Growth of the foraminifer *Nummulites venosus* in nature and culture

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Abstract Cultures of larger benthic symbiont-bearing foraminifers have been used for studying growth and reproduction by long term investigations taking several months. For getting results that can be interpreted as 'natural growth', the culture medium must represent the same environmental conditions like the sea at sampling time. Growth of *Nummulites venosus* specimens was tested where about 40 chambers have been built in the natural environment at the time of sampling. After exposition to cultures with the same environmental conditions as those at the sampling stations, growth in the culture was compared with 'natural growth' of the initial part. This was done calculating deviations of empirical data obtained in the culture from the Gompertz function established for the cell part constructed in the sea. Three reaction types could be detected: 1) strong (negative) deviations just after exposure to the culture; 2) continuing natural growth in the culture, starting deviation in later chambers; 3) throughout continuing natural growth. The chamber building rate, calculated by the Michaelis-Menten function as the second parameter determining growth, differs significantly between growth in the nature and culture, where the velocity of chamber building rates decrease immediately after exposure into the cultures. It must be concluded, that the culture medium, although approximating natural conditions rather perfectly, always impact growth, especially in the chamber building rate. Therefore, studies checking influences of environmental factors using cultures must be carefully interpreted.

Keywords Nummulites, Cell growth, Chamber building rate, Nature, Culture

Introduction

Growth studies on larger symbiont-bearing foraminifers were either based on population studies (e.g. Zohary et al. 1980; Sakai and Nishihira 1981; Fujita et al. 2000) or on individuals kept in laboratory cultures. Growth studies on single individuals used the test diameter (Hallock Muller 1974; Hallock 1981), diameter in combination with chamber drawings (Röttger 1972b) including the time of reproduction, or are based on estimated weight (ter Kuile and Erez 1984). The problem in culturing using seawater different from the sampling stations is the adoption of natural conditions in the disposable water like seawater from other regions or commercial artificial seawater (e.g. Röttger 1972a; Hallock 1981). Following

investigations primarily used seawater close to the field research stations where samples were cultivated, e.g. Heinz Steinitz Marine Biological Laboratory (Eilat, Israel), Sesoko Station (Okinawa, Japan), Australian Institute of Marine Sciences (Townsville, Australia).

The main problems for getting 'natural conditions' are environmental factors such as light and water movement. Nobes et al. (2008) solved the light problem using open air tanks filled with sea water pumped from the sea. The one were left open to the sunlight, the others were covered with shade cloths reducing light to 60 % and 90 %. Using this method, temperature control for deeper living organisms like *Nummulites venosus* (Fichtel and Moll, 1798) is impossible. Water movement is important for

shallow living species exposed to currents and waves. Flow-through systems with a direct connection to seawater pumps induce enough water movement in tanks for deeper living species, while extreme water movement could be handled using floating chambers (Hosono et al. 2013).

Beside long-term investigations on growth and reproduction, cultivations under ‘normal conditions’ were used as control groups for investigating a number of factors influencing growth, such as temperature (Reymond et al. 2011), photosynthetically active radiation (Ziegler and Uthike 2011), $p\text{CO}_2$ (Fujita et al. 2011; Schmidt et al. 2014), nutrients (Lee et al. 1991; Prazeres et al. 2016) or heavy metal pollution (Ben-Eliahu et al. 2020). Those investigations were mainly based on short-term investigations between 2 and 3 months.

Disturbed growth in the laboratory during long-term experiments has first been mentioned by Röttger (1972b) investigating *Heterostegina depressa* d’Orbigny, 1826, who differentiated between ‘Pluskammern’ (chambers

contributing to size) and chambers that did not lead to size acceleration. Based on chamber volumes, Eder et al. (2016) found significant differences in *H. depressa* specimens (collected by R. Röttger and R. Krüger from Hawaii and cultivated in the laboratory of the Kiel University) in proloculus size and chamber growth. Growth differences between nature and laboratory are also shown in Hohenegger et al. (2014).

Complete concordance between natural growth and growth in the laboratory is necessary when studying effects of environmental factors based on culture studies, which are supposed to represent natural conditions. To verify this assumption, it is necessary to investigate individuals grown in the sea with, after transfer into the laboratory, their subsequent growth in laboratory cultures supposed to represent ‘natural habit’ setups.

