MORPHOMETRICAL ANALYSIS OF EARLY DEVELOPMENT IN *Fucus spiralis* L. (Phaeophyta).

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ABSTRACT: López Rodas, V. & E. Costas. 1995. Morphometrical analysis of early development in *Fucus spiralis* L. (Phaeophyta). Revista de Biología Marina, Valparaíso, 30(1) :45-56

The main morphometrical parameters (volume, coefficient of form, surface/volume ratio, symmetry, growth equation and structural complexity) were analyzed in early embryos of *Fucus spiralis*. The bigger zygotes produced embryos with rhizoid outgrowth prior to cell division and the smaller zygotes produced embryos with cell division prior to rhizoid outgrowth. Polarization and asymmetry between the two earliest thallus cells is also necessary for normal development of embryos. In our experiments however *Fucus* embryos showed great plasticity in the early steps of development because different developmental patterns resulted in a normal embryo. Significative difference in growth equation and structural complexity appeared among the different developmental patterns of the embryos.

Key words: Morphometry; size; form; growth equation; early development; *Fucus spiralis*.

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Se analizaron distintos parámetros morfométricos (volumen, coeficiente de forma, relación superficie/volumen, simetría, ecuación de crecimiento y complejidad estructural) en los primeros estadios embrionarios de *Fucus spiralis*. En los zigotos grandes los embriones emitían rizoides antes de la división celular y los zigotos pequeños producen embriones que se dividen antes de que aparezca el rizoide. La polarización y la asimetría entre las dos células del talo son necesarias para el desarrollo normal de los embriones. Además los embriones de *Fucus* muestran una gran plasticidad en los primeros estadios de desarrollo y aparecen patrones distintos de desarrollo que dan como resultado embriones normales, aunque existen diferencias significativas en la ecuación de crecimiento y complejidad estructural entre los distintos patrones analizados.

Palabras claves: Tamaño; forma; ecuación de crecimiento; desarrollo temprano *Fucus spiralis*.

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INTRODUCTION

Ever since Thompson (1917) suggested that geometrical and physical laws can be pacemakers of the growth and form of living beings, much research has been performed to test these ideas. Huxley (1932) drew attention to the biological significance of the relative size and shape of living organisms through their development and evolution. Huxley's allometrical equations, as well as other mathematical tools recently developed have promoted our understanding of size changes as well as cellular differentiation and growth processes in animals and plants (Gould 1966, Pagel & Harvey 1989).

The marine seaweed *Fucus* is a favorable system to study early development and growth, with first reports dating back to the 19th century (Thuret 1878). Their gametes can be easily obtained and fertilization performed successfully in the laboratory. Since the pioneer work of Thuret's (1878) works, several hundred papers describing early development in many Fucales species have been published (reviewed by Fritsch 1965 and recently by Kropf 1992). During normal development, the young zygote of Fucoids usually displays no detectable asymmetry. It is a large, spherical and apolar cell. Yet a few hours after fertilization it generates a developmental axis that marks rhizoid and thallus poles. Over the following days of development the rhizoid elongates by apical growth and the embryo is divided into increasing smaller cells (Kropf 1992).

Fucus was also widely used to analyze the environmental effects on embryo development. The effect of illumination on rhizoids growth had been analyzed earlier (Fritsch 1965, Kropf 1992). Gradient of hydrogenion concentration, alkalinity, ultraviolet light and group-orientation play an important role on *Fucus* development (Whitaker & Lowrance 1937, 1940; Whitaker 1938, 1941). *Fucus* was used to study chemotaxis between spermatozoa and the eggs, as well as the chemical involved in sperm attraction by the eggs (Cook *et al.* 1948; Muller & Jaenicke 1973; Muller & Seferiadis 1977). Recently *Fucus* was used to elucidate the role play by cell wall in embryo development (Berger *et al.* 1994).

Although *Fucoids* have a long history as subjects for the examination of the processes that control early embryogenesis, morphometrical works characterizing early development of *Fucales* are scarce. Contrarily, morphometric characterization of early development has been widely performed in mammalian and human beings (Chan 1987, Cohen *et al* 1989. Goyanes *et al.* 1990) as well as in marine animals (Hay *et al.* 1987). Here we quantitatively characterized the early development of living embryos of *Fucus spiralis* L. using morphometrical procedures.

