

Regeneration rate after fission in the fissiparous sea star *Allostichaster capensis* (Asteroidea)

Tamara Rubilar¹, Pablo E. Meretta² & Maximiliano Cledón²

1. LAPEMAR, Centro Nacional Patagónico (CENPAT), B. Brown 2915, Puerto Madryn, Chubut, Argentina; rubilar@cenpat.edu.ar
2. IIMyC-Instituto de Investigaciones Marinas y Costeras, CONICET-UNMDP, Funes 3350, Mar del Plata 7600, Buenos Aires. Argentina; pabloemeretta@gmail.com; mcledon@yahoo.com.ar

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Abstract: Many studies have focused on the regeneration rate of arms in Asteroidea but no studies have focused on the regeneration rate after fission. *Allostichaster capensis* is a fissiparous sea star with a wide range of distribution. In Golfo Nuevo (42°46'49" S - 64°59' 26" W) sea stars undergo fission every spring and summer and regenerate the rest of the year. To analyze the regeneration rate, we conducted an experiment with sea stars collected just before fission. After sea stars underwent fission, the length of the three non-regenerating and the three regenerating arms were measured weekly. The arm length (regenerating and non-regenerating) was used in non-Linear Mixed Effect models in order to account for within-individual correlation in different models. The regenerating arms regenerate according to a Quadratic model, while the non-regenerating arms regenerate according to a linear model. In the regenerating arms, the regeneration rate was estimated to be 0.1 mm.week⁻¹ and in the non-regenerating arms, the growth rate was 0.004 mm.week⁻¹. Sea stars regenerate ca. 20 % of the arm in one month, and it takes about 5 months to be completely regenerated. At the beginning, the regeneration rate is fast generating the growth of the arms, once the pyloric caeca and gonads are present inside the arms the regeneration rate slows down probably due to allocation to gametes and pyloric caeca and arms. The factors that regulate the regeneration rate are unknown. However, food availability and energy storage seem to play an important role. Rev. Biol. Trop. 63 (Suppl. 2): 321-328. Epub 2015 June 01.

Key words: Regeneration rate, Asteroidea, fission, *Allostichaster capensis*, mixed-model effects.

Asexual reproduction by fission is the less common way of reproduction in echinoderms, only about 80 species are known to be able to reproduce asexually (Lawrence & Herrera, 2000; Mercier & Hammel, 2013). In Asteroidea there are three families that are able to use this type of reproduction: Asterinidae, Asteroiidae and Solasteridae (Mladenov & Burke, 1994). Fission is different from autotomy, which is the detachment of one or more arms, probably as a defense mechanism. During fission, the sea stars usually divide in two nearly equal halves, resulting in two animals that have to regenerate the missing parts (disc and new arms) (Emson & Wilkie 1980). This process is influenced

by photoperiod, nutritional condition, food availability, seawater temperature and density (Mladenov et al. 1986; Alves et al., 2002; Skold et al. 2002; Rubilar et al., 2005; Haramoto et al. 2007; Barker & Scheibling, 2008; Rubilar et al., 2011; Sterling & Shuster, 2011). Often fission occurs during a specific period of the year and the sea stars regenerate the new disc and arms during the rest of the year, until the individuals are practically symmetrical and fission occurs again (Mladenov et al., 1986; Achituv & Sher, 1991; Alves et al., 2002; Rubilar et al., 2005; Barker & Scheibling, 2008).

Studies on regeneration rate have been done in the sea star *Luidia clathrata* reporting



that the factors influencing this rate may be related to food availability, length of autotomize arm, number of arms and salinity (Dehn, 1980; Lawrence et al., 1986; Lawrence & Ellwood, 1991; Pomory & Lares, 2000; Lawrence & Pomory, 2008; Kaack & Pomory, 2011). However, no studies focusing on the regeneration rate after fission had been done yet. The aim of this work was to analyze the regeneration rate after fission of the fissiparous sea star *Allostichaster capensis* as part of its life story.