Material and methods

Sampling

Sampling was performed by Scuba diving at 50 m

Fig. 1 Location of sampling area and sampling sites (sample numbers in Table 1).

water depth around Sesoko Jima (Motobu, Kunigami district, Okinawa; Fig. 1) from 23 April 2014 to 14 July 2015 (Table 1). To prevent error due to clumped distributions, four samples with approximately 4-m distance apart were taken with plastic boxes. Temperature and photosynthetically active radiation (PAR) were measured at every sampling station using a WTW Multi350i, combined with a depth conductivity cell TA 197-LF and a LI-COR LI-250A light meter with a LI-192 underwater Quantum Sensor (Wöger et al. 2016).

At Sesoko Station, Tropical Biosphere Research Center, samples were poured into flat sorting trays, carefully washed with seawater and spread out to a thickness of

~0.5 cm (Fig. 2A). After resting for 24 hours, living foraminifera identifiable by their complete coloring due to symbiotic microalgae were picked, put into smaller boxes and identified to the species level. Half of the species sample, washed in freshwater and dried, was sent to the Vienna University for growth investigation using the ‘natural laboratory’ approach (Hohenegger et al. 2014, 2019; Kinoshita et al. 2017; Eder et al. 2019). From the remaining half put in larger petri dishes into the culture tank, the four largest and four smallest specimens were selected and separated into 4 small petri dishes combining one large and one small specimen.

Table 1 Parameters of sampling stations

Sample number	Date (d.m.y)	Longitude (E)	Latitude (N)	Depth (m)	Temperature (°C)	Number of investigated individuals
1	23.04.2014	127°51.388'	26°40.086'	56	22.7	2
2	02.05.2014	127°52.243'	26°37.126'	46	22.3	1
3	09.05.2014	127°51.331'	26°40.036'	50	21.8	1
4	30.05.2014	127°51.516'	26°40.220'	54	23.3	4
5	18.07.2014	127°51.532'	26°40.424'	57.5	23.6	
6	19.08.2014	127°51.467'	26°40.423'	56	26.2	
7	10.09.2014	127°51.528'	26°40.241'	54	27.2	2
8	03.10.2014	127°52.262'	26°37.425'	41	26.9	
9	10.11.2014	127°51.463'	26°37.351'	41	24.7	2
10	11.12.2014	127°51.517'	26°40.218'	47	23.5	4
11	16.01.2015	127°51.510'	26°40.214'	53.7	21	4
12	13.02.2015	127°51.508'	26°40.171'	57	20.1	5
13	04.03.2015	127°51.473'	26°40.267'	57	22	3
14	15.04.2015	127°51.454'	26°40.236'	58	23.5	1
15	18.05.2015	127°51.510'	26°40.276'	55	22.9	1
16	11.06.2015	127°51.620'	26°40.315'	56.5	24	
17	14.07.2015	127°51.514'	26°40.160'	50	27.4

Fig. 2 Laboratory setup. A. Sorting trays filled with washed samples, layers of ~0.5 cm thickness. B. Culture tanks for samples collected at 50 m (large) and 20 m (small).

Experimental setup

The size of the tank with samples from 50 m depth was $90 \times 135 \times 30$ cm illuminated by 2 Sudo Caribbean Blue S-3420 (20W) lightning tubes (Fig. 2B). Their timer was set to 10/14 hs (winter) and 13/11 hs (summer) light/dark cycles. Light intensity adjusted to field measurements were kept at $30\text{--}40 \mu\text{mol m}^{-2} \text{y}^{-1}$ from April to September 2014, then reduced to $15\text{--}20 \mu\text{mol m}^{-2} \text{y}^{-1}$. The water cooler GEX GXC-201 X 240W in combination with a Nisso IC Auto Neo Type 180 heater was used for temperature control. Temperature was adjusted to those measured in the field.

Filtered seawater available through a pipeline from 10 m depth in front of the station was further filtered using an ADVANTEC 10517P52 micro-pore filter ($5 \mu\text{m}$ and $1 \mu\text{m}$ cartridges). Commercial aquarium filters containing active charcoal were inserted to improve water circulation and reduce the amount of dissolved organic compounds. Frequent slow water changes enabled better temperature control, where water levels were kept at 15 cm and checked three times daily, together with temperature, salinity and pH. On average, 1/3 of the water volume was changed daily (Wöger et al. 2016).

Pictures of each specimen were taken every four days, mostly during night when photosynthesis was inactive, to document chamber building and shell growth by size (Kinoshita et al. 2017; Eder et al. 2019). This interval was chosen to prevent disturbance of the chamber building process by shorter intervals.

