Universidad del País Vasco / Euskal Herriko Unibertsitatea

ZIENTZIA ETA TEKNOLOGIA FAKULTATEA
FACULTAD DE CIENCIA Y TECNOLOGÍA

Gradu Amaierako Lana /Trabajo Fin de Grado
BIOLOGIA Gradua / Grado en BIOLOGÍA

Distribution of *Actinia equina* L. (Cnidaria, Anthozoa) in crevices and rock pools in Northern Spain (Cantabrian Sea, Biscay Bay): growth and spawning across winter to spring transition.

Egilea/Autor:
Unai Blanco Fundazuri
Zuzendaria/Director/a:
M. Mercedes Ortega Hidalgo

INDEX

ABSTRACT

In the littoral area, the sessile organisms that inhabit the intertidal zone undergo abrupt environmental variations due to their amphibious lifestyle. *Actinia equina* (L.) is a cosmopolitan species that can colonize upper shore levels (mussel fringe) occupying preferently sheltered areas such as rock crevices or intertidal pools to reduce extreme desiccation stress.

This study is complementary to the previous investigations that have been carried out with the community of *A. equina* from Sopelana, and its objectives are, on the one hand, to monitor in situ the anemones that inhabit both crevices and rock pools, also recording juvenile individuals, and on the other hand, to analyse in the laboratory the biometric parameters of individuals corresponding to rock pools. For this, periodic samplings have been carried out in the field and a relation has been established in the laboratory between the pedal disc area (PDA) and the live weight (LW) of the anemones.

In this way, it has been possible to observe a growth in the size of the individuals monitored in the field, and the effects of zonation and how anemones face temperature changes when they emerge have been determined. The number of offspring and the juveniles' growth have also been monitored. In the laboratory experiment, it has been concluded that the relation between pedal area and live weight does not differ significantly when rock pool anemones are submerged or after 3 hours of emersion. In addition, comparing with previous studies, it has been observed that the anemones of the cracks show a significantly equal relation, therefore it has been shown that biometry is a reliable method and that it is useful to determine the biomass of the anemones in situ.

Key words: *Actinia equina*, anemone, intertidal, biometry, monitoring, spawning, growth, distribution.

RESUMEN

En el área litoral los organismos sésiles que habitan en la zona intermareal sufren variaciones ambientales abruptas debido a su estilo de vida anfibia. *Actinia equina* (L.) es una especie cosmopolita capaz de colonizar zonas intermareales que generalmente opta por ocupar grietas o charcos en las rocas para evitar los cambios bruscos de condiciones ambientales.

Este estudio es complementario a las investigaciones previas que se han llevado a cabo con la comunidad de *A. equina* de Sopelana, y tiene como objetivo, por un lado, hacer un seguimiento in situ de las anémonas que habitan tanto en grietas como en charcos registrando también los individuos juveniles, y por otro lado, analizar en el laboratorio los parámetros biométricos de individuos correspondientes a los charcos. Para ello, se han realizado muestreos periódicos en el campo y en el laboratorio se ha establecido una relación entre el área pedal y el peso vivo de las anémonas.

De esta manera, se ha podido observar un crecimiento de tamaño de los individuos monitorizados en el campo y se han determinado los efectos que tienen la zonación y cómo afrontan las anémonas los cambios de temperatura cuando emergen. También se han monitorizado las crías y el crecimiento tanto en número como en tamaño. En el experimento de laboratorio se ha llegado a la conclusión de que la relación del área pedal y el peso vivo no difieren significativamente cuando las anémonas de los charcos están sumergidas o después de 3 horas de emersión. Además, comparando con estudios previos, se ha observado que las anémonas de las grietas muestran una relación significativamente igual, por lo que se ha demostrado que la biometría es un método fiable y que sirve para determinar la biomasa de las anémonas in situ.

1. INTRODUCTION

In the littoral area where tidal cycles occur, intertidal animals often suffer large and sudden perturbations related to amphibious life with temperature changes playing a determinant role (Cornelius 1972; Ortega et al 1984). Littoral Anthozoa are sessile and their ability to evade environmental stress is minimal being therefore a good model to study physiological responses to such short-term environmental changes (Navarro et al 1987).

Environmental conditions play a part in determining the distribution of benthic organisms such as sea-anemones (Gomes et al 1998). *Actinia equina* (L.), also known as the common beadlet sea anemone, is a cosmopolite species that is generally found on rocky shores (Monteiro et al 1997). This species has been extensively studied because of its abundance and ecological importance and remains a theme of considerable research concern (Orr et al 1982).

A. equina individuals are numerous in intertidal communities from Kola Peninsula (Russia), Atlantic coast of Europe (even in the British Isles) and West Africa (including isles along the African coast) in addition to the Adriatic, Mediterranean and Black Seas (Carling et al 2019; Chomsky et al 2009; Monteiro et al 1997; Ortega et al 1987). On exposed littorals the common beadlet is most abundant on the lower shore (in damp crevices or in rock pools) but it is also possible to found it on the middle and higher shore (Davenport et al 2011; Navarro et al 1981).

Living in intertidal zones involves emersion when tide level is low and *A. equina* is often susceptible to desiccation (Carling et al 2019). To avoid the abrupt changes of temperature, the anemones occupy rock pools and shaded crevices (Cornelius 1972) and they have a huge resistance of tissue desiccation (Griffiths 1977). Besides, the beadlet anemone is a sedentary (even if it is capable of slow movements) omnivorous passive suspension feeder (Chintiroglou & Koukouras 1992; Davenport et al 2011; Kruger & Griffiths 1996). Consequently, *A. equina* feeds on available material carried by wave and current action (Gomes et al 1998) and it is highly dependent on shore height and exposure (Davenport et al 2011).

Even if sexes in *A. equina* are separate (Chia & Rostron 1970), in a way majority of locations these anemones reproduce asexually (Chomsky et al 2009). It is also known that adult individuals may contain young anemones (irrespective of sex) and the colour of brooded juveniles generally coincide with their parents' colour (Cain 1974; Orr et al 1982). Nevertheless, they show capability for sexual reproduction although prepanula larval stages have been rarely found (Carter & Thorp 1979).

Several studies have been made in order to determine the distribution, growth, oxygen consumption and spawning of *A. equina* from Sopelana littoral (Biscay, Basque Country) (Barrenetxea 2015; Navarro et al 1981, 1987; Ortega et al 1984, 1987, 1988). The present study also deals with anemones from Sopelana shore and is complementary to the previous ones.

This work involves in situ monitoring and analysis of *A. equina* inhabiting rock crevices and rock pools. However, the study is chiefly focused on anemones from rock pools which were carried to laboratory in order to analyse biometrical parameters. Hence, these are the main objectives of this work:

- To find a relation between live weight and morphometric parameters of anemones from rock pools during submersion and emersion. In that way, it can be analysed whether this relation is the same in both conditions. The purpose of the laboratory experiment with individuals from pools is to compare results obtained with anemones from crevices by Ortega and Barrenetxea in 2016.
- The monitoring of anemones living in rock crevices and rock pools. Thus, biometrical characteristics of individuals inhabiting both habitats can be determined. Furthermore, monitoring allows even to compare individuals from different crevices or rock pools among them. Besides, influence of environmental temperature on anemones was analysed.
- The observation and monitoring of spawning in field and in laboratory in order to characterize the number of new individuals and their growth.

2. MATERIALS AND METHODS

2.1 Study area

The littoral area of Sopelana (Biscay, Basque Country) is the location of our study as well as in previously mentioned studies. The beaches in Sopelana are normally sandy and exposed (Navarro et al 1981), and an intertidal rocky area (loamy limestone) is characteristic of the shore (Barrenetxea 2015). Two nearby sites (200m separated) were selected for this study:

1. Three rock crevices were chosen in Atxabiribil beach (43°23'26.0"N 2°59'35.2"W) and 100cm of each crevice was monitored. They were given the name of ‘Rock Crack 1’, ‘Rock Crack 2’ and ‘Rock Crack 3’. Rock Crack 2 is located 100 cm above sea level and Rock Crack 3 and Rock Crack 1 are respectively 198 and 258 cm above sea level.
2. Three shallow rock pools (maximum depth = 7.9 cm) from Playa del Carbón (43°23'29.3"N 2°59'28.0"W) were selected for observation. They were named ‘Rock Pool 1’, ‘Rock Pool 2’ and ‘Rock Pool 3’.

Both sites belong to a similar tidal level (upper zone: 12-16 exposure hours per day; Barrenetxea 2015) and are well represented by *A. equina* mainly by red or green morphs as brownish individuals appear rarely.