The genus *Allostichaster* belongs to the Asteroiidae family. Almost all species are fissiparous and the process of fission has been described in *A. poliplax* (Emson, 1978) and *A. capensis* (Rubilar et al., 2006). The individuals usually show a distinctive asymmetry with two sets of arms of different sizes, although the asymmetry fades with the growth of the regenerating arms, it is always possible to distinguish the plane of fission. Prior to fission, the two sets of arms are located opposite to each other and they walk in opposite directions until fracturing the individual into two halves. In *A. capensis*, fission occurs every year during the late spring and early summer and regeneration takes place the rest of the year (Rubilar et al., 2005) however, the consequent regenerative growth was still unstudied.

MATERIALS AND METHODS

Fifty sea star with six arms were collected by scuba diving during the first week of December prior to the seasonal fission peak at Punta Cuevas (42°46'49" S - 64°59' 26" W), Golfo Nuevo, Chubut, Argentina. Sea stars were transported to the laboratory and kept in an aquarium filled with seawater, divided into 25 compartments by a mesh that allowed water flow. Two sea stars were randomly assigned to each compartment. An in situ Magnum 250 Marineland canister filter was continuously used to maintain water quality, circulating oxygenated water. Additional air pumps were used to ensure water aeration. Twenty five percent of the water volume was changed weekly. An air-driven box filter containing filter floss and

activated charcoal was additionally used to maximize aeration and filtration. The experiment was conducted under a 12 h light: 12 h dark schedule at constant temperature (15 °C), a similar situation to natural conditions during fission season (Rubilar et al., 2005). Sea stars were fed *ad libitum* with the mussel *Aulacomya atra atra*. After fission, 45 sea stars were size 1 (11 - 20 mm) and 55 were size 2 (21 - 30 mm). After sea stars underwent fission, the length of the three non-regenerating and the three regenerating arms were measured weekly in the resulting one hundred sea stars to the nearest 1mm with a digital caliper. Arm length measured from the disk to the arm tip is usually the parameter used to measure the growth of the arms since this measure is easy to obtain and it is not invasive. At the end of the experiment, sea stars were returned to their natural environment. Due to mortality of 11 sea stars and no regeneration on 7 sea stars, 82 sea stars were used for the statistical analysis.

The experimental data were obtained from the regenerating sea stars that were measured repeatedly through time. Since there was temporal pseudo-replication (repeated measurement) (Pinheiro & Bates 2000), non-Linear Mixed Effect models were used in order to account for within-individual correlation in all models. To this, "individuals" were included as a random effect (Littell et al., 2000). To analyze the growth of regenerating arms, a set of six candidate mixed-effects models were fitted to the regenerating and no-regenerating data (Table 1). For each data set, all growth models were fitted using Maximum Likelihood. The performance of each model was assessed by Information-Theoretic procedures (IT). To quantify the plausibility of each model given the data and the set of models the Akaike information criteria (AIC), differences in AIC (Δ_i) and AIC weights (w_i) of all possible models were obtained (Burnham et al., 2011; Symonds & Moussalli, 2011). Under this criterion the model with the lowest AIC and highest w_i is the one that best represents the data. To supplement parameter likelihood evidence, 95% confidence intervals were also calculated for all estimated

TABLE 1
Alternative growth models fitted to weekly sea star radius data. R is the radius (mm) at time t . Letters a , b and c represent model parameters

Model	Equation	Model/source	Parameters significance
m_1	$R = a \times (1 - e^{-b \times (t-c)}) + er$	von Bertalanffy (1938)	a is the size reached after an infinite time of growth, b is the growth constant, c is the age at which size would be zero and d determines the shape of the curve.
m_2	$R = a \times e^{-e^{-b \times (t-c)}} + er$	Gompertz (1825)	
m_3	$R = \frac{a}{1 + e^{-b \times (t-c)}} + er$	Logistic (Ricker, 1975)	
m_4	$R = a \times t^2 + b \times t + c + er$	Quadratic	a is the deceleration of growth, b is the regeneration rate and c is the initial size
m_5	$R = a + b \times t + er$	Linear	a is the age at which size would be zero and b determines the slope of the line
m_6	$R = a + er$	Null	