Measurements

For measuring chamber volumes, computing tomography was applied using the high energy Skyscan1173 at the Department of Paleontology, Vienna University. The volume of each extracted chamber was measured using the dedicated software Amira 5.4.3 VSG working on the three-dimensional models obtained (Briguglio et al. 2013, 2014; Eder et al. 2016). Sometimes, chamber volumes could not be measured according to weak preservation of chamber walls. To obtain cell growth based on the cumulative distribution of chamber volumes, the problem of lacking volumes could be statistically solved by the first derivative of the applied growth function (table 1 in Hohenegger 2018,).

Statistical analyses

In a first step, volumes as nonlinear measurements were transformed to linearity calculating their sixth root (Table S1). Starting with the first chamber after the nepiont, which consists of a proto- and deuteroconch, the first derivative of the Gompertz function

$$v_{\text{chamber}} = L_{\infty} b c e^{b e^{c n}} \quad (1)$$

n = chamber number, L_{∞} = upper limit of cell volume

was calculated for chamber volumes v at the shell part, which has been constructed in the sea before sampling. Fitting this function (red line in Fig. 3C) by statistical programs is possible, although some chamber volumes could not be measured due to poor preservation.

Volumes of lacking chambers, necessary for calculating the empirical cell volumes as cumulative frequencies of chamber volumes, could be replaced estimating their values using equation 1, then retransformed by the sixth power.

After completing the empirical chamber volumes, the theoretical cell growth function V under natural conditions, based on the sixth power of the Gompertz function

$$V_{\text{cell}} = L_{\infty} e^{b e^{c n}} \quad (2)$$

(red line in Figs. 3C, D) could be compared with empirical growth functions of the shell portion constructed under natural (blue columns in Figs. 3C, D) and laboratory conditions (ochre columns in Figs. 3C, D).

Growth under natural conditions and in the laboratory were compared using residuals of the empirical cell growth function at chamber number n to the theoretical function that is based on natural growth (red lines in Figs. 3C, D)

$$Res_n = |V_{n,\text{empirical}} - V_{n,\text{theoretical}}| \quad (3)$$

Because residuals increase exponentially with cell growth, they have to be standardized by

$$Res_{n,\text{standardized}} = |V_{n,\text{empirical}} - V_{n,\text{theoretical}}| / V_{n,\text{theoretical}} \quad (4)$$

Cell growth in nature and laboratory were compared between residuals of the shell part constructed in the nature and residuals of the shell part constructed in the laboratory. Significant (negative) differences between both empirical functions, checked by Student-t test based on equal or unequal variances, demonstrate hampered

Fig. 3 Chamber and cell growth of specimen 04_07 kept in culture after collection from the natural habitat. A. Chamber volumes grown under natural conditions. B. Chamber volumes added under laboratory conditions. C. Frequencies of chamber volumes, fit by Gompertz function based on frequencies in the natural habit. D. Frequencies of cell volumes, fit by Gompertz function based on frequencies in the natural habit.

growth in cultures (Table 2).

A mean chamber-building rate under natural conditions depending on time (in days) was estimated for *N. venosus* specimens using the Michaelis-Menten function

$$n = L_{\infty} t / (k + t) \quad (5)$$

t = day, L_{∞} = upper limit of cell number,
 k = number of days where $L_{\infty}/2$

with $L_{\infty}=109.4$ and $k=156.2$ days (standard deviation=71.05 days) measured on population means in the nature during the investigation period (Kinoshita et al. 2017; Fig. 7 in Hohenegger et al. 2019.). Using the inverse of the Michaelis-Menten function (Hohenegger 2018)

$$t = kn / (L_{\infty} - n), \quad (6)$$

Table 2 Parameters of cultured individuals. In probabilities for equality in standardized residuals $p(H_0)$, italics determine significant differences. Constant k of the Michaelis-Menten function appoints to the days, when half of the growth limit $L_\infty/2=54.7$ chambers is reached.