2.2 Field monitoring

Anemones from the three crevices in Atxabiribil beach and the three rock pools in Playa del Carbón beach were monitored 8 times between December 16th, 2019 and June 2nd, 2020 (last sampling date before the pandemic was March 14th and first measurement after the confinement was on May 29th). 10 specimens of each crevice and rock pool were randomly selected for monitoring (except for the site named 'Rock Pool 1' where there were only 7 individuals) for a total of n=57 anemones (30 in crevices and 27 in rock pools). Pictures were taken with a mobile phone (16MP) and a meter rule was placed next to the anemones as a reference. Then, pedal disk area (PDA, mm^2) was calculated employing ImageJ software (available at: http://rsb.info.nih.gov/nih-image/). A frequency distribution of this morphometric parameter was made in crevices (grouping Rock Crack 1, Rock Crack 2 and Rock Crack 3) and rock pools (grouping Rock Pool 1, Rock Pool 2 and Rock Pool 3).

For the purpose of comparing both habitats and determine the potential growth in each site, mean PDA was calculated. In addition, equality among the mean PDA of each rock pool was analysed with ANOVA and Tukey tests. The same was done to test the PDA mean equality among the three rock cracks. Besides, effects of zonation were measured by testing whether height above the sea level influences size of anemones inhabiting crevices. For that, mean PDA of all samplings in each rock crevice was calculated and a graphic representation was created facing PDA means against crevices' height.

Additionally, air temperature was measured as well as the corporal temperature of anemones emerged in rock crevices. Thus, difference between anemones' and air temperature was calculated on different sampling dates.

2.3 Biometry in laboratory

The March 3rd, 2020 anemones were carried from Playa del Carbón site for biometrical analysis in laboratory. 20 individuals from which 12 were red, 6 were green and 2 were brownish were carefully collected from rock pools. In the laboratory, anemones were maintained in an aquarium with constant aeration and re-circulating sea water and they were regularly feed with imitation elvers (fish protein).

The temperature of the aquarium was kept constant on 17ºC throughout the experiment in order to match sea temperature (the day anemones were collected sea temperature was 17.1ºC). In the aquarium, each specimen was placed in a different tagged container to allow individual monitoring. Once anemones were acclimated and attached to their new substrate, biometry experiment was started. From March 3rd to May 8th the specimens were individually weighed in two different conditions: when anemones were taken out of water (t0) and after three hours of exposure (t3). Thus, 10 weight measurements at t0 were done as well as 6 measurements for t3.

Pictures of specimens were taken at the beginning (t0) and after three hours of exposure (t3) with a mobile phone (16MP) each time weight was measured. Images were processed with ImageJ to obtain the pedal disk area (PDA, mm^2) of each anemone. When both parameters (Live Weight and PDA) were obtained, the log-log relation between them was determined. For that, regression lines of measurement results in t0 and t3 corresponding the equation Log (LW) = $a + b \cdot$ Log (PDA) were created. In order to determine whether the value b of the slope was significantly different for t0 and t3 ANCOVA test was applied.

2.4 Spawning

The crevice ‘Rock Crack 2’ from Atxabribil beach and the rock pool named ‘Rock Pool 3’ from Playa del Carbón beach were selected for the monitoring of the spawning. Images obtained from field monitoring were used for spawning analysis. A monitoring of all juveniles in both sites was carried out and their PDA was calculated employing ImageJ software. Only two young anemones were found in ‘Rock Crack 2’ and they disappeared from a sampling day to the following one. Therefore, they were not considered and only ‘Rock pool 3’ juveniles were subjected to PDA analysis. A frequency distribution of this morphometric parameter allowed to determine the growth of the offspring.

Spawning was also expected in laboratory. Anemones were placed in tagged individual flasks in order to determine the number of juveniles coming from each adult.

2.5 Statistical analysis

For ANOVA and Tukey tests Past software (available in: https://folk.uio.no/ohammer/past/) was used. ANCOVA tests and regression lines were made with Statview software (not available) and frequency distributions and regression lines were calculated and plotted with Microsoft Excel software (not free, available in: https://www.microsoft.com/en-us/microsoft-365/excel).

3. RESULTS

3.1 Field monitoring

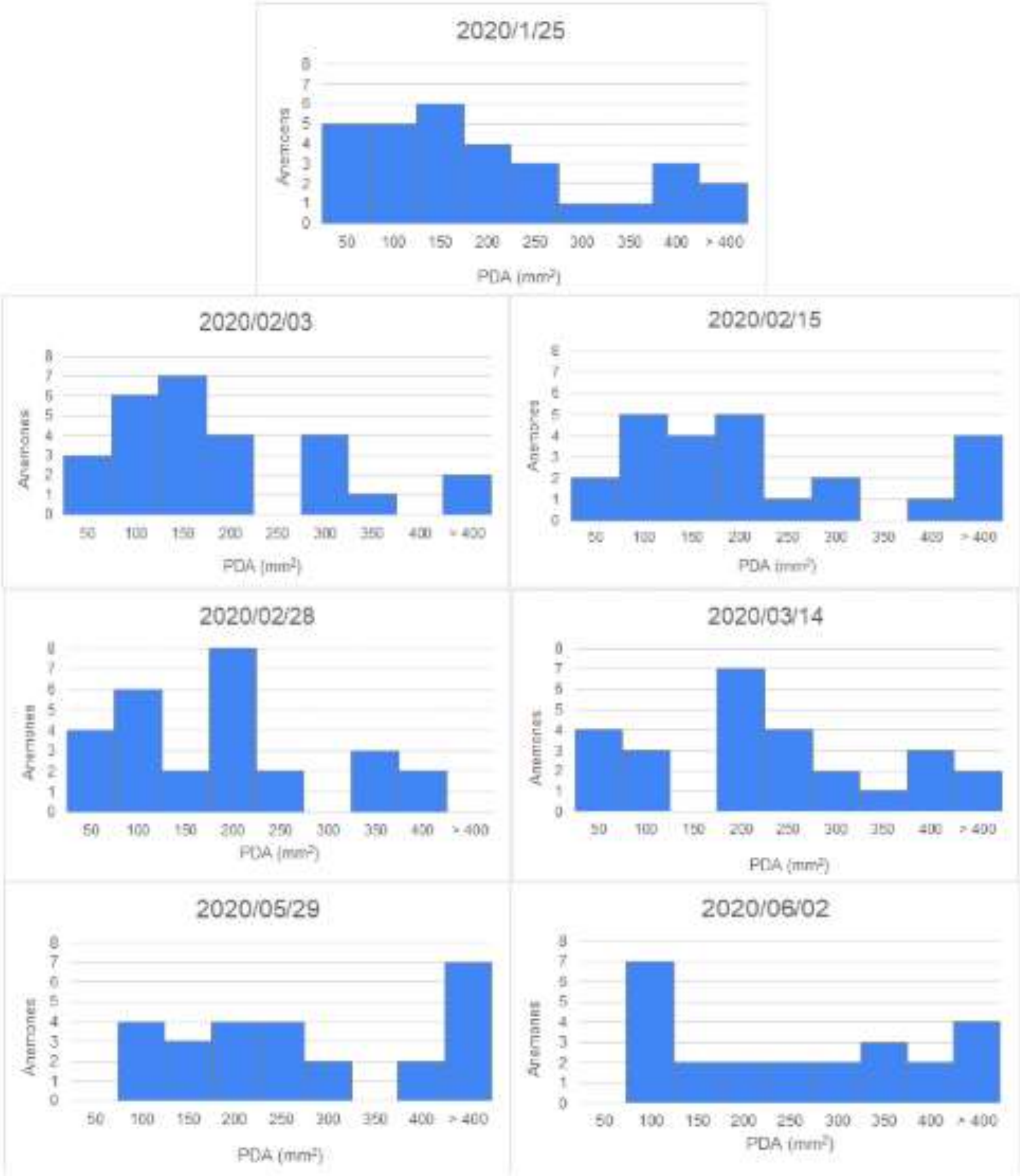

Figure 1: frequency distribution in rock crevices (Rock Crack 1, Rock Crack 2 and Rock Crack 3 grouping) depending on Pedal Disk Area (mm^2) between January 25th and June 2nd, 2020.

