellschaft in Zürich; 190 Stück, 1988. 100 p.: ill., ports.

Tardent P 1993. The Cnidaria – in spite of their highly innovative achievements – an evolutionary dead end. Lecture given at the 5th International Workshop on Hydroid Development, Schloss Reisenburg, Germany, 22-26 September, 1993, 26 p.

Thompson D'AW 1895. A Glossary of Greek Birds. Clarendon Press, Oxford 204 p.

Thompson D'AW 1910. The Works of Aristotle. Vol IV. Historia Animalium. Translated into English. Clarendon Press, Oxford.

Thompson, D'AW 1947. A Glossary of Greek Fishes. Oxford University Press, London, 302 p.

Trembley A 1744. Mémoires, pour servir à l'histoire d'un Genre de Polypes d'eau douce à Bras en forme de Cornes. Jean & Herman Verbeek, Leiden, 324 p.

Reçu le 24 mai 2002; received May 24, 2002
Accepté le 24 juillet 2002; accepted July 24, 2002

VIE MILIEU, 2002, 52 (4) : 223-228

From Marine Ecology to Developmental Biology
In Honour of Pierre Tardent (1927-1997)

PIERRE TARDENT: A PASSION FOR MARINE BIOLOGY

R. HONEGGER, T.G. HONEGGER***

**Institut für Pflanzenbiologie, Universität Zürich, Zollikerstrasse 107, 8008 Zurich, Switzerland*
***Zoologisches Institut der Universität Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland*

correspondence: thho@zool.unizh.ch

ABSTRACT. – The Swiss zoologist Pierre Tardent, professor at the University of Zurich, was a leading expert on the biology and ecology of Cnidaria. As a generalist he had a deep interest in marine life and accumulated an impressive knowledge ever since his postdoctoral studies at the Stazione Zoologica of Naples and at the Friday Harbor Marine Laboratories of the University of Seattle. At Naples he was as an assistant of Reinhard Dohrn and soon became director of the zoology department. Pierre Tardent was an excellent university teacher in zoology for biology and medical students. Over a time span of 30 years he annually came to the Laboratoire Arago in Banyuls-sur-Mer to teach marine biology classes and courses for advanced students of the University of Zurich. His colleagues, collaborators, former students and friends remember Pierre Tardent as an outstanding scientist, an inspiring mentor and a dear friend.

RÉSUMÉ. – Le zoologiste suisse Pierre Tardent, professeur à l'Université de Zurich, était un éminent spécialiste de la biologie et de l'écologie des Cnidaires. En tant que généraliste, il s'intéressait profondément à la vie marine, et depuis ses études post-doctorales qu'il avait menées à la Station Zoologique de Naples et aux Friday Harbor Marine Laboratories de l'Université de Seattle, il avait accumulé un savoir impressionnant. A Naples il commençait comme assistant auprès de Reinhard Dohrn, pour devenir ensuite directeur du Département de zoologie. Pierre Tardent était un excellent enseignant de zoologie pour les étudiants de biologie et de médecine. Pendant trois décennies, il a séjourné au moins une fois par an au Laboratoire Arago pour donner des cours de biologie marine aux étudiants de deuxième cycle et des cours plus spécialisés aux étudiants avancés de l'Université de Zurich. Ses collègues, ses collaborateurs, ses anciens étudiants et tous ses amis gardent le souvenir du scientifique de grande qualité, animateur de la recherche, ami cher à tous.

INTRODUCTION

Pierre Tardent, surrounded by his students of the University of Zurich, relaxed and satisfied with a rich harvest of marine life, enjoyed returning from a field trip on the "Nereis", a boat of the Laboratoire Arago in Banyuls-sur-Mer (Fig. 1). This is one of the many happy memories we treasure from the numerous marine biology courses we were able to teach with him at the marine biology station of the Université Pierre et Marie Curie (Paris) in the western Mediterranean. Unforgettable for everyone was Pierre's excitement when the dredge poured its contents over the deck like a marine horn of plenty. He immediately started to pick up invertebrates and fish, sort them out and explain what they were, handing them to students for safe storage in special containers for later investigation in the classroom. However, the majority of students were unable to fully appreciate his generous offers, they were too seasick to bend over the floor of the small, rolling boat where these marine treasures emitted a smell that gave the decisive turn to the already tortured stomach. Although he was born and grew up in Switzerland, the little country in the centre of Europe with no access to the sea, Pierre never suffered from nausea at sea, at least not in the many years we knew him. Why was this ? And how could a Swiss become an expert on marine life and write a textbook on marine biology ?