specimen	date of exposure (y/m/d)	chamber number		days in		standardized residuals					constant k
		nature	culture	nature	culture	mean nature	mean culture	variance nature	variance culture	$p(H_0)$	halftime days
01_01	2014/4/23	43	13	−100	157	0.035	−0.040	0.004	0.002	<i>0.003</i>	245
01_02		32	10	−64	170	0.024	−0.040	0.112	0.109	<i>0.002</i>	375
02_04	2014/5/2	44	21	−104	237	0.048	−0.188	0.125	0.198	<i>8.00E-06</i>	233
03_01	2014/5/9	36	24	−76	166	0.027	−0.135	0.007	0.006	<i>2.31E-08</i>	199
04_03	2014/5/30	31	23	−61	146	0.019	−0.135	0.003	0.014	<i>2.52E-16</i>	213
04_04		44	13	−104	141	0.018	−0.097	3.51E-04	0.002	<i>3.67E-08</i>	225
04_07		51	19	−135	133	0.012	−0.091	2.99E-04	0.002	<i>1.25E-08</i>	151
04_08		49	12	−126	141	0.047	−0.112	0.004	0.002	<i>4.92E-07</i>	211
07_03	2014/9/10	33	9	−67	121	0.006	−0.126	8.03E-05	0.004	<i>1.75E-04</i>	301
07_08		66	9	−235	269	−0.009	−0.086	1.93E-04	0.001	<i>1.85E-09</i>	231
09_01	2014/11/10	36	22	−76	242	0.004	0.004	0.004	0.001	0.968	282
09_06		63	9	−210	213	−0.002	−0.054	0.004	2.61E-04	<i>1.86E-06</i>	220
10_01	2014/12/11	40	22	−89	214	0.007	−0.111	0.001	0.002	<i>5.49E-12</i>	232
10_04		37	23	−79	214	0.004	0.010	2.53E-04	0.002	0.458	241
10_06		34	9	−70	190	0.007	−0.012	0.006	2.05E-04	0.102	401
10_07		48	10	−121	190	−0.007	0.026	0.005	0.002	0.156	276
11_02	2015/1/16	38	18	−82	178	0.009	−0.015	0.005	0.002	0.119	248
11_03		28	24	−53	178	0.021	0.022	0.004	0.002	0.921	255
11_04		33	18	−67	178	0.027	0.004	0.006	0.001	0.157	280
11_07		47	14	−117	178	−2.71E-04	0.006	0.002	0.001	0.657	234
12_02	2015/2/13	33	20	−67	148	0.003	−0.038	0.009	0.013	0.241	229
12_03		29	25	−56	148	0.016	0.008	0.002	0.001	0.464	209
12_04		35	20	−73	148	0.018	−0.036	0.003	0.004	<i>0.001</i>	218
12_07		54	12	−151	148	0.006	0.011	0.004	0.001	0.680	196
12_08		51	13	−135	148	0.022	−0.043	0.002	0.001	<i>1.96E-05</i>	201
13_02	2015/3/4	37	12	−79	128	0.033	0.030	0.015	2.57E-04	0.888	255
13_03		43	11	−100	128	0.026	−0.025	0.005	0.005	0.040	234
13_04		38	8	−82	128	0.044	0.006	0.011	0.001	0.059	290
14_02	2015/4/15	36	10	−76	89	0.024	−0.040	0.004	0.002	0.003	227
15_01	2015/5/18	26	9	−48	54	0.013	−0.006	0.002	0.001	0.268	217

the chamber number n at the day, when the specimen has been transferred from the sea to the laboratory, approximates the number of days the specimen has lived under natural conditions. Proving changes in chamber building rates in the culture, the number of days using the parameters for the chamber building rate under natural conditions can be compared with the number of realized days. Taking $L_\infty=109.4$ as the upper limit of cell numbers, the constant k , indicating velocity of the chamber building

rate, can be measured by

$$k = t(L_\infty - n) / n \quad (7)$$

where increasing values of k determine decreasing chamber building rates.

Finding natural groups was performed by UPGMA cluster analysis (Sneath and Sokal 1973) using ‘Normalized Euclidean Distances’ (Orlaci 1966), named ‘Chord’ in PAST 4.02 (Hammer 2020). The correctness of classi-

fication was checked by discriminant analysis (Fischer 1936) where only three cases were positioned to another group based on Jackknifing (PAST 4.02).

All complex calculations used the program packages IBM SPSS Statistics 27 and PAST 4.02, while simpler calculations were done in Microsoft EXCEL.

Results

A total of 128 individuals (gamonts/schizonts) (Fig. 4) from 15 sampling dates (Table 1) were cultured starting with 4 small and 4 large specimens from the first sampling date at 23/04/2014 and ending on 16/07/2015, when the experiment was finished. From 56 individuals cultured at sampling periods 23/04/2014 to 10/09/2014, only 2 specimens (3.6%) survived until the end of the experiment. This is partially caused by the much longer culture time compared to the remaining sampling stations from 08 (04/10/2014) to 15 (18/05/2015). Here, 31 from 64 individuals (48.4%) lived from the start of the exposure until the end of the experiment, obviously caused by shorter and decreasing time intervals (Fig. 4).