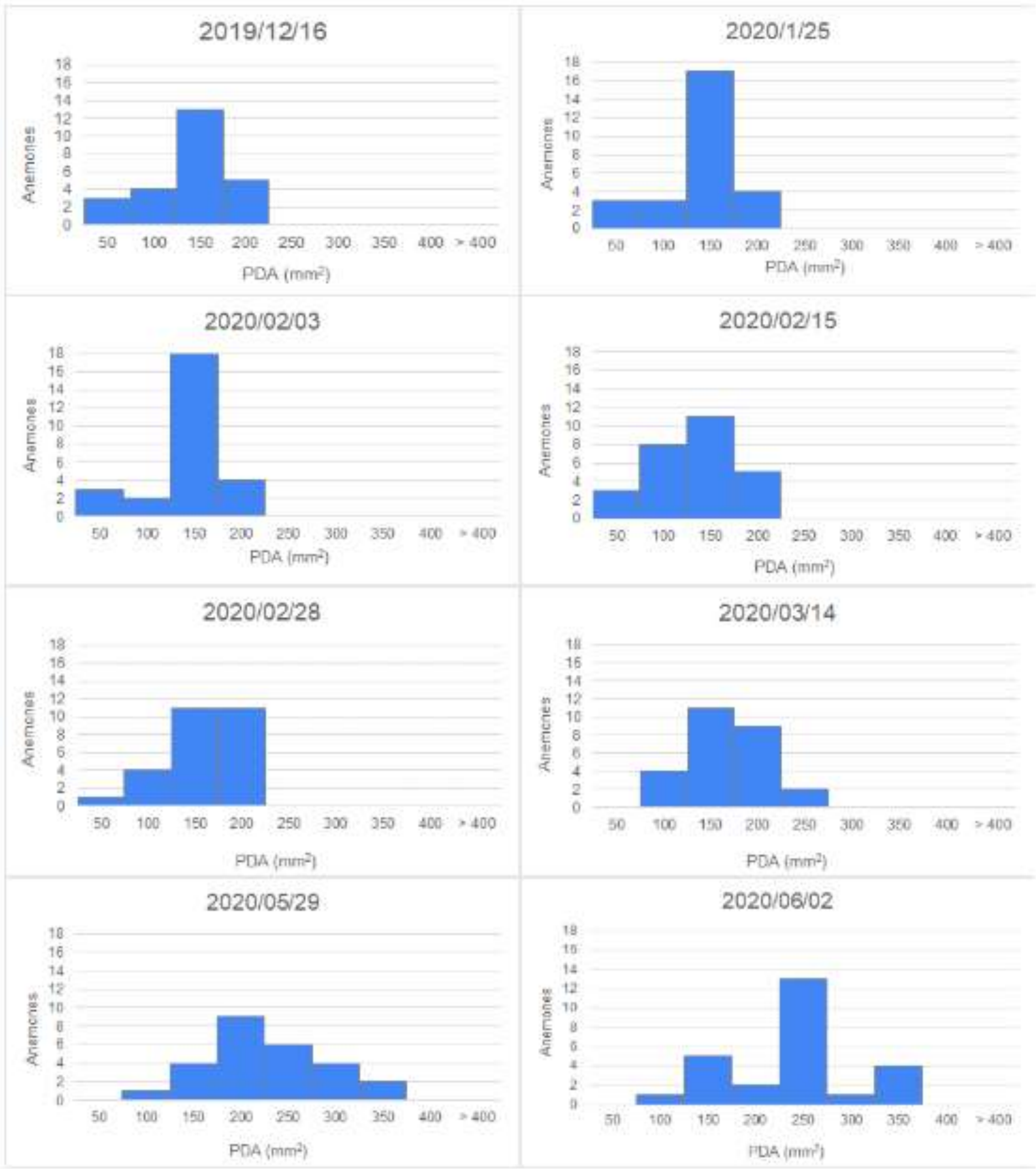

Figure 2: frequency distribution in rock pools (Rock Pool 1, Rock Pool 2 and Rock Pool 3 grouping) depending on Pedal Disk Area (mm^2) between December 16th, 2019 and June 2nd, 2020.

In rock crevices (*Figure 1*), the first measurement on January 25th, 2020 values range from <50 to >400 mm^2. Next date the frequency distribution is similar yet on February 15th it can be noticed that number of anemones with PDA ranging from 100 to 150mm^2 lessens whereas there are more individuals that reach sizes between 150 and 250mm^2. This tendency can be observed until March 14th where almost 50% of anemones have a PDA size between 150 and 250mm^2.

After the interruption due to COVID-19 pandemic, no more individuals with a PDA <50 were observed. Besides, on May 29th and June 2nd individuals measuring >300mm^2 represent more than a third of the sample, and they are the only dates where this percentage is surpassed.

In the case of rock pools (*Figure 2*), in the first three measurements between December 16th, 2020 and February 3rd, 2020, more than a half of individuals have a PDA between 100 and 150mm^2. On February 15th the number of individuals with a PDA between 100 and 150mm^2 lessens while those ranging from 150 to 200mm^2 increase. On the next two dates most specimens' PDA are found to be between 100 and 200mm^2.

A change in the distribution can be observed in the samplings after the COVID-19 pandemic. On the one hand, on May 29th the PDA of more than a half of individuals range between 200 and 300mm^2. On the other hand, on June 2nd the PDA of 50% of anemones measure between 250 and 300mm^2. It must be underlined that in both measurements there are individuals that reach PDA values beyond 300mm^2 for the first time.

When comparing PDA frequency distribution of rock crevices against rock pools (*Figures 1 and 2*) it can be observed that in crevices values are more spread: individuals' sizes range from <50 to >400mm^2 in the majority of samplings as in rock pools anemones' PDA is found to be between <50 and 250mm^2. Additionally, anemones living in rock cracks reach >400mm^2 of PDA in all dates excepting February 28th while individuals from rock pools rarely pass from 300mm^2 (this only occurs in the last two measurements and individuals do not reach 400mm^2).

Rock Cracks

	2019/12/16	2020/1/25	2020/02/03	2020/02/15	2020/02/28	2020/03/14	2020/05/29	2020/06/02
Mean PDA (mm2)	234,141	185,687	165,136	208,368	163,813	209,868	308,534	280,728
Standard deviation	143,794	149,010	118,920	167,641	107,387	131,875	237,974	233,217
Variation coefficient	0,614	0,802	0,720	0,805	0,656	0,628	0,771	0,831

Test for equal means (crevices)

	Sum of sqrs	df	Mean square	F	p (same)
Between groups:	301196	2	150598	37,4	1,75E-07
Within groups:	80538,2	20	4026,91	Permutation p (n=99999)	
Total:	381734	22	1,00E-05		

Table 1: Mean PDA (mm^2), Standard Deviation and Variation coefficient from rock crevices on sampling dates (above) and ANOVA test for equal means comparing Rock Crack 1, Rock Crack 2 and Rock Crack 3 with 0,05 significance level (below).

Regarding the evolution of PDA mean from rock cracks along sampling time (*Table 1*), it can be observed that during the first 6 sampling episodes the PDA mean value keeps unvarying around 150 and 200mm2. In the last two measurements (days 165 and 169 from the start of measurements), however, mean PDA values approach 300mm2. However, ANOVA test proves that means of the three subgroups (Rock Crack 1, Rock Crack 2 and Rock Crack 3) differ significantly among them (p-value = 1,75E-07; *Table 1*), therefore analysis along time considering crevices as a group cannot be made.

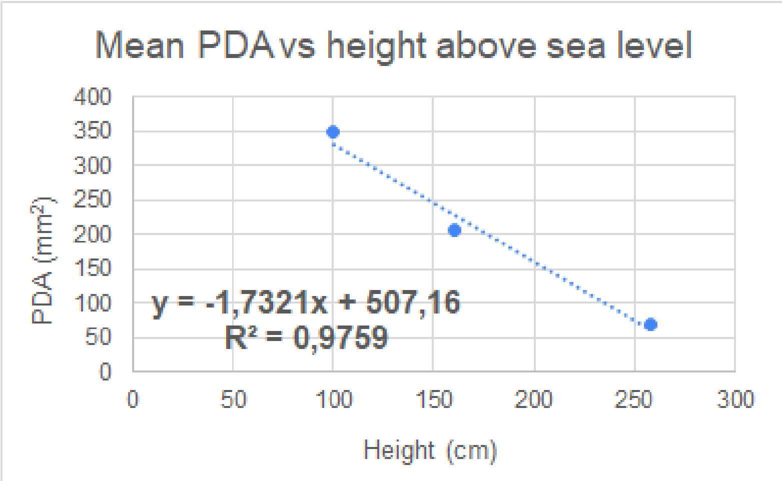

Figure 3: mean PDA (mm²) of each crevice against its height (cm). From left to right, the points correspond to: Rock Crack 2, Rock Crack 3 and Rock Crack 1.

When relating mean PDA of anemones living in crevices with the height of the crevice itself (*Figure 3*), it can be noticed that a greater elevation above the sea level anemones' PDA lessen. The equation y = -1,7321x + 507,16 shows the linear relationship between height (x) and the corresponding expected PDA for a given anemone (y). Besides, this linear regression predicts that no *A. equina* individuals are expected to be found above 293cm.