parameters in each analyzed model. The Quadratic function model (m_4) was the best fit to the data for the regenerating arms and the Linear function model (m_5) was the best fit to the data for the non-regenerating arms (Table 2); therefore, these models were used to make the comparison between sizes (Burnham et al., 2011; Symonds & Moussalli, 2011). Models with all possible combinations of variables were considered, including the null model (without any of the independent variables) and the full model (with all independent variables). All statistical analyses were performed with the Open Access Software R 3.0.2 (R Development Core Team 2013). Non-linear mixed-effects models were fitted using Maximum Likelihood with the “nlme” package (Pinheiro and Bates

2000). The library “*bbmle*” (Bolker 2013) was also used to generate 95 % confidence intervals to each estimated parameter.

RESULTS

The mixed linear model analysis used to determine the growth pattern of the regenerating arms and the non-regenerating arms of the seas stars revealed that the regenerating arms regenerate according to a quadratic model, while the non-regenerating arms adjusted to a linear model (Table 2). In the regenerating arms, the regeneration rate was estimated to be 0.1 mm.week⁻¹ (Table 3); the term *time* explained 36.05 % of the total variation, while differences among individuals explained 63.95

TABLE 2
Non-Linear Mixed-Effects Models selection for sea star radius data in regenerating and non-regenerating individuals. In bold type the best models

Model	Regenerating arms			Non-regenerating arms		
	AIC	Δ_i	w_i	AIC	Δ_i	w_i
m_1	356.58	174.72	< 0.001	963.90	10.50	0.002
m_2	240.27	58.42	< 0.001	956.40	3.00	0.10
m_3	242.25	60.39	< 0.001	956.40	3.00	0.10
m_4	181.86	0.00	0.97	956.21	2.81	0.11
m_5	188.83	6.97	0.03	953.40	0.00	0.45
m_6	1041.69	859.83	< 0.001	954.75	1.35	0.23

$N^\circ par_i$ = number of parameters, AIC_i = Akaike’s information criterion, Δ_i = AIC differences, w_i = normalized weights of AIC_i .

TABLE 3
Non-Linear Mixed-Effect Model parameter estimates and 95 % confidence limits (brackets) corresponding to the Quadratic function model for regenerating individuals and the Linear function model for non-regenerating

Treatment	Parameters		
	<i>a</i>	<i>b</i>	<i>C</i>
Regenerating arms	- 0.003 (- 0.005 to -0.002)	0.1 (0.08 to 0.12)	0.29 (0.16 to 0.40)
Non-regenerating arms	1.97 (1.90 to 2.03)	0.004 (0.002 to 0.006)	–

% of the total variation. Size was important in determination of the regeneration rate, bigger individuals had a slower regeneration rate (0.095 mm.week⁻¹) than smaller ones (0.104 mm.week⁻¹) (Fig. 1) (Table 4).

In the non-regenerating arms, the growth rate was very slow (0.004 mm.week⁻¹) (Table 3); the term time explained 38.39 % of the total variation, while differences among individuals explained 61.61 % of the total variation. Size was important in determination of the regeneration rate, bigger individuals had negligible growth while smaller individuals grew slightly (Fig. 1) (Table 4).

DISCUSSION

Regeneration after fission is quite a different process than regeneration after autotomy. During fission, the individuals break up the internal organs of the disc (jaws, stomach,

nervous system, etc.) and afterwards the wide wound must be closed and these organs must be regenerated as well as three new arms. Since arms are necessary for locomotion, feeding, energy storage (in the pyloric caeca) and reproduction (by production of gametes in the gonads); fission and regeneration impose functional constraints to the individuals (Lawrence & Larrain, 1994; Lawrence, 2010). Sea stars that have suffered multiple arm loss have their foraging capabilities diminished (Bingham et al., 2000; Ramsay et al., 2001; Díaz-Guisado et al., 2006; Barrios et al., 2008). Recently split *A. capensis* individuals have a reduced capacity to feed. Until the individuals are able to feed again, their survival after fission and success regeneration of the new arms depends on the nutrients stored in the pyloric caeca (Rubilar et al., 2011). The variability observed in the regeneration rate among individuals in this study may be related to the nutritional state of