Pierre Tardent studied zoology at the University of Bern (Switzerland). His professors were the developmental physiologist Fritz E Lehmann and the developmental biologist Fritz Baltzer. He received his PhD in 1953 for a thesis on the distribution of interstitial cells and their role in regeneration of the

freshwater polyp *Hydra* and the marine hydropolyp *Tubularia*. Baltzer was a former student of Theodor Boveri, the eminent German zoologist who had carried out his classical experiments on the fertilization of sea urchin eggs at the Stazione Zoologica in Naples (SZN) from 1889 onwards. Baltzer used the sea urchin egg as a model system for exploring nuclear-cytoplasmic interactions. Like Boveri he kept close connections to the SZN throughout his professional life. Pierre Tardent enthusiastically took up Baltzer's suggestion of postdoctoral studies at the Stazione Zoologica in Naples, the world-renowned marine biology institution at the Mediterranean in Southern Italy.

Stazione zoologica di napoli

Founded in 1872 as a private institution by the German zoologist Anton Dohrn and presided over by members of the Dohrn family until 1967 (Fantini 2002) the Stazione Zoologica offered lab space and research facilities including chemicals, equipment, ships and an outstanding library to visiting scientists. In the second half of the 19th century marine organisms became the centre of interest among many scientists who explored their diversity or found interesting model systems for experimental research. Thus the SNZ soon became an important working and meeting place of leading scientists from all over Europe and beyond. Similar institutions, usually linked to universities, were founded in many other places: Ostende at the Northsee being the first (1843), Concarneau at the Atlantic (1850), Roscoff and Wimereux at the Channel (1873), Banyuls-sur-Mer in the Western Mediterranean (1881). Marine biology stations were also founded in England, Germany, the United States and Japan. In the second half of the 19th century many Swiss investigators realised that they had to collaborate with such institutions in order to have access to marine research facilities. Already in 1882 a Committee was founded under the auspices of the Swiss government, which rented a table at the Station Biologique de Roscoff in Brittany and at the Stazione Zoologica in Naples, i.e. paid certain amounts of money per year to these institutions and thus guaranteed lab space and access to the facilities for visiting Swiss scientists. These contracts were annually renewed, with interruptions during the very difficult periods of World War I and II. Switzerland and Sweden were among the first to renew their contracts with the SZN after World War II.

Pierre Tardent became an assistant of the director, Reinhard Dohrn, at the Stazione Zoologica in the post-war period when the Institute and the whole region slowly recovered from the severe disturbances. His small salary was paid from a grant donated by the Elie Lilly Company on a three months basis. His duties were to supply the numerous visiting scientists with the marine organisms they needed for their research, a unique chance for the highly motivated young zoologist not only to meet investigators from all over the world with interesting research projects, but also to learn a lot about biodiversity and ecology of marine organisms. Later Pierre respectfully acknowledged the immense knowledge and skills of the fishermen of the Stazione whom he regularly accompanied on their collecting trips. Assistance was also needed in secretarial and editorial work related to the series Pubblicazioni della Stazione Zoologica di Napoli. Pierre certainly fulfilled his duties with utmost care, reliability and commitment and was appointed director of the zoology department of SZN in 1956, only three years after having received his PhD. In this post he continued his scientific studies on the regenerative capacity of *Tubularia*.

Before leaving Switzerland for Naples Pierre Tardent married Ruth Siegfried, a graduate of a commercial school (Fig. 2). Pierre and Ruth were a devoted couple throughout their married life and their beautiful friendship lasted until his untimely death. With her professional training and skills Ruth was Pierre's right hand. She was a constant source of encouragement and understanding. An excellent secretary, she had the overview of his agenda and typed innumerable letters and manuscripts not only in breathtaking speed, but also with hardly any typing errors. In the lab she patiently took care of cnidarians and other animals in culture. All this work was done voluntarily; only in the last two years before Pierre's retirement did she have a paid position. Both considered their years in Naples in the stimulating atmosphere of the Stazione Zoologica as a very good and interesting period. The young couple was happy to announce the birth of their first daughter Claudine. Pierre was a proud and warm-hearted father. His beloved family was the centre of his life.

In 1959 Pierre Tardent and his family moved to the United States where, with a grant from the Rockefeller Foundation, he did postdoctoral studies first with Professor Arthur Whitley at the University of Seattle and its marine laboratory in Friday Harbor on San Juan Island, then in the Carnegie Institute in Baltimore. In spring 1960 the family moved back to Naples. In 1962 Pierre was appointed Senior Research Assistant ("Oberassistent") by Professor Ernst Hadorn at the Zoological Institute of the University of Zurich, Switzerland. Here he became an assistant professor in 1963 and a full professor in 1968.