MATERIAL AND METHODS

GAMETE RECOVERY, FERTILIZATION AND EARLY DEVELOPMENT

Fucus spiralis L. var. platycarpus (Thuret) Batt. was collected in La Coruña Bay (N-W Spain) and washed for two hours in f/2 medium without silicate (Sigma) with 150 mg l^{-1} penicilin and 100 mg l^{-1} streptomycin to eliminate bacteria. Afterwards the plants were maintained in flasks with f/2 medium without silicate (Sigma), under 12:12 hours light-dark cycles of 50 µmol photon $m^{-2}s^{-1}$ at 14 ± 1°C during two days. Afterwards, plants were treated with a hipotonic solution (20% CINa, 30 min) to induce gamete liberation. Liberate zygotes were collected with a Zeiss-Eppendorf micromanipulator-microinjector

and deposited in microplate wells (Nunc) with 1 ml of f/2 medium (Sigma). These cultures were maintained under 12:12 hours light-dark cycles, 50 µmol photon $m^{-2}s^{-1}$ at 14±1°C. Once every hour, the early embryos were observed in a Zeiss 35 Axiovert Invertoscope and a video recording was performed with a high resolution (Sony) videotape coupled to the invertoscope during the first 60 hours. Afterwards, embryos were observed weakly. Only the embryos which were able to successfull develop young plants were considered in this study. More details on handling the *Fucus* gametes and early embryos are given in Costas *et al.* (1994).

MORPHOMETRICAL ANALYSIS ON LIVING EARLY EMBRYOS.

All the measurements were performed on video images of early embryos at different stages of development. The number of samples analyzed in each stage was estimated by using the progressive mean technique (Williams 1977) with a confidence limit of 5%. Perimeters were estimated by a curvimeter, and the area of the profiles on micrographs was estimated by point counting and planimetry (Baak & Oort 1983).

Absolute volume (V) was measured by the morphometrically procedure of Suciu (1985).

$$V = 4/3\ (lw/4)^{1.5}$$

Where l is the longest diameter and w the diameter perpendicular to l. This procedure is applicable to both spherical and non spherical shapes.

The coefficient of form (CF) (Renau-Piqueras & Cervera 1983, Renau-Piqueras *et al.* 1985) was determined using the expresion:

$$CF = 4\ area\ /\ (perimeter)^2$$

The surface/volume ratio (S/V) was estimated according to the Miyamoto *et al.* (1988) equation:

$$S/V = 4P/a$$

Where P is the mean profile perimeter and a is the mean profile area. The coefficient of variation (CV) were used as a measure of the relative dispersion of the morphometrical parameters. The Mann-Whitney U test was used as a homogenity test because most parameters analyzed do not have a normal distribution.

Also, the allometric growth equation of rhizoids was calculated following Huxley's (1932) equation:

$$y = a\ x^b$$

Where y is the length of the embryo, including rhizoid, and x is the width of the embryo. Then, taking logarithms and performing a linear regression analysis the allometric coefficients a and b were calculates.

Brillouin (1962) index ($H = 1/N \log 2\ N!/Na! + Nb! + ... + Ns!$) was employed in an attempt to estimate the evolution of structural complexity during the early development.

Methods in morphometric analysis of living oocytes and early embryos as well as morphometric analysis on cells, have been previously described in great detail (Costas *et al.* 1988, 1992; Goyanes *et al.* 1990).

RESULTS

Figure 1 shows the developmental sequence of early *Fucus spiralis* embryos in our experiments. Around 77% of the early embryos polarize with the germination of a rhizoid outgrowth prior to cell division. This feature is considered the normal pattern in Fucoids (Knoll 1992). Nearly of 23% of the early embryos showed cell division before rhizoid outgrowth. A low percentage of the embryos (<1%) showed cell division producing 3 equal cells before rhizoid outgrowth. Figure 2 shows a photographic recording of this scarce developmental sequences.

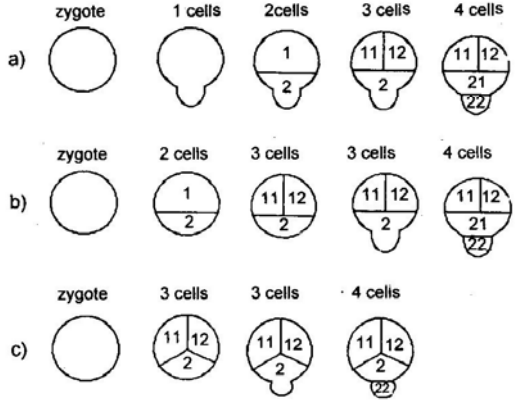

Figure 1. Scheme of early development in *Fucus spiralis*. Each blastomere was numbered according to current nomenclature of embriology. Only frontal views are shown but embryos were moved by micromanipulator to ensure the correct observation of other views.

a) Developmental steps of embryos with rhizoid outgrowth before cell division.
b) Developmental steps of embryos with cell division before rhizoid outgrowth.
c) Developmental steps of embryos with 3 similar cells before rhizoid outgrowth.
In this scheme embryo cells were numbered. This numeration is employed in morphometrical tables.