TABLE 4
Non-Linear Mixed-Effect Models selection for sea star radius variation in each body size classes.
In bold type the best models

Models	N° par	AIC	Δ_i	w_i %
Regenerating individuals				
<i>a</i> ~ <i>fac</i> , <i>b</i> ~ <i>fac</i> , <i>c</i> ~ <i>fac</i>	10	161.47	4.98	0.04
<i>a</i> ~1, <i>b</i> ~ <i>fac</i> , <i>c</i> ~ <i>fac</i>	9	159.79	3.30	0.09
<i>a</i> ~ <i>fac</i> , <i>b</i> ~1, <i>c</i> ~ <i>fac</i>	9	159.44	2.95	0.11
<i>a</i> ~ <i>fac</i> , <i>b</i> ~ <i>fac</i> , <i>c</i> ~1	9	159.45	2.96	0.11
<i>a</i> ~ <i>fac</i> , <i>b</i> ~1, <i>c</i> ~1	8	158.60	2.11	0.17
<i>a</i> ~1, <i>b</i> ~ <i>fac</i> , <i>c</i> ~1	8	156.49	0.00	0.48
<i>a</i> ~1, <i>b</i> ~1, <i>c</i> ~ <i>fac</i>	8	166.70	10.21	0.006
<i>a</i> ~1, <i>b</i> ~1, <i>c</i> ~1	7	181.86	25.37	<0.001
Non-Regenerating individuals				
<i>a</i> ~ <i>fac</i> , <i>b</i> ~ <i>fac</i>	7	- 1834.46	0.00	0.77
<i>a</i> ~1, <i>b</i> ~ <i>fac</i>	6	- 755.37	1079.09	< 0.001
<i>a</i> ~ <i>fac</i> , <i>b</i> ~1	6	- 1832.08	2.38	0.23
<i>a</i> ~1, <i>b</i> ~1	5	953.40	2787.85	< 0.001

$N^\circ par_i$ = number of parameters, AIC_i = Akaike's information criterion, Δ_i = AIC differences, w_i = normalized weights of AIC_i .