The knowledge, skills and expertise Pierre Tardent had accumulated at the Stazione Zoologica in Naples served him later in research, in teaching and in his duties as a member of the Commission of Oceanography and Limnology (COL) of the Swiss Academy of Science, which he presided over from 1972 to 1975, and as a member of the Scientific

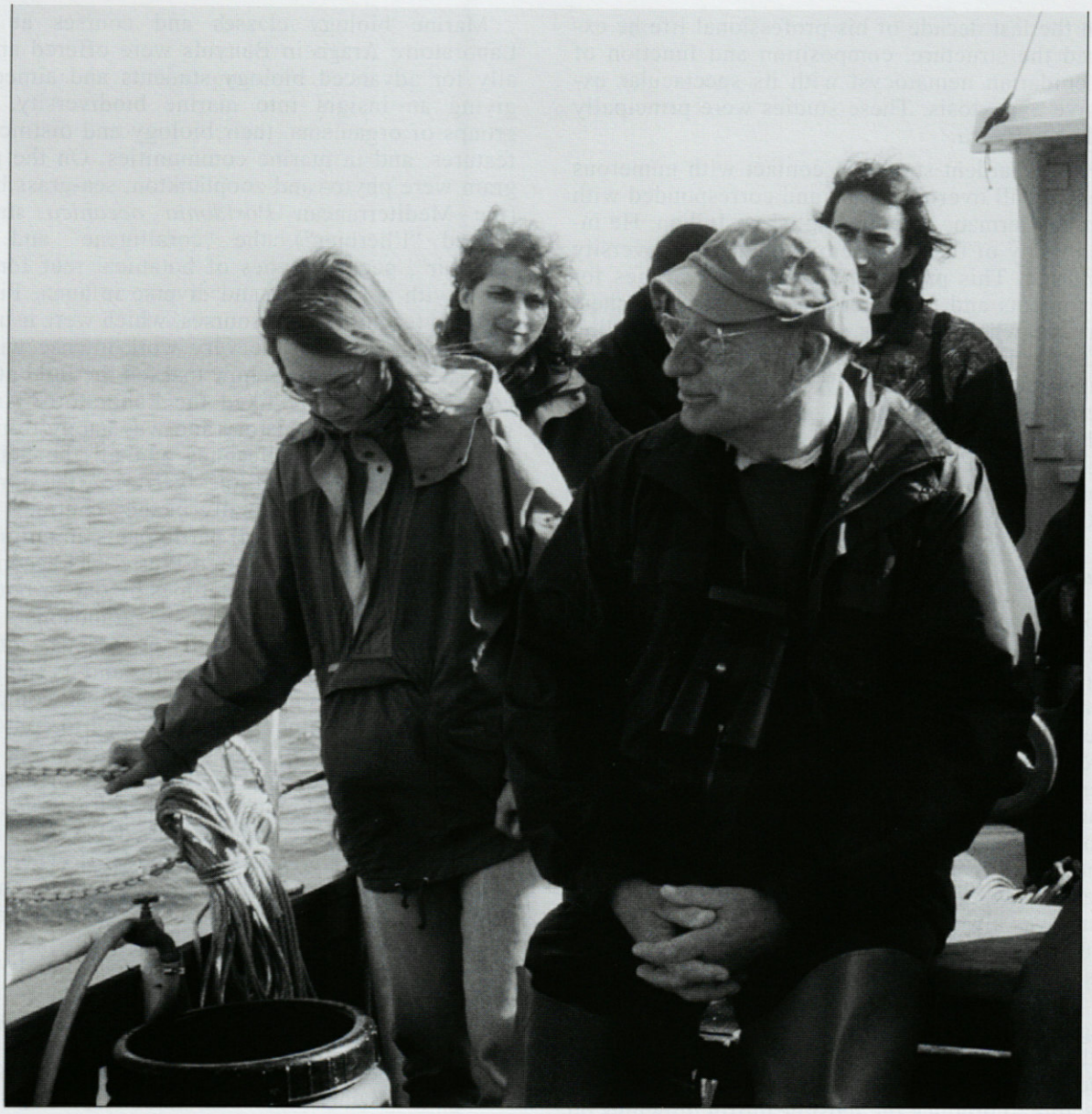


Fig. 1. – Pierre Tardent and students of the University of Zurich on the "Nereis" (boat of the Laboratoire Arago) after a successful collecting trip near Banyuls-sur-Mer (1994). Photograph by Thomas Honegger.

Advisory Board of the Stazione Zoologica from 1988 to 1991. His textbook on marine biology "Meeresbiologie" (Tardent 1979, 1993) is at least partly a fruit of Pierre Tardent's manifold activities at the Stazione Zoologica in Naples.