Apparently the pattern of early development in *Fucus spiralis* is correlationated with the zygote volume (Table 1). Zygotes which produced embryos with cell division before rhizoid outgrowth were statistically smaller ($p < 0,01$, Mann-Whitney U test) than zygotes which produced embryos following the normal pattern of rhizoid outgrowth before cell division. In addition, the zygotes which produced embryos with cell division in 3 similar cells before rhizoid outgrowth were the smallest ($p<0,01$, Mann-Whitney U test).

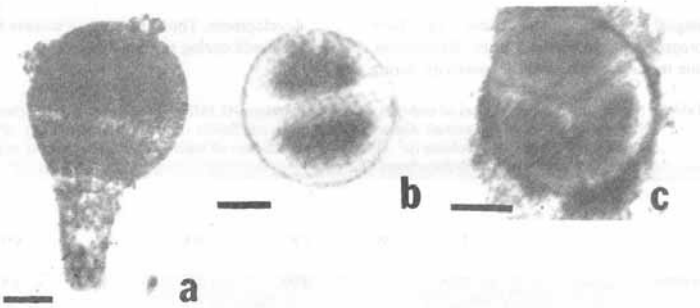

Figure 2. Types of early development in fucus spiralis.
a) Polarized embryos with rhizoid outgrowth (normal pattern) Scale bar= 50 μ.
b) Embryos with cell division before rhizoid outgrowth. Scale bar= 40 μ
c) Embryos with 3 similar cells before rhizoid outgrowth. Scale bar= 30 μ
The three types of embryos were able to originate normal young plants.

Table 1. Volume (mm3) of Fucus zygotes classiffied in 3 groups according their pattern of early development:
a) Rhizoid outgrowth before cell division (normal pattern).
b) Cell division before rhizoid outgrowth.
c) Cell division producing 3 similar cells before rhizoid outgrowth.
**) Statistically significant differences (p<0,01, Mann-Whitney U test).

	number of analyzed zigotes	mean	±sd
a	71	1162	±45
b	23	806	±39
c	9	572	±12

EMBRYOS POLARIZED WITH RHIZOID OUTGROWTH PRIOR TO CELL DIVISION

The main features of these embryos are morphologically characterized in Table 2. These zygotes, which follow the normal pattern are bigger. They grow out prior to cell division, then show a clear polarization in an vertical axis. Afterwards, they divide asymmetrically into an upper and a lower cell (lower cell about 18% less in volume than in upper cell). There was a clear polarization in the vertical axis with considerable asymmetry between upper and lower cells. The upper cell divided into two simmilar cells. Contrary to the vertical axis, the embryos were symmetrical in right-left axis. The lower cell grows out followed by an horizontal division whereby a small rhizoid-cell is formed. This cell is the smallest cell of the early embryo (around 42 time less than the total embryo).

The volume of the whole embryo progressively increased. Coefficient of variation of volume increases during development, suggesting a different speed of growth among the different development

stages embryos. Coefficient of form progressivèly decreased after fecundation, due to the embryos loss of sphericity during development. Thus, the surface/volume ratio increased during segmentation.

Table 2. Morphometrical parameters of embryos with rhizoid outgrowth before cell divison. I. = longitudinal diameter (μm); W = transversal diameter (μm); CF= coefficient of form (adimensional); S/V = surface/volume ratio; V = volume (μ^3 . 10^3); CV = coefficient of variation (%). The number asigned to each cell corresponds with the schema of Figure 1.

	I	W	CF	S/V	V	CV
zygotes	150	109	0.90	0.72	1162	3.9
1 cell growing out	151	111	0.82	0.84	1241	5.3
2 Cells						
Cell 1	-	-	0.71	1.49	715	
Cell 2	-	-	0.78	1.12	605	
Total embryo	151	114	0.77	0.97	1321	8.4
3 Cells						
Cell 11	-	-	0.70	1.46	398	
Cell 12	-	-	0.71	1.47	395	
Cell 2	-	-	0.70	1.45	614	
Total embryo	152	116	0.74	0.94	1407	7.6
4 Cells						
Cell 11	-	-	0.71	1.43	393	
Cell 12	-	-	0.72	1.46	391	
Cell 21	-	-	0.70	1.43	632	
Cell 22	-	-	0.52	1.77	35	
Total embryo	152	117	0.73	0.96	1451	8.9

EMBRYOS WITH CELL DIVISION PRIOR TO RHIZOID OUTGROWTH.