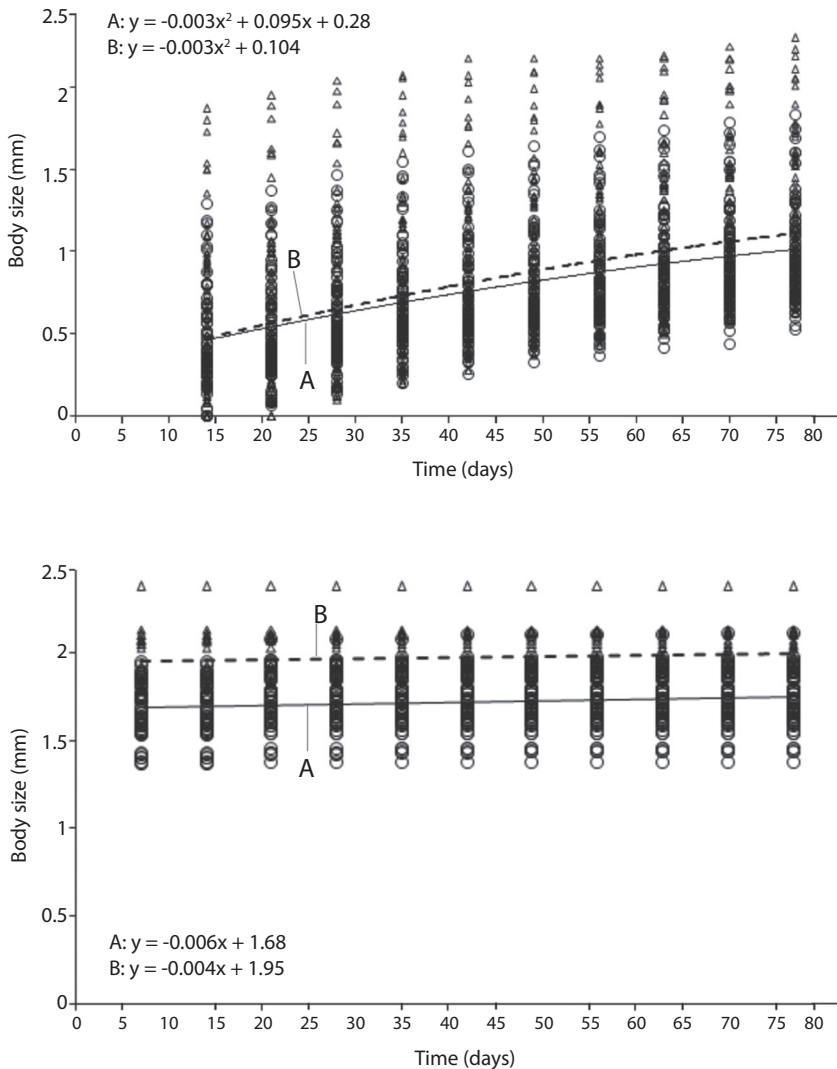


Fig. 1. Sea stars arms growth related to days at two sizes in regenerating (upper panel) and in non-regenerating (lower panel) arms. In both cases: A, individuals at size 1 (solid line); B, individuals at size 2 (dotted line). Estimated parameters values for each size are shown in each graph. The lines indicate the estimated mean of non-linear mixed-effect quadratic model for regenerating arms (upper panel) and the estimated mean of non-linear mixed-effect linear model for non-regenerating arms (lower panel).

each individual before fission occurs. Those individuals with reserve of nutrients in their pyloric caeca probably will have more chances of surviving and a faster regeneration rate. The new arm buds appear ca. 7 days after fission, while in *L. clathrata* ca. 8 days after arm loss regardless of the where the arm loss occurs (Lawrence & Pomory, 2008).

According to the mixed linear model analysis, the regeneration data had the best fit to a quadratic model in *A. capensis*. Regeneration rate was rapid at first and slowed down as the regenerating arms near the length of the intact arms. The same scenario was observed for the regeneration rate of *L. clathrata* regardless of the where the arm loss occurs and the number

of arms that had been regenerate (Lawrence & Ellwood, 1991; Pomory & Lares, 2000; Lawrence & Pomory, 2008). *A. capensis* regenerates ca. 20 % of the arm in one month, therefore after 4 - 5 month the arms would be completely regenerated. Even though arm length may not be an ideal indicator of growth, since most of the growth represents incorporation of biomass through body thickening rather than increasing length, it can be used to compare with other sea stars species. Despite the fact that autotomy and fission are different processes; the rate of the arm regeneration can be compare. *Asterias rubens* with two autotomized arms regenerates ca. 7 % in one month and with three autotomized arms regenerates ca. 9 % in one month, taking 8 - 9 month to completely regenerate in length (Ramsay et al., 2001). In the field, *L. clathrata* regenerates ca. 8 % of an arm in one month in individuals with two regenerating arms and it takes about 12 - 13 month to completely regenerate in length (Pomory & Lares, 2000). However, the regeneration rate varies according to the position of arm loss, the rate was inversely related to the position of arm loss. Autotomized arms proximal to the disc regenerate ca. 11 % in one month, autotomized arms in a medial position to the disc regenerate ca. 7.5 % and distal to the disc ca. 3.9 % (Lawrence & Pomory, 2008). One would expect that a fissiparous sea star that produces gametes and undergoes fission every year has to have a high regeneration rate to the arms reach the length to undergo fission again. Initially, the regeneration rate is fast generating the growth of the arms in length. Once the arms are about 40 - 50 % of the total length (2 - 3 months) the pyloric caeca and gonads are present inside the arms and gametogenesis begins in all the arms of the sea stars (Rubilar et al., 2005). Afterwards, the regeneration rate slows down probably due to energy allocation to gametes and pyloric caeca and to growth of the arms in length. The regeneration rate between sizes was different, larger individuals had a higher value than smallest ones. This may be related to the size that has to be regenerate, since the regeneration time frame is the same for all. The non-regenerating