Research

Pierre Tardent was a zoologist through and through, a generalist with a very broad knowledge of systematics and ecology, general biology and cell biology. He was interested in almost all animals from elephants to minute plankton species and mentioned that he would have loved to become a director of a zoo. He was a passionate birdwatcher. Pets of the Tardent family were tortoises, some of which had already been kept by Pierre's father. However, his main scientific interest was attracted by certain classes of marine invertebrates, top favourites being the cnidarians with their astonishing lifestyles, shapes and colours and their immense beauty. Pierre Tardent's research activities (reviewed by Galliot & Schmid 2002) focused on the development and cell biology of cnidarians, especially on the generation of morphogenetic fields and their regulatory properties, the induction of organization centres, interspecific histocompatibility between cells and tissues, and the regenerative potential. As a leading expert in his field Pierre Tardent completed the volume on *Cnidaria* in the series "Morphogenese der Tiere" (Tardent 1978)

In the last decade of his professional life he explored the structure, composition and function of the cnidarian nematocyst with its spectacular explosive exocytosis. These studies were principally made on *Hydra*.

Pierre Tardent stayed in contact with numerous scientists all over the globe and corresponded with them in German, French, English or Italian. He invited many of them for seminars at the University of Zurich. This provided unique opportunities for his students and collaborators to meet researchers at a time when congress attendance and travelling to far destinations was much more exceptional for young people than today. A highlight was the 4th International Coelenterate Congress in 1979, splendidly organized by Pierre and Ruth Tardent in Interlaken (Switzerland). The contributions were published as "Developmental and Cellular Biology of Coelenterates" (Tardent & Tardent 1980).

Teaching

Pierre Tardent was a charismatic teacher. His enthusiasm was contagious, he liked teaching and he liked students, altogether an ideal constellation. In our university system full professors have to teach at all levels of education, and Pierre took this burden very seriously and spent lots of time and energy in carefully preparing his lectures and courses. He was not only teaching zoologists, but also first year medical students, and there were hundreds of them every year. As a consequence Pierre, having taught a whole generation of physicians, was always warmly greeted and very kindly treated when ill, especially at hospital where he was immediately recognised by medical doctors of all hierarchical levels. Whether lecturing in the biggest lecture hall of the University of Zurich or in the classroom at a marine station: Pierre had his special style. As a gifted painter and illustrator he was able to produce the most beautiful drawings on the blackboard in no time. Not only were all proportions perfect, the size of the drawing was adjusted to the size of the lecture hall, and so was the writing. By varying the thickness of the line Pierre could almost generate 3D effects. Step by step was the drawing completed, often with different colours, and the different parts neatly labelled. To protect his decent suits from the dust of all these different crayons Pierre used to wear a white lab coat when lecturing. While taking their notes the students absorbed the matter, and although their professor was pretty fast, they could follow him. Today students are bombarded with lots of sophisticated computer presentations, colourful and very rich in data, and some professors are astonished to see that this wealth of information adheres to their student's minds no better than water to the plumage of waterfowl.

Marine biology classes and courses at the Laboratoire Arago in Banyuls were offered annually for advanced biology students and aimed at giving an insight into marine biodiversity, i.e. groups of organisms, their biology and distinctive features, and in marine communities. On the program were phyto- and zooplankton, sea-grass beds (the Mediterranean *Posidonia oceanica* stands termed "l'herbier"), the "coralligene" and the "trottoir", peculiar types of botanical reef formations with a very rich and diverse infauna. Pierre enjoyed teaching these courses, which were usually held in spring after the very work-intense winter semester and thus somehow marked the light at the end of a tunnel. He liked the Laboratoire Arago with its interesting history and was happy to meet many friends and colleagues and all the helpful people in the secretariat, the "verrerie", the library, the aquarium and especially in the "cantine" who made these courses for all participants an unforgettable experience. Very often Pierre was accompanied by his wife Ruth, sometimes by one or both of their daughters, later even by grandsons. Surrounded by his beloved family at the coast of the Mediterranean with all its treasures and its rich cultural heritage meant for Pierre almost complete happiness. From time to time we tried to convince him that we ought to take the students to the Station de Biologie Marine at Roscoff in Brittany in the Northwest of France, which we love and visit regularly ever since our students days, especially enjoying beach collecting at low tide. However, Pierre's affection was with the Mediterranean, and as with his human friendships he was a faithful man.