Test for equal means (rock pools)

	Sum of sqrs	df	Mean square	F	p (same)
Between groups:	4979,03	2	2489,51	1,423	0,2634
Within groups:	36750,1	21	1750,01	Permutation p (n=99999)	
Total:	41729,2	23	0,2636		

Table 2: and ANOVA test for equal means considering Rock Pool 1, Rock Pool 2 and Rock Pool 3 with a significance level of 0,05.

ANOVA test (*Table 2*) confirms that mean PDA of anemones living in each rock pool is not significantly different from the rest (p-value = 0,2634). Therefore, the three rock pools can be analysed as if it were one.

Rock Pools

	2019/12/16	2020/1/25	2020/02/03	2020/02/15	2020/02/28	2020/03/14	2020/05/29	2020/06/02
Mean PDA (mm2)	113,927	116,095	119,998	108,432	132,670	146,180	200,950	212,080
Standard deviation	42,568	40,433	37,223	40,269	43,228	46,251	58,347	67,340
Variation coefficient	0,374	0,348	0,310	0,371	0,326	0,316	0,290	0,318

Table 3: Mean PDA (mm^2), Standard Deviation and Variation coefficient from rock pools on sampling dates.

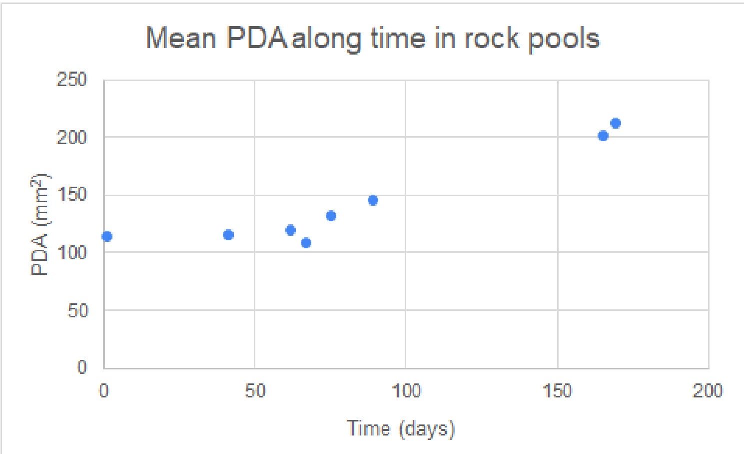

Figure 4: mean PDA (mm^2) along time of anemones living in rock pools. Day 0 corresponds to first sampling on December 16th, 2019 and day 169 corresponds to last sampling on June 2nd, 2020.

Test for equal means (rock pools)

	Sum of sqrs	df	Mean square	F	p (same)
Between groups:	301707	7	43101	18,83	2,91E-19
Within groups:	464719	203	2289,26	Permutation p (n=99999)	
Total:	766426	210	1,00E-05		

Significance of mean among sampling dates (rock pools)

	25/01/2020	2020/02/03	2020/02/15	2020/02/28	2020/03/14	2020/05/29	2020/06/02
16/12/2019	1	0,9998	0,9999	0,8512	0,2439	1,77E-08*	1,55E-10*
25/01/2020		1	0,999	0,9079	0,3051	2,19E-08*	1,78E-10*
2020/02/03			0,9869	0,9777	0,4902	1,08E07*	9,99E-10*
2020/02/15				0,579	0,08401	8,26E-10*	5,19E-12*
2020/02/28					0,9698	1,37E-05*	2,01E-07*
2020/03/14						0,001366*	3,93E-05*
2020/05/29							0,9906

**Significant*

Table 4: ANOVA test for equal PDA means of anemones living in rock pools on different sampling dates (above) and Tukey test of mean PDA among sampling dates in rock pools (below). Both tests have a significance level of 0,05.

The mean PDA of anemones living in rock pools (*Table 3*, *Figure 4*) is relatively constant between 100 and 150 mm^2 the first 89 days. Although a slightly increase can be observed, Tukey test rejects significant mean PDA differences among the first six sampling dates (*Table 4)*. However, the last two sampling episodes differ significantly from the previous ones which coincides with the measurements after COVID-19 pandemic. Therefore, mean PDA values before and after the pandemic were calculated (*Table 5*).

It can be noticed in *Table 5* that mean PDA of anemones living in rock pool increases more than $80mm^2$ from before to after the confinement. Besides, ANOVA test with 0,05 significance level proves that the increase of size is significant during the interruption of measurements due to COVID-19.

	Before Covid-19	After Covid-19
Mean PDA (mm2)	122,634	206,515
Standard deviation	43,044	62,636
Variance coefficient	0,351	0,303

Test for equal means

	Sum of sqrs	df	Mean square	F	p (same)
Between groups:	275271	1	275271	116,6	7,19E-22
Within groups:	490975	208	2360,46	Permutation p (n=99999)	
Total:	766246	209	1,00E-05		

Table 5: mean PDA (mm^2), Standard deviation and variance coefficient for rock pool anemones before and after the pandemic (above) and ANOVA test for equality od means before and after the confinement with a 0,05 significance level (below).

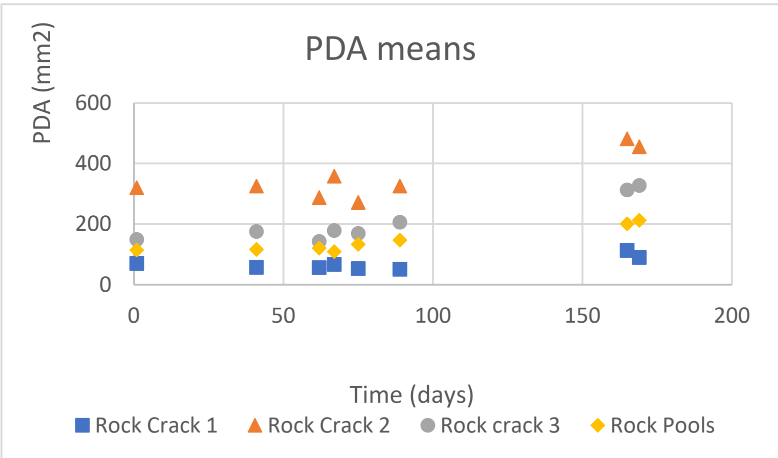

Figure 5: mean PDA (mm^2) of each sampled rock crack and the grouping of rock pools along time.

As three rock pool sites do not differ significantly yet rock crevices show significant differences, it was decided to compare them (*Figure 5*). Hence, it can be observed that Rock Crack 1 anemones' mean PDA is smaller than PDA od individuals from rock pools. On the contrary, when contrasting Rock Crack 2 with rock pools, average size of individuals living in crevices is larger (sometimes almost the double). Rock Crack 3 also presents bigger mean PDA values compared to rock pools, but smaller than Rock Crack 2. Additionally, ANOVA and Tukey test of the four sites show significant differences among them irrespective of time (*Table 6*).

Test for equal means

	Sum of sqrs	df	Mean square	F	p (same)
Between groups:	3,27E+06	3	1,09E+06	111,7	6,38E-53
Within groups:	4,01E+06	411	9766,78	Permutation p (n=99999)	
Total:	7,29E+06	414	1,00E-05		

Significance of mean among sampling sites

	Rock Crack 2	Rock Crack 3	Rock Pools
Rock Crack 1	0*	0*	2,61E-06*
Rock Crack 2		0*	0*
Rock Crack 3			0,0002864*

**significant*

Table 6: ANOVA test for equal PDA means of anemones living in rock pools and the three rock crack sites on different sampling dates (above) and Tukey test of mean PDA among sampling dates in rock pools and the three rock crack sites (below). Both tests have a significance level of 0,05.

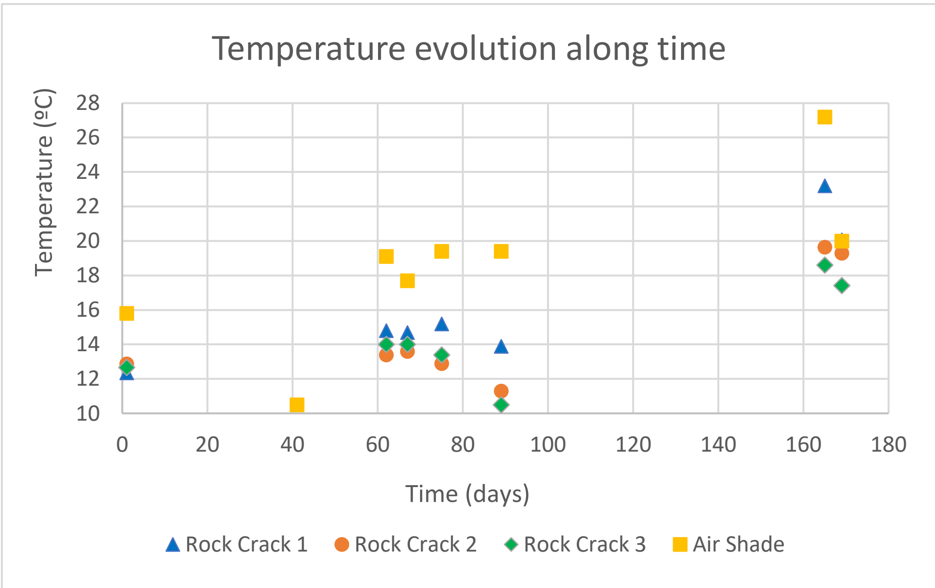

Figure 6: temperatures (ºC) registered in anemones from crevices and the respective air temperature on shade.