Due to complex measuring, 30 specimens were selected for investigation of chamber volumes growth (Table 1; red bars in Fig. 4). Beside chamber volume measurements (Table S1) separated in natural and laboratory growth parts, the theoretical growth functions gained from the test part grown in nature were calculated using equations 1 and 2. Standardized residuals (Equation 4) measured departures of empirical growth function (nature and laboratory) from the theoretical growth function based on chambers constructed in the sea. Their means were tested for every specimen searching for significant differences between natural and culture growth (Table 2).

The three characters ‘chamber number grown under natural conditions’ (ChNnat), ‘mean of standardized residuals under natural conditions’ (mResNat) and ‘mean of standardized residuals under laboratory conditions’ (mResLab) (Table 2) were used to find significant groups by classification analyses. The character ‘chamber number grown under laboratory conditions’ (ChNlab) could not be used because of the different exposure times in the laboratory.

The UPGMA cluster analysis, followed by discriminant analysis, resulted in 5 clusters (Figs. 5, 6; Table S2).

Differences between clusters in the character ChNnat are insignificant as proven by ANOVA accepting the 0-Hypothesis with $p(H_0)=0.1476$, but a weak significant difference could be found between clusters 2 and 5 as proven by the a-posteriori Duncan test (Table S2). In mResNat characterizing residuals in natural growth, no significant differences are indicated by ANOVA supported with weak probability of $p(H_0)=0.0636$. This weakness is caused by strong significant differences between clusters 4 and 5 proven by the Duncan test (Table S2). In contrast, ANOVA based on the character mResLab shows strong significant differences with $p(H_0)=9.77E-14$. Clusters 1, 2 and 3 do not differ in this character with extremely low means that characterize strong deviations from the theoretical growth functions, while Clusters 4 and 5, not differing in this character, demonstrate concordance with theoretical growth functions (Table S2).

These relations are strengthened by discriminant analysis (Fig. 5). With 96.04% of explained variance, the first axis is the dominant discriminator between clusters based on the character mResLab with the loading ratio Axis1/Axis2=46.2. Axis 2 representing only 3.06% of explained variance is loaded by the character ChNnat with Axis2/Axis1=21.3 and by the character mResNat with loading ratios Axis2/Axis1=13.3. Therefore, the separation between cluster 4 and 5 is not expressed in the first axis, because differences in mResLab are insignificant. Axis 2 separates individuals with higher chamber numbers in the initials part (e.g. specimen 07_08, 09_06), additionally marking the weak significant differences between clusters 4 and 5 in mResNat (Fig. 5).

Consequences of this grouping can be retraced in frequency diagrams of cell growth (Fig. 6). Specimens in Clusters 1 and 2 show strong negative deviation from the theoretical growth function obtained in the sea just after their exposure in cultures. Specimens of cluster 3 first continue the growth in the sea after becoming exposed, then deviate negatively in later parts (Fig. 6). In clusters 4 and 5, cell growth strictly continues the functions obtained in the sea.

With the knowledge of chamber numbers in the test part constructed in the sea, the end of lifetime (~550 days; Kinoshita et al. 2017; Hohenegger et al. 2019)

Fig. 4 Lifespan of cultured *Nummulites venosus* specimens, artificially finished by the investigation end on 16.07.2015. Red bars determine the investigated individuals, grey bars mark individuals lost during investigation period.

Fig. 5 Discriminant analysis based on the variables ChNnat, mResNat and mResLab (see text) using clusters obtained by UPGMA based on normalized Euclidean Distances.

under undisturbed conditions can be calculated for each specimen using Equation 6. In the sampling interval from 23/04/2014 to 10/09/2014, lifetime in the cultures is strongly reduced (Fig. 7), coupled with a significant negative deviation in cell growth, all belonging to Clusters 1, 2 and 3 (Tab. 2; Fig. 6). In the remaining period from 10/09/2014 until 18/05/2015 only 2 specimens (07_01, 10_03) combine restricted growth with strong negative deviations in cell growth (light green in Fig. 7), while in 6 specimens the significant weak deviation cannot be combined with restricted cell growth due to the limited exposure time (middle green in Fig. 7). From the 14 remaining specimens with cell growth continuing chamber growth in the sea, 2 specimens (10_06, 10_07) were dying before the end of exposure (Fig. 7).