In courses we were always fascinated by Pierre's almost incredible patience. Students could ask unbelievably stupid questions, but Pierre never became angry and he explained the same matter again and again if required, always with the same kindness and enthusiasm. This was surprising since he was, in many aspects of life, not a patient person at all. Unforgettable is how he used to walk nervously up and down the pier in front of the Laboratoire Arago early in the morning when plankton was ordered from the fishermen of the Institute for the day's program, and Pierre assumed that they would bring it in around eight am although everyone knew that this would never happen before nine in this part of the world. Once the plankton was there Pierre started to explore it with great excitement. Armed with a dissecting microscope, pipettes, depression slides and small Petri dishes he isolated specimens and shouted, like at a market stand: "a trochophora, who wants a trochophora larva ? Oh, and here we have a beautiful *Obelia*, who wants an *Obelia* medusa ?" Students were queuing up to get the samples for closer examination.

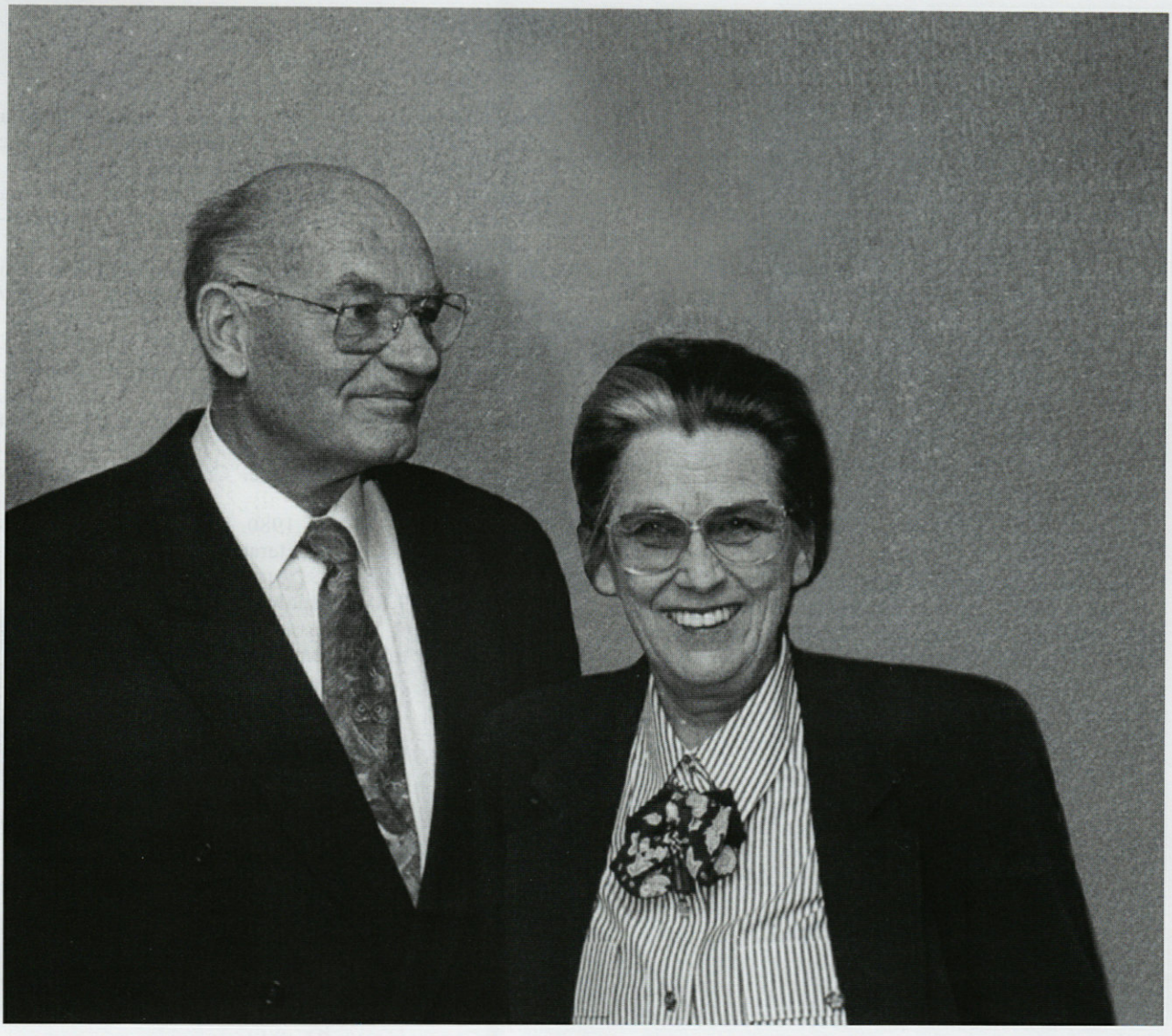


Fig. 2. – Pierre and Ruth Tardent in spring 1994 at a reception offered by the Laboratoire Arago to celebrate Pierre's retirement. Photograph by Jean Lecomte.