Temperature measurements show that A. equina from the three rock cracks have similar temperature values and rarely reach temperatures higher than 20ºC. Indeed, they only surpass that value once, and in the first 6 samplings anemones' temperature is always below 16ºC. Additionally, it is observed that excepting last date anemones are between 3 and 6ºC cooler than air temperature on shade.

3.2 Biometry in laboratory

Anemones were weighed and PDA was measured right after being submerged (t0) and after 3h of exposure (t3). Thus, regression lines which show the logarithmic relationship between PDA and Live Weight (LW) of anemones living in rock pools were created. An equation was originated for each moment:

t0: Log LW = 3,0689 + 1,1264 · Log PDA

t3: Log LW = 3,3405 + 1,0946 · Log PDA

ANCOVA test with significance level of 0,05 was made to prove equality between *b* values of t0 and t3. The ANCOVA test proved that both slopes are significantly equal (p-value = 0,8276), therefore a new relationship including t0 and t3 whose value for *b* is 1,1125 was originated.

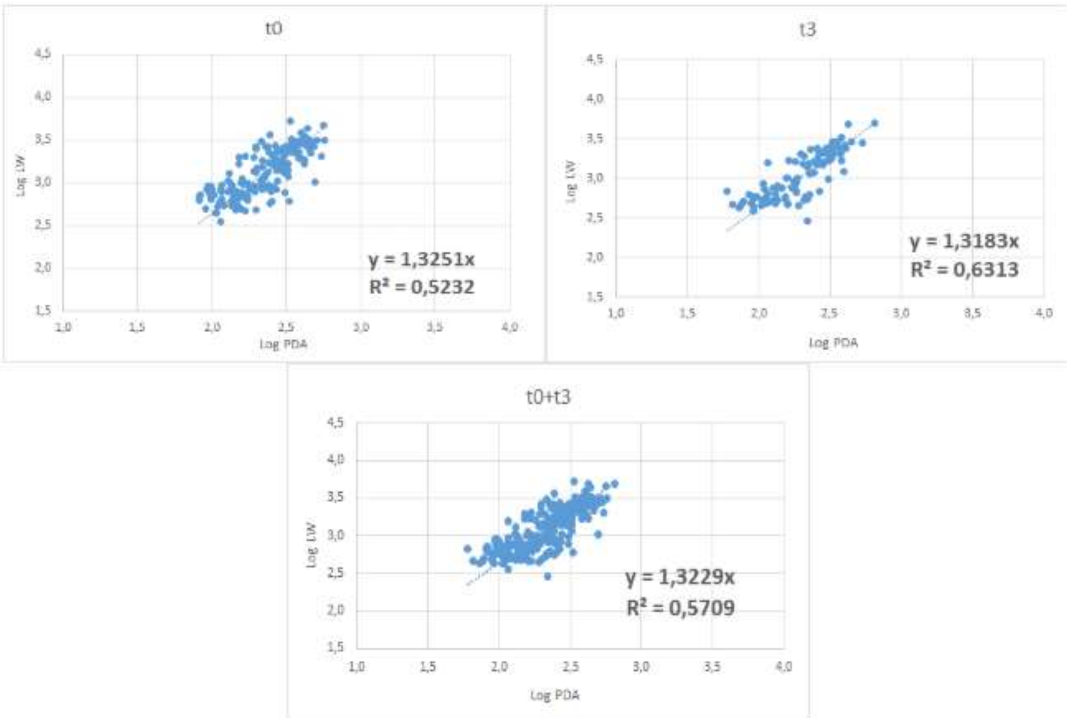

Figure 7: graphic and regression lines relating Log Live Weight (LW) and Log Pedal Disk Area (PDA) of anemones from rock pools at t0 (top left), t3 (top right) and t0+t3 (below). LW corresponds to 'y' *and PDA is identified as* 'x'.

Confidence Intervals
LW pools t0 vs. LPDA pools t0

	Coefficient	95% Lower	95% Upper
LPDA pools t0	1,325	1,313	1,337

Table 7: b *value for slope in t0 and corresponding 95% confidence interval.*

When *a* value is 0 and equations are readjusted, it can be noticed that *b* value in the relationship between LW and PDA suffers variation. Hence, the new equations relating LW and PDA are:

t0: Log LW = 1,3251 · Log PDA or $LW(mg) = PDA(mm^2)^{1,3251}$

t3: Log LW = 1,3183 · Log PDA or $LW(mg) = PDA(mm^2)^{1,3183}$

Value for *b* in t3 (1,3183) is inside the 95% confidence interval of *b* obtained in t0 (1,313 - 1,337; *Table 7*). Therefore, since there are not significant differences between slopes in t0 and t3 they can be united to obtain a relation for both moments. Thus, relationship between LW and PDA in t0 and t3 with *a* value adjusted to zero can be expressed as: LW = 1,3229 · PDA or $LW(mg) = PDA(mm^2)^{1,3229}$ (*Figure 7*). Accordingly, size data here presented in terms of pedal area evolution (mm2) can be readily translated into biomass (Live weight).

3.3 Spawning

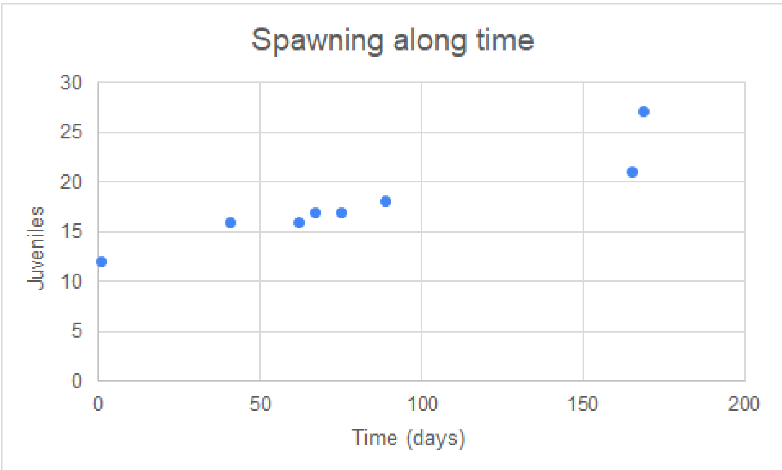

Figure 8: recording of number of young anemones from Rock Pool 3 site registered in each sampling.

Regarding the spawning in Rock Pool 3 (*Figure 8*) it can be observed that from one sampling date to the next one young *A. equina* number increases or keeps constant. Thus, from the first to the last monitoring date the number of juveniles registered is more than the double.