The main features of these embryos are morphometrically characterized in Table 3. Zygote of these embryos are around 30% less volume than zygotes with rhizoid outgrowth prior to cell division. The first wall divides the zygote into an upper and a lower cell. The upper cell is bigger than the lower one. Polarization appears during the first division. Soon afterwards, the upper cell divides into two similar cells. The lower cell grows out prior to the appearance of any septa. Their coefficient of form decreased and contrarily, their surface/volume ratio increased. This outgrowth is followed by an horizontal division cutting a small rhizoid-cell out at the base. This rhizoid-cell is about 50 times smaller than the total embryo. The coefficient of variation is higher in the late phases of segmentation, thus indicating that variation of volume increases during development.

Table 3. Morphometrical parameters of embryos with cell division before rhizoid outgrowth. l= longitudinal diameter; W= transversal diameter (um); CF= coefficient of form (adimensional); S/V = surface/volume ratio; V= volume ($um^3.10^3$); CV = coefficient of variation (%). The number asigned to each cell correspond with the scheme of Figure 1.

	l	W	CF	S/V	V	CV
Zygotes	119	112	1.05	0.69	805	4.8
2 Cells						
Cell 1	-	-	0.79	1.26	399	
Cell 2	-		0.81	1.27	365	
Total embryo	116	111	0.90	0.77	765	
3 Cell						
Cell 11	-	-	0.94	1.58	234	
Cell 12	-	-	0.93	1.56	243	
Cell 2	-	-	0.81	1.06	394	
Total embryo	115	107	0.90	0.77	871	11.8
3 Cells growing out						
Cell 11	-	-	0.95	1.58	238	
Cell 12	-	-	0.97	1.57	235	
Cell 2	-	-	0.69	1.27	438	
Total embryo	118	111	0.83	0.81	911	13.6
4 Cells (n=17)						
Cell 11	-	-	0.96	1.47	240	
Cell 12	-	-	0.98	1.48	232	
Cell 21	-	-	0.53	1.68	465	
Cell 22	-	-	0.53	1.68	18	
Total embryo	121	116	0.72	0.96	955	17.5

EMBRYOS WITH CELL DIVISION PRODUCING 3 SIMILAR CELLS BEFORE RHIZOID OUTGROWTH.

The main features of these embryos are morphometrically characterized in Table 4. The zygote of these embryos are around 50% less volume than zygotes with outgrowth prior to cell division. This smaller zygotes divides into three similar cells. Afterwards, one of these cells grows out and increases its volume about 20%. Their coefficient of form decreased and their surface/volume ratio increased. this growth is followed by an horizontal division whereby a small rhizoid-cell is cut out at the base. This rhizoid-cell, which is about 44 times smaller than the total embryos, showed a similar volume, surface/volume ratio and coefficient of form than the rhizoid-cell of normal embryos.

Table 4. Morphometrical parameter of embryos with cell division producing 3 similar cells before rhizoid outgrowth. l= longitudinal diameter; W= transversal diameter (um); CF= coefficient of form (adimensional); S/V = surface / volume ratio; V= volume ($um^3.10^3$); CV= coefficient of variation (%). The number asigned to each cell correspond with the scheme of Figure 1.

	l	W	CF	S/V	V	CV
	105	101	1.01	0.72	571	2.1
3 equal cells						
(n=5)						
Cell 11	-	-	0.76	1.48	239	
Cell 12	-	-	0.76	1.49	237	
Cell 2	-	-	0.75	1.48	248	
Total embryo	114	109	1.09	0.66	725	1.4
3 equal cells growing out						
Cell 11	-	-	0.74	1.47	251	
Cell 12	-	-	0.75	1.47	249	
Cell 2	-	-	0.70	1.65	305	
Total embryo	117	114	0.83	0.74	806	3.2
4 Cell						
Cell 11	-	-	0.76	1.48	252	
Cell 12	-	-	0.77	1.47	247	
Cell 21	-	-	0.71	1.46	298	
Cell 22	-	-	0.51	1.70	18	
Total embryo	118	114	0.75	0.99	816	4.3

GROWTH EQUATION AND STRUCTURAL COMPLEXITY.