Family and friends

Pierre Tardent was a man of great politeness and correctness, and he had lots of style. As customary among professors of his generation in German-speaking countries he kept considerable distance from his students and co-workers, but at the same time he was a warm-hearted mentor and very much concerned about the well-being of everyone around him. Life-long friendships resulted and not only with companions of his professional life. The hospitality of Ruth and Pierre Tardent in their beautiful home was legendary. Among their best friends from their times in Naples who came to see them regularly was Ilona Richter, the renowned scientific illustrator from Szeged, Hungary, who created the overwhelmingly beautiful colour plates of marine invertebrates for two monographs of the *Fauna e Flora del golfo di Napoli*, "Anthomedusae-Athecatae (Hydozoa, Cnidaria) of the Mediterranean" (Brinckmann-Voss 1968) and "Opisthobranchia des Mittelmeers" (Schmekel & Portmann 1982). Pierre and Ruth greatly admired her for her skills and dedication and had a deep understanding for her professional and private situation. Pierre himself greatly enjoyed drawing, both free and scientific, and he was a very talented painter. His book on marine biology (Tardent 1979, 1993) is one of the few textbooks in which the beautiful line drawings are from the same hand as the text. To his and Ruth's great pleasure their first daughter Claudine became an artist and arts teacher, the younger, Josiane, a zoologist and biology teacher.

Among the people whom Pierre Tardent always mentioned with great affection was a farmer's family from the Swiss Alps in the very steep valley of Gadmen in the Bernese Oberland. Already his grandparents had regularly spent their summer vacations in the old wooden house of this family, and from his early childhood to the end of his life Pierre loved this place. He went there regularly with his own family and was very pleased to see how happy his own children and grandchildren were at this impressively beautiful site. He wished his ashes to be buried there, in the colourful meadow under the larch tree where he had spent so many happy hours with his beloved wife Ruth, their two daughters and four grandsons.

We very respectfully and gratefully remember Pierre Tardent as an outstanding scientist, as a stimulating mentor and dear friend with whom we shared so many pleasures in our professional and private lives.

ACKNOWLEDGEMENTS. – Our sincere thanks are due to our friend Ruth Tardent for valuable information, to Jean Lecomte for providing Figure 2, and to Jean-Jacques Pittet for his competent help with the artwork.

REFERENCES

Brinckmann-Voss A 1970. Anthomedusae-Athecata (Hydrozoa, Cnidaria) of the Mediterranean. *Fauna e Flora del Golfo di Napoli* 39: 1-96.

Fantini B 2002. The history of the Stazione Zoologica Anton Dohrn. An outline. Published by Stazione Zoologica Anton Dohrn Napoli, 37 p.

Galliot B, Schmid V 2002. Cnidarians as a model system for understanding evolution and regeneration. *Int J Dev Biol* 46: 39-48.

Schmekel L, Portmann A 1982. Opistobranchia des Mittelmeeres: Nudibranchia und Saccoglossa. *Fauna e Flora del Golfo di Napoli* 40; Springer, Berlin, 410 p. (Awarded as the most beautifully illustrated book of the year 1982).

Tardent P 1978. Coelenterata. Cnidaria. VEB Gustav Fischer Jena.

Tardent P, Tardent R eds 1980. Developmental and cellular biology of Coelenterates. Elsevier /North Holland Biomedical Press Amsterdam. 499 p.

Tardent P 1979 Meeresbiologie. Thieme, Stuttgart.

Tardent P 1993. Meeresbiologie. 2. neubearbeitet und erweiterte Aufl. Thieme, Stuttgart: 304 p.

VIE ET MILIEU: Volume 52

TABLE DES MATIÈRES – *TABLE OF CONTENTS*

Les évaluateurs suivants ont examiné les manuscrits publiés dans le tome 52. La rédaction leur exprime sa reconnaissance pour leurs analyses critiques.