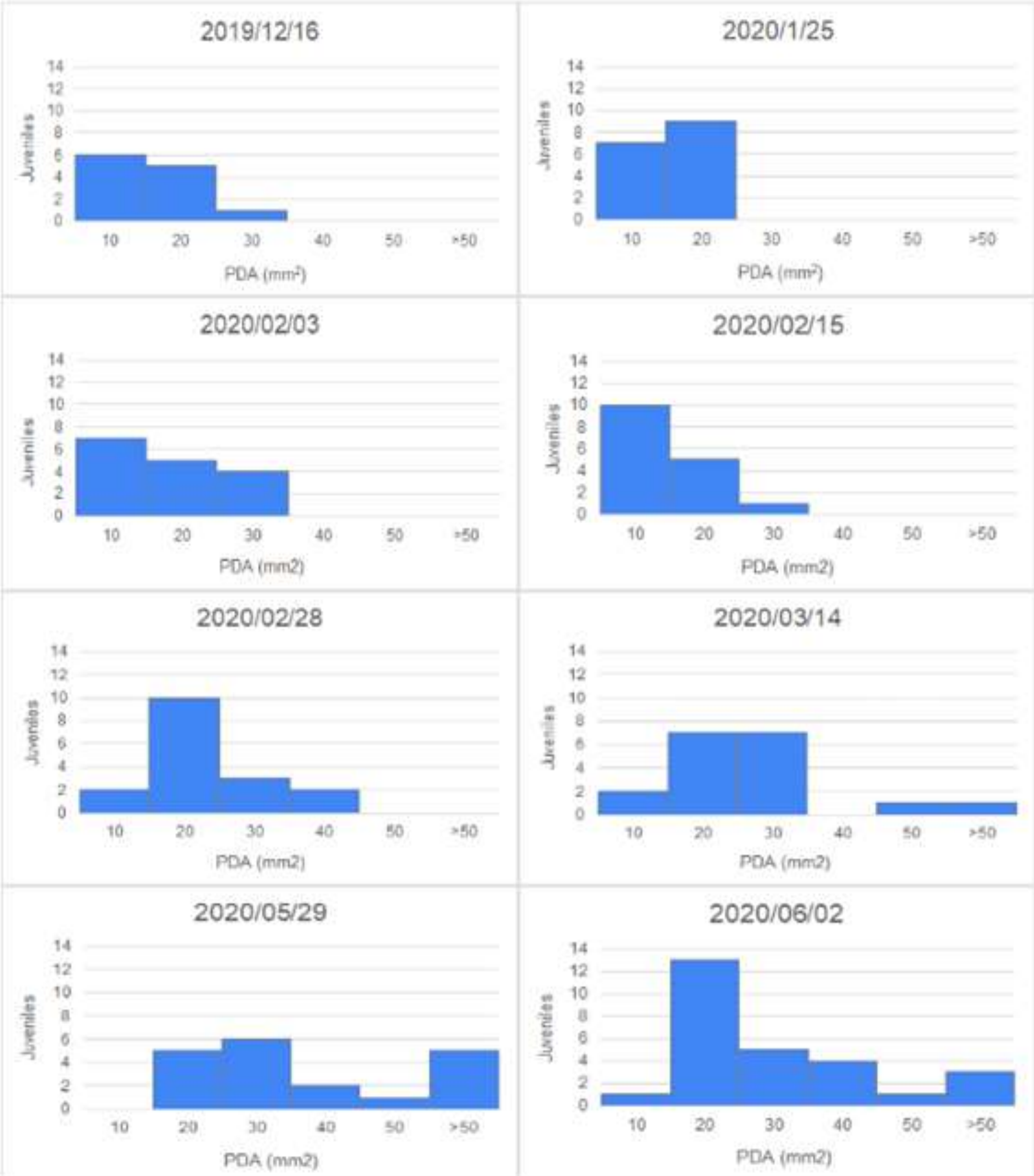

Figure 9: frequency distribution of juvenile A. equina *in Rock Pool 3 depending on PDA (mm^2) between 16th December 2019 and 2nd June 2020.*

In respect of frequency distribution of young anemones (*Figure 9*), it can be noticed that in the first four measurements most of the juveniles' PDA range between <10 and 20mm^2. On February 28th although most individuals' PDA can be found between 10 and 20mm^2, first young *A. equina* with a PDA superior to 30mm^2 are observed. In the next two samplings juveniles bigger than 40mm^2 appear but the PDA size of most individuals is in the 10-30mm^2 frequency range. Finally, on June 2nd frequency distribution is relatively homogeneous except for 10-20mm^2 range which stands out due the fact that almost a half of the juveniles are that size.

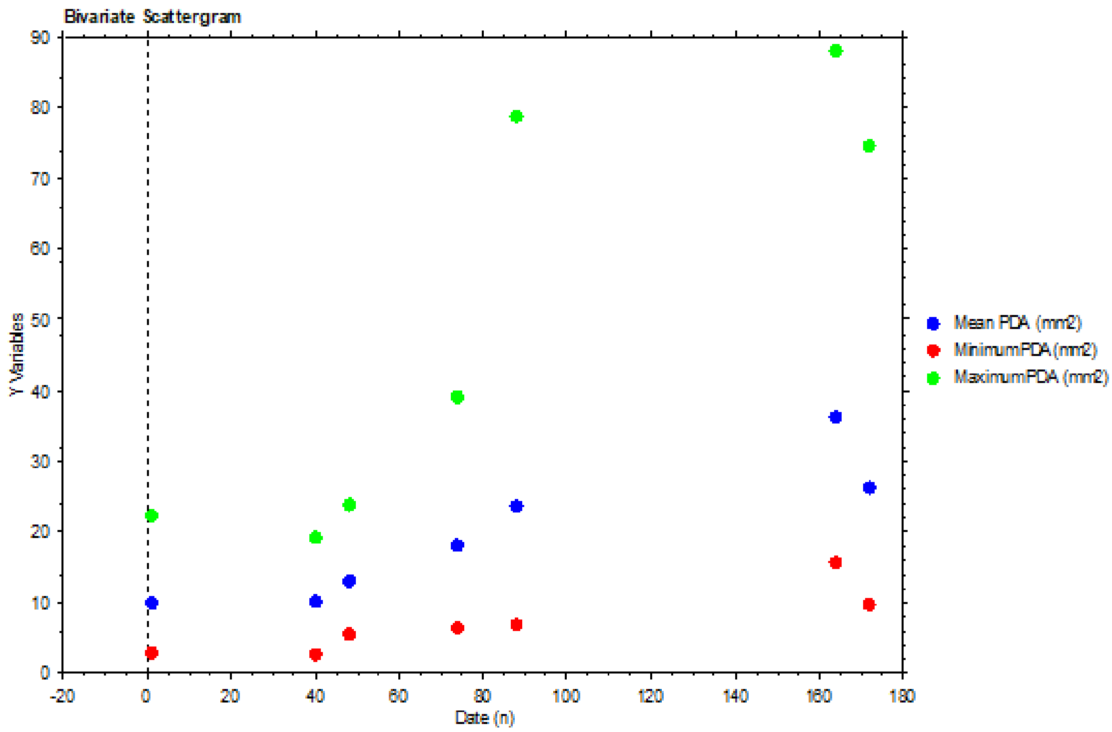

Figure 10: minimum (red), mean (blue) and maximum (green) PDA (mm^2) registered in juveniles in the sampling dates.

Test for equal means

	Sum of sqrs	df	Mean square	F	p (same)
Between groups:	11222,9	7	1603,27	8,696	9,07E-09
Within groups:	24520,4	133	184,364	Permutation p (n=99999)	
Total:	35743,3	140	1,00E-05		

Significance of mean among sampling dates

	25/01/2020	2020/02/03	2020/02/15	2020/02/28	2020/03/14	2020/05/29	2020/06/02
16/12/2019	1	0,999	1	0,7567	0,1392	1,67E-05*	0,01757*
25/01/2020		0,9988	1	0,696	0,0872	2,44E-06*	0,006445*
2020/02/03			0,9997	0,9607	0,3287	4,12E-05*	0,05182*
2020/02/15				0,7687	0,1173	4,3E-6*	0,01001*
2020/02/28					0,9366	0,00264*	0,5436
2020/03/14						0,09351	0,9983
2020/05/29							0,2159

*significant

Table 8: ANOVA test of mean PDA (mm^2) equality among the sampling dates (above) and Tukey test of mean PDA considering different measurement dates of juveniles from Rock Pool 3. Both tests have a significance level of 0,05.

Regarding the maximum PDA values (*Figure 10*), the first three samplings they maintain between 20 and 25 mm^2. After the day 75 they increase to values around 80mm^2. On the contrary, minimum PDA values do not undergo such increases although sizes of juveniles from the last two samplings nearly double previous PDA values. In the case of mean PDA values, the first two samplings are constant around 10mm^2. However, it starts to increase and in the last two dates the highest mean PDA values for young anemones is registered. Indeed, Tukey test with a significance level of 0,05 confirms that the mean PDA registered in the last two samplings differs significantly from the previous dates (*Table 8*).

4. DISCUSSION

4.1 Field monitoring

Growth can be measured in multiple ways and size determination gains strength when the researcher wants to indirectly determine the weight of the animal with photographs (Aguirre 2018). Size changes can be measured by looking at the pedal disk of the anemone (Sebens, 1983). Since the start of monitoring, frequency distribution (*Figure 1*), PDA means show that this morphometric parameter of anemones living in rock crevices continuously increases (*Table 1*). This fact demonstrates that individuals have growth during field monitoring. In the last sampling, a small decrease in anemones´ PDA can be noticed. This fall could be due to a shrinkage caused by stress (Chomsky et al 2004) as that day spring tides took place.

Increase of PDA observed in *A. equina* from crevices is also present specimens living in rock pools (*Figures 2 and 4)*. Besides, mean PDA growth registered after the confinement is statistically more significant than in rock pools (*Table 3 and 4*) and a sampling without interruptions would allow to watch a continuous PDA increase.