The growth equation (in the allometric form $y=ax^b$) of early embryos with: a) rhizoid outgrowth prior to cell division normal pattern, b) cell division in two cells to rhizoid outgrowth and c) cell division in three cells prior to rhizoid outgrowth, are summarized in Table 5. Embryos which followed the normal pattern show a significative different growth equation from embryos which divided prior to rhizoid outgrowth. A tentative approach to estimate the variation of structural complexity during the early steps of development is summarized in Figure 3. Initial steps are structurally simplest in embryos which follow the normal pattern. Contrarily, the smaller embryos follow the structurally complex pattern of early segmentation. At the third developmental step all the embryos show a similar structural complexity.

DISCUSSION

Fucus spiralis was a perfect experimental model to estimate quantitative variation of the cellular organization during early embryo development. The ova volume is correlated with the pattern of early development of *Fucus spiralis* embryos. The bigger ova followed the normal pattern of

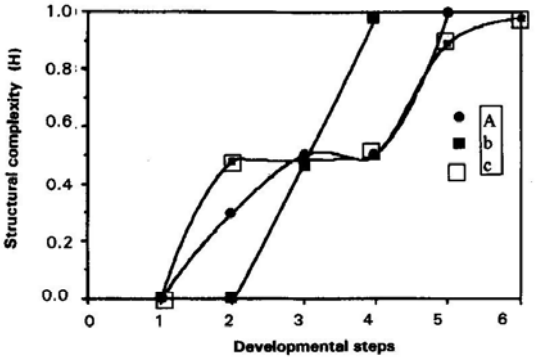

Figure 3. Variation of structural complexity during the early development of *Fucus spiralis*. The development steps correspond with the events indicates in Fig.1.
a) Embryos with rhizoid outgrowth prior to cell division.
b) Embryos with cell division into two cells prior to rhizoid outgrowth
c) Embryos with cell division into three cells prior to rhizoid outgrowth

Table 5. Growth equations in allometric form ($y = ax^b$) of embryos which polarized with rhizoid outgrowth prior to cell division (A) embryos which divided into two cell prior rhizoid outgrowth (B), and (C) embryos which divided into three simmilar cells prior rhizoid outgrowth. W= transversal diameter (um); l= longitudinal diameter (um).

A	$W = 2{,}7 \cdot 10^{-10} \; l^{5{,}33}$
B	$W = 0{,}13 \cdot l^{1{,}41}$
C	$W = 0{,}56 \cdot l^{1{,}12}$

segmentation with rhizoid outgrowth prior to cell division, and the smaller ova develop with cell division prior to rhizoid outgrowth.

Both zygotes with and without rhizoid outgrowth reached the same developmental patterns before few developmental steps. On the other hand, it is known that most mRNA and proteins are inherited maternally but zygotic transcription and translation are necessary for completion of early development (Koehler & Linskens 1967, Quatrano 1968). These facts suggest that bigger ova (which follow normal pattern of early development) have a complete set of maternal RNA and proteins, but smaller ova (which follow abnormal developmental patterns) perhaps lack some of the maternal proteins which are synthetized during the first steps of development.

Polarization between cells seem to be necesary for normal development. In normal embryos, polarization appears before cell division whith the rhizoid outgrowth

which constitutes the first obvious morphological expression of the inherent polarity. In embryos which divide prior to rhizoid outgrowth, polarization appears just after the first segmentation with a smaller upper cell and a bigger lower cell, and the rhizoid outgrowth always comes from the bigger cell. Perhaps the light ovoicity of zygotes which follow a normal pattern of segmentation can be interpreted as earlier polarization. In this regard a recent paper (Costas *et al.* 1994) shows that early zygotes of *Fucus* are polarized for glycan moieties of cell surface prior to rhizoid growth and cell division.

Recent studies suggest that volume and form of human and ape zygotes has not influence neither developmental pattern nor their viability (Mohr *et al.* 1983, Humeau 1985, Plachot *et al.* 1987. Cohen *et al.* 1989, Goyanes *et al.* 1990, Maneiro *et al.* 1991). However, early development of human embryos is conditioned by asymmetry between the first cells (Goyanes *et al.* 1990) just as *Fucus* do.

Recent paleontological data suggest that macrophytes were one of the organism which first reached an embryo development (Knoll 1992). An approach using seaweeds, perhaps one of the first multicellular organism on Earth, could help to understand the ultimate aim of embryology: the mathematical derivation of the adult from the distribution of growth in the germ.

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