The following persons have reviewed manuscripts published in volume 52. Their constructive comments have been valuable for the authors and are greatly appreciated by the editorial board:

Akesson B., Göteborg, Sweden ; Amouroux J.M., Banyuls/Mer, France ; Bachelet G., Arcachon, France ; Baltanás A, Madrid, Spain ; Bauchot ML, Paris, France ; Blackstone N.W., Dekalb, USA ; Boletzky S.v., Banyuls/Mer, France ; Bouchereau JL., Pointe à Pitre, Guadeloupe ; Boutin C., Toulouse, France ; Boyle PR., Aberdeen, Scotland ; Briarty G., Nottingham, England ; Buergin T, St Gallen, Suisse ; Byrne M., Sydney, Australie ; Calder D., Toronto, Canada ; Clamens A., Montpellier, France ; Coineau N., Banyuls/Mer, France ; Collins M., Aberdeen, Scotland. ; de Bovée F, Banyuls/Mer, France ; Dejean A., Toulouse, France ; Desrosiers G., Rimousky, Canada ; Elinson R, Toronto, Canada ; Faust M., Suitland, USA. ; Fiers F., Bruxelles, Belgique ; Gaudy R., Marseille, France ; Gumbrich T., Stockholm, Sweden ; Hily C., Plouzané, France ; Honegger T., Zurich, Suisse ; Kjesbu O.S., Bergen, Norway ; Kress A., Basel, Suisse ; Lescher-Moutoué F, Paris, France ; Martin J.L, Montpellier, France ; Mather J., Lethbridge, Canada ; Mesleard F, Arles, France ; Mielke W., Göttingen, Germany ; Morte S., Valencia, Spain ; Motos L., Pasaia, Spain ; Panigada S., Milan, Italy ; Pearse V., Santa Cruz, USA ; Pernet B., Friday Harbor, USA ; Picard A., Banyuls/Mer, France ; Pospisil P., Vienne, Autriche ; Reid J.W., Martinsville, USA ; Robson E., Reading, UK ; Rodriguez-Prieto C., Girona, France ; Sala E., La Jolla, USA ; Sasal P., Perpignan, France ; Schmid V., Bâle, Suisse ; Sella G., Torino, Italy ; Sleigh M.A., Southampton, U.K. ; Thiebaut E., Paris, France ; Turon X., Barcelona, Spain ; Verdhuijzen P., Amsterdam, The Netherlands ; Villanueva R., Barcelona, Spain ; Winsor M.P., Toronto, Canada.

Directeur de la publication : G. BOEUF

Printed in France *Dépôt légal 4e trimestre 2002* *Commission paritaire no 22217*

Imprimerie Louis-Jean, 05002 GAP cedex — Dépôt légal 940 — Décembre 2002

INSTRUCTIONS AUX AUTEURS

TEXTE

Les manuscrits, dactylographiés en double interligne sur le recto seulement des feuilles numérotées (ne pas excéder 20 pages) sont présentés en trois jeux complets, sous leur forme définitive.

Le titre du manuscrit doit être le plus court possible; il est suivi du prénom et du nom de l'auteur (ou de chacun des auteurs) ainsi que de l'adresse (ou des adresses) du Laboratoire dans lequel a été effectué le travail.

Chaque manuscrit comportera :
— un résumé en français de 15 lignes maximum figurant en début d'article, suivi de sa traduction en anglais,
— des mots clés en français et anglais (6 au maximum) permettant un traitement rapide par des revues analytiques,
— un titre abrégé pour haut de page (60 signes et espaces au plus),
— la traduction anglaise du titre de l'article,
— une liste hors-texte des légendes des illustrations et leur traduction en anglais,
— une liste hors-texte des légendes des tableaux numérotés en chiffres romains et traduites en anglais.

Les noms scientifiques (genres, espèces, sous-espèces) figurent en italiques ou soulignés d'un seul trait.

Les références bibliographiques des auteurs cités dans le texte sont regroupées à la fin du manuscrit dans l'ordre alphabétique des noms d'auteurs; elles doivent être conformes aux modèles suivants :

Park GS, Park SY 2000. Long-term trends and temporal heterogeneity of water quality in tidally mixed estuarine waters. *Mar Poll Bull* 40 (12): 1201-1209.

Edvardsen B, Paasche E 1998. Bloom dynamics and physiology of *Prymnesium* and *Chrysochromulina. In* Anderson DM, Cembella AD & Hallegraeff GM eds, The Physiological Ecology of Harmful Algal Blooms. Springer Verlag, Heidelberg: 193-208.

Le titre des périodiques doit être abrégé d'après les règles internationales (World list of Scientific Periodicals).

Les notes infrapaginales et les remerciements seront aussi brefs que possibles.

ILLUSTRATIONS

Les figures au trait doivent être exécutées à l'encre de chine sur papier calque assez fort, bristol, carte à gratter, papier millimétré bleu. Il est exigé des lettres et chiffres « transfert » par caractères autocollants pour le lettrage et la numérotation, dont la taille tient compte de la réduction à supporter. Les figures sont regroupées au maximum en planches dont la justification pleine page est de 17 × 24,35 cm une fois réduites (penser à retrancher la légende de ces dimensions); largeur d'une colonne : 8,1 cm. Méthode des « rectangles homologues » pour la réduction : tracer les diagonales d'un rectangle de 17/24,35 cm, les prolonger; tout rectangle admettant ces prolongements comme diagonales correspondra à la justification après réduction. Indiquer le numéro d'ordre des figures en chiffres arabes et le nom de l'auteur au crayon bleu au recto ou au dos. Ne pas inscrire de légende sur les illustrations.