The effect of zonation is also demonstrated in this study. Monitored *A. equina* live in similar tide zone rock pools, therefore size of individuals does not significantly differ from a pool to another (*Table 4*). On the contrary, as each crevice is in a different tide level, anemones' PDA decreases when height above sea level increases (*Figure 3)*. As anemones are opportunistic feeders (Chintiroglou & Koukouras 1992; Navarro et al 1987), they are strongly dependent on shore exposure and height for feeding availability (Davenport et al 2011). Therefore, this result agrees with the study of Carling in 2019 which claims that emergent habitats host smaller anemones owing to they are associated with lower food resources.

However, anemones monitored in pools were in general smaller than those from crevices (*Figure 5, Table 6*). This fact occurs probably because the three sites selected in Playa del Carbon are in a higher tidal level than Rock Crack 2 and Rock Crack 3 sites. Even if anemones from rock pools do not undergo exposure, they feed on materials delivered by wave and current action (Davenport et al 2011; Kruger & Griffiths 1996; Ortega et al 1988).

Finally, regarding to temperature (*Figure 6*) is was observed that anemones almost never reach a body temperature above 20ºC. Furthermore, their body temperature is normally found to be around 5ºC than air temperature in shade. On 1984 Ortega et al demonstrated that anemones acclimate at 10-20ºC in winter and between 15 and 20ºC on summer. Therefore, they have an evaporation mechanism for maintaining temperature below the acclimation limit. When tide recedes and anemones get emerged, they close in order to retain water in the coelenteron (Aguirre 2018). In that way, they have a water reservoir to fight against desiccation and to maintain body temperature fresh enough.

However, during samplings it was noticed that sometimes water temperature in rock pools was around 30ºC (mostly in the samplings after the pandemic). At such temperatures anemones living in rock pools may not be able to acclimate to temperatures within annual range and their metabolism rate may be increased (Ortega el al 1984).

4.2 Biometry in laboratory

Finding a constant in an anemone body becomes an arduous task as their appearance is highly variable (Aguirre 2018). After exposure of three hours, obtained linear regression equations (log-log transformations) do not differ significantly from the equations obtained in t0. Thus, a common equation was originated adding t0 and t3. When elevation adjusted to zero, slopes in t0 and t3 continue not to have a significant difference (*Table 7*) and another joint equation was originated: LW = 1,3229 PDA (*Figure 7*).

As anemones in our laboratory experiment live in rock pools, it was decided to compare whether LW and PDA relation also corresponds to that obtained with anemones from rock cracks of Sopelana (Ortega & Barrenetxea 2016). It can be observed that relation between LW and PDA can be expressed as $LW(mg) = PDA(mm^2)^{1,3106}$ where *b* value is 1,3072 (*Figure 11*).

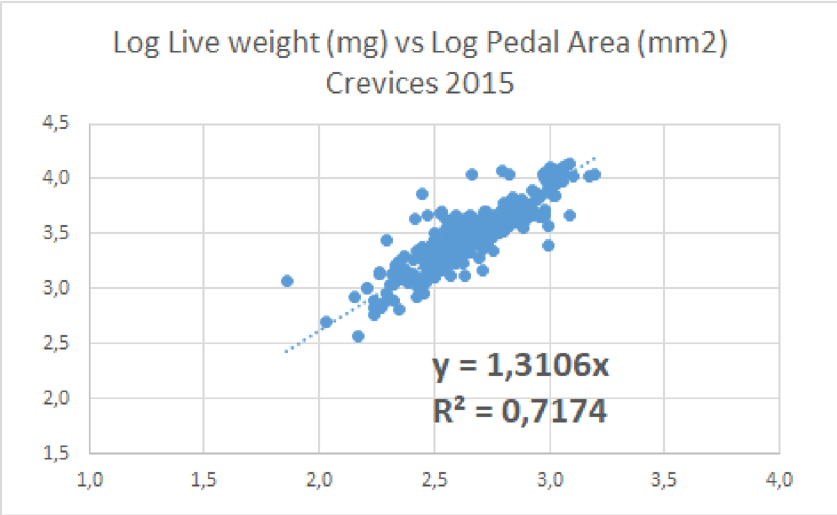

Figure 11: graphic and regression line relating Log Live Weight (LW) and Log Pedal Disk Area (PDA) of anemones from rock crevices after 3 hours of exposure (Ortega & Barrenetxea 2016).

Confidence Intervals
LW pools t0 vs. LPDA pools t0

	Coefficient	95% Lower	95% Upper
LPDA pools t0	1,325	1,313	1,337

Confidence Intervals
LW Crevices t3 vs. LPDA crevices t3

	Coefficient	95% Lower	95% Upper
LPDA crevices t3	1,311	1,305	1,316

Table 9: 95% confidence intervals and slope b *value for* A. equina *of our experiment (left) and the obtained on 2015 by Barrenetxea (right).*

When comparing coefficient 95% confidence intervals of both studies (*Table 9*), it can be observed that they overlap. Therefore, slopes obtained in this work and in the study made by Barrenetxea can be considered equals and it is possible to originate a common regression line including measurements of both experiments (*Figure 12*).

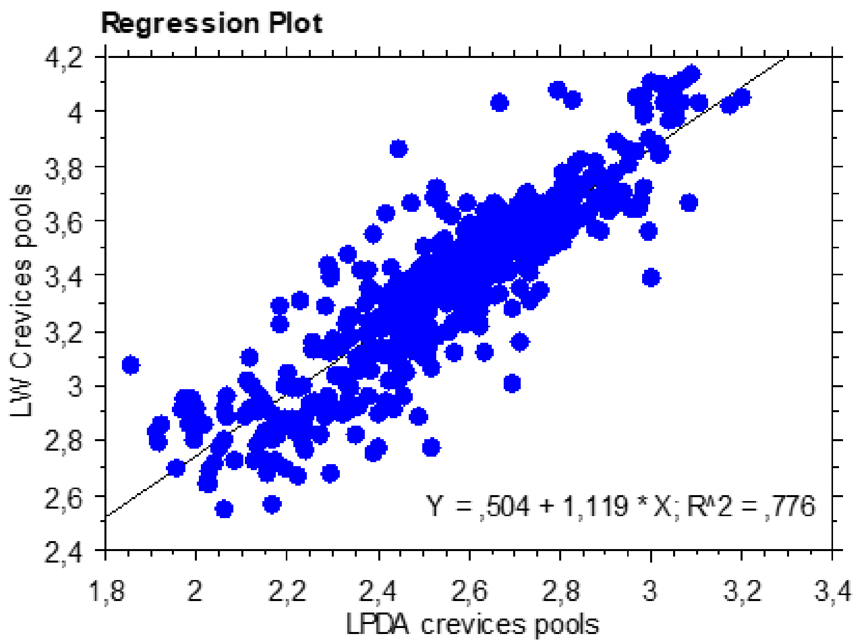

Figure 12: graphic and regression lines relating Log PDA and Log LW obtained from the grouping of Ortega & Barrenetxea (2016) and the present studies' measurements irrespective of habitat or exposure lapse.

Regression Coefficients
LW Crevices pools vs. LPDA crevices pools

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
LPDA crevices pools	1,314	,003	1,035	503,828	<,0001

Confidence Intervals
LW Crevices pools vs. LPDA crevices pools

	Coefficient	95% Lower	95% Upper
LPDA crevices pools	1,314	1,309	1,320

Table 10: regression coefficient irrespective of habitat or exposure lapse relating Log PDA and Log LW when a *value adjusted to zero and 95% confidence interval for the mentioned coefficient (below).*

Finally, the *a* value adjusted equation obtained for relating LW against PDA is: LW(mg) =PDA(mm^2)^{1,314} (*Table 10*). This result seems to be a structural feature not related to size range since most anemones grew during three months of maintenance in laboratory. In conclusion, pedal disk area appears as a robust parameter to analyse biomass in long term surveys and proves that biometry is a trustworthy method. Besides, this PDA-LW relation serves to obtain easily field anemones' biomass only by calculating their pedal disk diameter.

4.3 Spawning

27 young *A. equina* were registered during 6 months in Rock Pool 3 site. Juvenile frequency distributions (*Figure 9*) and spawning along time (*Figure 8*) show that both anemones' size and number increases as time goes by. Number of young anemones suddenly increases between the last two samplings. This can be explained because of bad quality of pictures taken May 29th as such an abrupt offspring increase is not probable.

Juveniles grow rapidly (Chomsky et al 2004) and it is demonstrated owing to that in less than 100 days from the first sampling date offspring's mean PDA is doubled (*Figure 10*). Additionally, in this work it can be noticed that new offspring appear in the late fall (12 juveniles were already identified on December 16th) and in spring (mostly on March and June). This fact coincides with the periods when gametes are more abundant in mesoglea of the gonadal mesenteries (Chia & Rostron 1970).