arms growth data had the best fit to a lineal model, the non-regenerating arms practically did not increased in size during the experiment; only the smallest individuals showed a slight growth. According to Rubilar et al. (2005) recently split individuals have gonads negligible in size and in recovery stage, therefore, no energy is invested in gonads during the first stages of regenerations. This supports the idea that energy it is allocated in regenerating the new arms, pyloric caeca and to produce gametes immediately thereafter in regenerating and non-regenerating arms. According to Rubilar et al. (2005) sea stars bigger than 50 mm are rare, most individuals do not exceed the 30 mm. The absence of growth in the non-regenerating arms would limit the size of the individuals of this species.

The factors that regulate the regeneration rate are not known. However food availability seems to play an important role in non-fissiparous sea stars (Lawrence & Ellwood, 1991; Ramsay et al., 2001), while nutrient storage in fissiparous species (Rubilar et al., 2011). Salinity seems to be another important factor to determine regeneration rate. For instance, *L. clathrata* exposed to low salinity (20 %) presented lower regeneration rates than in normal conditions (Kaack & Pomory, 2011). There are no studies on the effect of salinity in the regeneration rate of other sea stars. Temperature may also be important, but no studies focus on the effect of temperature in regeneration rate. There are no data of other populations in the wide distribution range of *A. capensis* and therefore it would be interesting to study if the regeneration rate varies with different environmental conditions. There is a need on research on the factors controlling the regeneration rate in fissiparous and non-fissiparous sea stars for predicting the future of these species under the climate change scenario.

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RESUMEN

Tasa de regeneración luego de la fisión en la estrella de mar fisipara *Allostichaster capensis* (Asteroidea). Numerosos estudios se han enfocado en estudiar la tasa de regeneración de brazos en Asteroidea, sin embargo ninguno se han centrado en la tasa de regeneración después del proceso de fisión. *Allostichaster capensis* es una estrella de mar fisipara con un amplio rango de distribución. En el Golfo Nuevo (42°46'49" S - 64°59'26" W) las estrellas de mar se fisieron cada primavera y verano y se regeneran durante el resto del año. Para analizar la tasa de regeneración, se realizó un experimento con estrellas de mar colectadas justo antes de la fisión. Después de estrellas de mar se fisieron, se midió la longitud de los tres brazos en regeneración y de los tres brazos originales semanalmente. Se utilizaron modelos no lineales de efectos mixtos para analizar la correlación dentro de individuos del largo de los brazos (regenerados y que no regeneran). Los brazos en regeneración, regeneraron sus brazos de acuerdo con un modelo cuadrático, mientras que los brazos originales ajustaron a un modelo lineal. En los brazos de regeneración se estimó que la tasa de regeneración era de 0.1 mm/semana; mientras que en los brazos originales, la tasa de crecimiento fue de 0.004 mm/semana. Las estrellas de mar se regeneran un 20 % aproximadamente del brazo en un mes, y tardan alrededor de cinco meses en estar completamente regenerado. Al principio, la alta tasa de regeneración genera el crecimiento rápido de los brazos en longitud, una vez que el ciego pilórico y las gónadas están presentes en el interior de los brazos; la tasa de regeneración disminuye, probablemente debido a la asignación de energía en la producción de gametas, en el ciego pilórico y en los brazos. Los factores que regulan la tasa de regeneración son desconocidos. Sin embargo, la disponibilidad de alimentos y el almacenamiento de energía parecen jugar un papel importante.

Palabras clave: Tasa de regeneración, fisión, *Allostichaster capensis*, efecto de modelos mixtos.

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