Regrouper les similis (photographies, lavis...) en planches. Employer une échelle graphique qui sera réduite avec la figure, et un lettrage par transfert. Tableaux et planches similis sont numérotés de I à N en chiffres romains. Limiter le nombre des tableaux et ne retenir que ceux qui sont indispensables à la compréhension du travail.

La revue publie gratuitement 2 planches au trait pleine page et 1 seule planche en simili; les illustrations supplémentaires ou en couleurs sont facturées aux auteurs.

ÉPREUVES ET TIRÉS À PART

Un jeu d'épreuves accompagné du manuscrit est envoyé à l'auteur qui doit retourner l'ensemble après correction et indication de la place des illustrations dans le texte, dans un délai maximum de 15 jours.

Les tirages à part, facturés directement par l'imprimeur, doivent être commandés dès réception de l'imprimé spécial joint aux épreuves.

INSTRUCTIONS TO AUTHORS

TEXT

The manuscript should be typed double-spaced on one side of white paper, format A4, pages numbered (max. 20 pp), and sent in definitive form, in triplicate, to the editor.

The title of the paper should be as concise as possible; it is followed by the first and second name of the author(s) and address of the laboratory at which the work was done.

Each manuscript should include:
— a french summary of 15 lines max., followed by the english translation,
— key words in french and english (6 max.) allowing treatment by abstracting journals,
— short version of title (60 signs and spaces max.),
— english translation of the full title,
— a separate list of figure legends in english and french,
— a separate list of numbered tables with their legends in english and scientific names (genera, species, sub-species) should be written in italics or underlined once.

References of papers cited in the text should be listed at the end of the manuscript in alphabetical order, according to the following models:

Park GS, Park SY 2000. Long-term trends and temporal heterogeneity of water quality in tidally mixed estuarine waters. *Mar Poll Bull* 40 (12): 1201-1209.

Edvardsen B, Paasche E 1998. Bloom dynamics and physiology of *Prymnesium* and *Chrysochromulina. In* Anderson DM, Cembella AD & Hallegraeff GM eds, The Physiological Ecology of Harmful Algal Blooms. Springer Verlag, Heidelberg: 193-208.

The title of a journal must be abbreviated according to the rules set by the World list of Scientific Periodicals.

Footnotes and acknowledgments should be as brief as possible.

ILLUSTRATIONS

Line drawings should be prepared with India ink on heavy tracing paper, bristol board, white drawing-cardboard or graph paper (blue grid). Letters and numbers on figures should be large enough to be easily readable after reduction; use letter transfer equipment. As far as possible, figures should be grouped in plates of 17 × 24.35 cm final size (generally the explanations are included in this frame); the width of a single column is 8.1 cm. Prepare figures using the "homologous rectangles" rule; indicate the number of the figure and the author's name with blue pencil on the back of the figure. Do not write explanations on the figure.

Photographs and half-tone drawings must always be grouped as plates. Scale bars are reduced with the figures. Number plates and tables in roman numbers on the back. Limit total number of tables to an absolute minimum of items indispensable for clear presentation.

The journal publishes free of charge two plates with line drawings and one plate of half-tone figures per article. Additional illustrations and colour plates are charged to the author(s)

GALLEY-PROOFS AND REPRINTS

One set of galley-proofs is sent with the manuscript to the author(s); both proofs and manuscript must be returned to the editor with indications on indispensable corrections and insertion of figures within two weeks.

Reprints can be ordered by using the form enclosed with the galley-proofs.

VIE ET MILIEU, 2002, 52 (4)

Life & Environment

From Marine Ecology to Developmental Biology – In Honour of Pierre Tardent (1927-1997)

edited by S.v. Boletzky, J.-P. Féral, D.K. Hofmann, H.-J. Marthy

SOMMAIRE – *CONTENTS*

Indexé dans / *indexed in* : **Current Contents**/Agriculture, Biology & Environmental Sciences, Animal Behavioural Abstracts, Aquatic Sciences and Fisheries Abstracts (ASFA), Biological Abstracts, Bowker international Serials Data base, Current Awareness in Biological Sciences, Elsevier / Geo Abstracts, Marine Science Content Tables, NISC FishLit data base - South Africa, Pascal (INIST-CNRS), Science Citation Index, Zoological Record (BIOSIS).