It was observed that some juveniles disappeared from a sampling to another. It is known that to avoid desiccation anemones retain water in their gastrovascular cavity (Aguirre 2018). Besides, big anemones are able to retain more water and their surface/volume ratio smaller than in young ones. Thus, juveniles have more difficulties to fight desiccation and this may be one reason of offspring mortality. Therefore, a minimum size may be required to avoid desiccation when emerged for a successful juvenile. In addition, a shaded rock pool (to avoid emersion and to facilitate acclimation) seems to be the ideal place for offspring viability but more research is needed to prove it.

Unfortunately, no offspring was observed in laboratory even if it was expected as in previous studies with *A. equina* from Sopelana (Barrenetxea 2015). The reason why could be that the aquarium was moved during COVID-19 confinement in order to follow the experiment.

In the new emplacement anemones lived under presence of artificial light. Kruger & Griffiths in their work of 1996 proved that illumination intensity and temperature are among the key factors in anemones' oxygen consumption rate determination. Hence, individuals of this study may have undergone metabolic alterations due to insufficient illumination intensity and therefore reproduction could have been altered. In addition, it is proved that temperature disrupts anemones' metabolism (Gadelha et al 2017). In any case, we encourage future works to analyse the effect of illumination intensity in anemones, that could provoke alterations in their metabolism and reproduction.

5. ACKNOWLEDGMENTS

I would like to acknowledge Mertxe for all the help and support she gave to me despite the difficult and abnormal situation caused by COVID-19. A week after the anemones were collected from rock pools, confinement was called and Mertxe carried home the aquarium with all the necessary equipment. In addition, she has made the weight measurements during confinement. These acts taught me her involvement in this work and the passion she shows for anemones.

Finally, I deeply thank my parents and my friend Joseba Trinidad for accompanying and helping me in the samplings.

6. BIBLIOGRAPHY

Aguirre, S. (2018). Metabolic and digestive parameters of the subtidal *Actinia equina* (Cantabrian Sea, Spain). Bachelor thesis. Euskal Herriko Unibertsitatea (EHU/UPV).

Barrenetxea, M. (2015). Distribution, growth and recruitment of *Actinia equina* (Cnidaria, Anthozoa) in a population of the Cantabric Sea. Master thesis. Euskal Herriko Unibertsitatea (EHU/UPV).

Cain, A.J. (1974). Breeding System of a Sessile Animal. *Nature*, *247*, 289–290. doi: 10.1038/247289a0.

Carling, B., Gentle, L. K. & Ray, N.D. (2019). Several parameters that influence body size in the sea anemone *Actinia equina* in rock pools on the Yorkshire coast. *Journal of the Marine Biological Association of the United Kingdom, 99*, 1267–1271. doi: 10.1017/S0025315419000171.

Carter, M. A. & Thorp, C. H. (1979). The reproduction of *Actinia equina* L. var. *mesembryanthemum*. J*ournal of the Marine Biological Association of the United Kingdom*, *59*, 989-1001**.** doi: 10.1017/S0025315400036985.

Chia, F. S., & Rostron, M. A. (1970). Some aspects of the reproductive biology of *Actinia equina* (Cnidaria: Anthozoa). *Journal of the Marine Biological Association of the United Kingdom*, *50*(1), 253-264. doi: 10.1017/S0025315400000758.

Chintiroglou, C., & Koukouras, A. (1992). The feeding habits of three Mediterranean sea anemone species, *Anemonia viridis* (Forskål), *Actinia equina* (Linnaeus) and *Cereus pedunculatus* (Pennant). *Helgoländer meeresuntersuchungen*, *46*(1), 53. doi: 10.1007/BF02366212.

Chomsky, O., Douek, J., Chadwick, N. E., Dubinsky, Z. & Rinkevich, B. (2009). Biological and population-genetic aspects of the sea anemone *Actinia equina* (Cnidaria: Anthozoa) along the Mediterranean coast of Israel. *Journal of Experimental Marine Biology and Ecology*, *375*, 16-20. doi: 10.1016/j.jembe.2009.04.017.

Chomsky, O., Kamenir, Y., Hyams, M., Dubinsky, Z., & Chadwick-Furman, N. E. (2004). Effects of feeding regime on growth rate in the Mediterranean Sea anemone *Actinia equina* (Linnaeus). *Journal of Experimental Marine Biology and Ecology*, *299*(2), 217-229. doi: 10.1016/j.jembe.2003.09.009.

Cornelius, P. F. S. (1972). Thermal acclimation of some intertidal invertebrates. J*ournal of Experimental Marine Biology and Ecology*, *9*(1), 43-53. doi: 10.1016/0022-0981(72)90005-6.

Davenport, J., Moloney, T. V., & Kelly, J. (2011). Common sea anemones *Actinia equina* are predominantly sessile intertidal scavengers. *Marine Ecology Progress Series*, *430*, 147-156. doi: 10.3354/meps08861.

Gadelha, J. R., Jesus, F., Gomes, P. B., Von Osten, J. R., Morgado, F. & Velho da Maia Soares, A. M. (2017). Temperature Tolerance Test Exposition with Temperate Sea Anemone *Actinia equina*, a Climatic and Environmental Changes Simulation. *Open Access Library Journal*, *04*(03), 1-24. doi: 10.4236/oalib.1103360.

Griffiths, R. J. (1977a). Temperature acclimation in *Actinia equina* L. (Anthozoa). Journal of Experimental Marine Biology and Ecology, *28*(3), 285-292. doi: 10.1016/0022-0981(77)90097-1.

Gomes, P. B., Belém, M. J. & Schlenz, E. (2008). Distribution, abundance and adaptations of three species of Actiniidae (Cnidaria, Actiniaria) on the intertidal beach rock in Carneiros beach, Pernambuco, Brazil. *Miscel·lània Zoològica, 21*(2), 65-72.

Kruger, L. M. & Griffiths, C. L. (1996). Sources of nutrition in intertidal sea anemones from the south-western Cape, South Africa. *South African Journal of Zoology*, *31*, 110-119.

Monteiro, F. A., Solé-Cava, A. M., & Thorpe, J. P. (1997). Extensive genetic divergence between populations of the common intertidal sea anemone *Actinia equina* from Britain, the Mediterranean and the Cape Verde Islands. *Marine Biology*, *129*(3), 425-433. doi: 10.1007/s002270050183.

Navarro, E., Ortega, M. M., & Madariaga, J. (1981). Effect of body size, temperature and shore level on aquatic and aerial respiration of *Actinia equina* (L.) (Anthozoa). *Journal of experimental marine Biology and Ecology*, *53*(2-3), 153-162. doi: 10.1016/0022-0981(81)90016-2.

Navarro, E., Ortega, M. M., & Iglesias, J. I. P. (1987). An analysis of variables affecting oxygen consumption in *Actinia equina* L. (Anthozoa) from two shore positions. *Comparative Biochemistry and Physiology Part A: Physiology*, *86*(2), 233-240.

Orr, J., Thorpe, J. P., Carter, M. A. (1982). Biochemical Genetic Confirmation of the Asexual Reproduction of Brooded Offspring in the Sea Anemone *Actinia equina. Marine Ecology Progress Series*, *7*(2), 227-229. doi: 10.3354/meps007227.

Ortega, M. M., & Barrenetxea, M. (2016) Distribution, growth and recruitment of *Actinia equina* (Cnidaria, Anthozoa) in the Cantabric Sea: crevices vs. rock pools. *Book of Abstracts of XV International Symposium on Oceanography of the Biscay Bay (Isobay15). Changing Ecosystems: Natural vs anthropogenic effects* (pp. 56). Bilbao (Spain).

Ortega, M. M., Iglesias, J. I. P., & Navarro, E. (1984). Acclimation to temperature in Actinia equina L.: effects of season and shore level on aquatic oxygen consumption. *Journal of experimental marine biology and ecology*, *76*(1), 79-87. doi: 10.1016/0022-0981(84)90018-2.

Ortega, M. M., Iglesias, J. I. P, & Navarro, E. (1987) Efecto de la aclimatación experimental a la temperatura sobre el metabolismo respiratorio en *Actinia equina* (L.) (Cnidaria, Anthozoa). *Cuad. Marisq. Publ. Téc. 11*, 213-223.

Ortega, M. M., Lopez de Pariza, J. M. & Navarro, E. (1988). Seasonal changes in the biochemical composition and oxygen consumption of the sea anemone *Actinia equina* as related to body size and shore level. *Marine Biology*, *97*, 137-143. doi: 10.1007/BF00391253.

Sebens, K. P. (1983). Population dynamics and habitat suitability of the intertidal sea anemones *Anthopleura elegantissima* and *A. xanthogrammica. Ecological Monographs*, 53(4), 405-433. doi: 10.2307/1942646.