The chamber building rates in the laboratory based on natural growth could be compared with their realization (Fig. 7). All specimens except individual 04_07 show a strong deceleration in chamber building rates. This is documented by velocity parameters k (Equations 5 to 7) where its distribution parameters $\bar{x}=244.4$ days ($sd=$

50.6) are significantly higher than the estimated mean in the nature ($\bar{x}=156.5$ days, $sd=71.05$), documenting the reaction of all specimens due to the exposition in the culture by the reduced chamber building rate.

Discussion

The main result is that cell growth of specimens is affected in different degrees by the transfer from the natural habit into cultures, although the environmental parameters temperature, salinity, pH, duration and intensity of light exposure with changing day/night cycles were kept close to measurements going with the sampling stations. Contrary to the concurrently investigated *Operculina complanata* and *Heterostegina depressa*, added sediment did not affect growth in *N. venosus* (Wöger et al. 2016).

After sampling, specimens were disturbed in their growth by preparation in the laboratory, from washing with sea water, pouring into flat trays, resting 24 hours, picking and sorting into smaller petri dishes before putting into the culture tank. This takes at least 2 days, where specimens were strongly exposed to unfavorable condi-

Fig. 6 Cell growth diagrams of the investigated individuals, ordered by clusters obtained by UPGMA (specimen's title colours correspond to clusters in Fig. 5). Reclassification of individuals 07_08, 13_03 and 12_3 by discriminant analyses following initial classification.

tions.

By cell growth, groups react in different ways to the exposure. In the first group, cell growth is reduced just after exposition (Cluster 1 and 2 in Fig. 6). While in some specimens the diminution in cell growth continues until life end (e.g. specimen 02_04 in Fig. 6), the rest shows an approximation to cell growth realized in the sea that can be observed after some time (e.g. specimens 04_04, 10_01 in Fig. 6). Both growth forms can be found in the time interval from 23/04/2014 to 10/09/2014 (Figs. 4, 7),

obviously caused by light intensity. All environmental factors with the exception of light were adjusted to conditions at the sampling stations. Only light conditions have been adjusted to $30\text{--}40\ \mu\text{mol m}^{-2}\ \text{y}^{-1}$ from April to September 2014, then reduced to $15\text{--}20\ \mu\text{mol m}^{-2}\ \text{y}^{-1}$ (Wöger et al. 2016). This may be the reason, why some specimens tend to approximate the original growth in later parts, where light conditions became better.

In the second group, after exposure, cells continue growth carried out in the sea, later becoming reduced (e.g.

Fig. 7 Lifespan of the investigated individuals, on the one side keeping in culture the chamber building rate of the sea, on the other showing the effects of the reduced chamber building rate in days.

specimens 12_02, 12_04 in Fig. 6). This growth is rare in the sampling period from April to September 2014, but it is the only form of restricted growth from October 2014 to July 2015 (Fig. 7). An explanation for this type of growth restriction may be the insufficient adaptation to culture conditions, also expressed in the shortened lifetime.

Specimens of the third group seems to be unaffected by the transition from the sea to the laboratory. In culture, they continue growth realized in the sea, sometimes with positive deviations (e.g. specimens 09_01, 10_04, 11_03 in Fig. 6). Due to their short exhibition period (Fig. 7), it cannot be assured that they would be affected in their later lifetime by culture conditions.

The chamber building rate approximated by the Michaelis-Menten function (Equation 5) is strongly affected by the transition from the sea to the laboratory (Fig. 7), but demonstrates constantly increasing time intervals in chamber construction. The cell growth rate, approximated by the Gompertz function (Equation 1), in contrast, directly shows the influence of environmental

factors in cultures by deviations of empirical from theoretical functions obtained in the sea (Hohenegger 2018).

In summary, it could be demonstrated, that culturing in the laboratory cannot substitute natural conditions in growth studies, although conditions are set closest to the nature by the following: 1) the laboratory is located at the coast close to the sampling stations, 2) filtered sea-water was used through a pipeline starting in front of the station at 10 m depth, 3) 1/3 of the water mass continuously moving in the tanks was changed daily, and 4) the environmental parameters for culturing (temperature, salinity, pH, light intensity and day/night cycles) were adjusted going with conditions at the sampling stations. Not included in cultural investigation was bacterial food (*N. venosus* does not feed on algae and detritus), water quality, pressure and wavelength.

The main result of this investigation is that all works in the laboratory carried out under “natural conditions” must be carefully interpreted, especially when the investigation period is short (e.g. 2–3 months).

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Electronic supplementary material

ESM Table. S1-S2 can be downloaded from the J-STAGE website: https://doi.org/10.3755/galaxea.G2021_S12O

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