Mediterranean Marine Science

Vol 22, No 1 (2021)

Vol 22, No 1 (2021)

Phenology of Anemonia viridis and Exaiptasia diaphana (Cnidaria: Anthozoa) from marine temperate ecosystems

GIOIA CRISTINA Di CAMILLO, SILVIA AROSSA, DANIELA PICA, BASTARI AZZURRA, FABRIZIO TORSANI, CARLO CERRANO

doi: 10.12681/mms.24600

To cite this article:

Di CAMILLO, G. C., AROSSA, S., PICA, D., AZZURRA, B., TORSANI, F., & CERRANO, C. (2021). Phenology of Anemonia viridis and Exaiptasia diaphana (Cnidaria: Anthozoa) from marine temperate ecosystems. *Mediterranean Marine Science*, *22*(1), 40–50. https://doi.org/10.12681/mms.24600

Research Article

Mediterranean Marine Science
Indexed in WoS (Web of Science, ISI Thomson) and SCOPUS
The journal is available on line at http://www.medit-mar-sc.net
DOI: http://dx.doi.org/10.12681/mms.24600

Phenology of *Anemonia viridis* and *Exaiptasia diaphana* (Cnidaria: Anthozoa) from marine temperate ecosystems

Cristina Gioia Di CAMILLO[1], Silvia AROSSA[2], Daniela PICA[1], Azzurra BASTARI[1], Fabrizio TORSANI[1] and Carlo CERRANO[1,3]

[1] Marche Polytechnic University, Department of Life and Environmental Sciences, Ancona, Italy
[2] King Abdullah University of Science and Technology (KAUST), Red Sea Research Centre (RSRC) and Computational Bioscience Research Center (CBRC), Thuwal, 23955, Saudi Arabia
[3] Stazione Zoologica Anton Dohrn, Villa Comunale, 80121, Napoli, Italy & Fano Marine Center, The Inter-Institute Center for Research on Marine Biodiversity, Resources and Biotechnologies, viale Adriatico 1/N, 61032 Fano, Pesaro Urbino, Italy

Corresponding author: silvia.arossa@kaust.edu.sa

Contributing Editor: Agnese MARCHINI

Received: 28 August 2020; Accepted: 6 November 2020; Published online: 8 January 2021

Abstract

Population dynamics in lower metazoans are strongly regulated by environmental factors. The current climate crisis could affect regularity of life strategies; therefore, studying life cycles and reproductive patterns of benthic species may provide key information on effects of environmental changes in temperate seas. The North Adriatic Sea is a peculiar basin due to high food availability and high fluctuations of physical parameters. Here, cnidarians are one of the most abundant taxa, and sea anemones are the largest representatives. Aim of this study is to assess the life history and the reproductive biology of two potential species models: the sea anemones: *Anemonia viridis* and *Exaiptasia diaphana*. The present research is the first quantitative study on the variations in abundance and reproductive biology of the two species. We reported new insights about abundance fluctuations throughout the year and about the gametogenesis of the two sea anemones. In particular, data on spermatogenesis of *A. viridis* from the Mediterranean Sea were supplied for the first time.

Keywords: Sea anemones; Adriatic Sea; *Anemonia viridis*; *Exaiptasia diaphana*; reproductive biology; life history.

Introduction

Climate change is leading to shifts in abundance, distribution and phenology in terrestrial and aquatic organisms (Cohen *et al.,* 2018; Kharouba *et al.,* 2018) with consequent asynchronies between cycles of interacting species (Parmesan, 2006). Especially at higher latitudes, a lengthening of the vegetative growing period was described for several plants (Parmesan 2006 and references therein).

Concerning the marine environment, the increased ocean heat content (Cheng *et al.,* 2019) together with anthropogenic pressures (Poloczanska *et al.,* 2016) are driving geographic and bathymetric variations in distribution, seasonal timing, composition and abundance of marine organisms (Poloczanska *et al.,* 2013).

Temperate marine benthic species with a well-defined growing period are generally considered ‘seasonal’; however, sudden and sharp variations in physical parameters due to thermal anomalies could affect life cycles even in marine organisms (Basile *et al.,* 2009; Puce *et al.,* 2009). Thanks to a peculiar plasticity in their life cycles, ephemeral, fast-growing species can adapt to environmental variabilities showing flashing growth and reproduction when relative environmental conditions are in their optimum (Reusch, 2014 and references therein). On the contrary, cycles of long-living species may be even more affected by unstable environmental conditions: enduring species could show irregular regression/growth phases and, above all, a dramatic alteration in gamete production, with unpredictable consequences on biogeochemical fluxes, ecosystem functioning, and species diversity (Dulvy *et al.,* 2008). Phenology of indicator species could supply information on the response of benthic communities to climatic anomalies (Birchenough *et al.,* 2011, 2015; Corriero *et al.,* 2015). Knowing how species may adapt to environmental changes may be crucial for Marine Spatial Planning as well (Rilov *et al.,* 2019).

However, studies on life-cycle traits of marine benthic organisms (both plants and animals) have received scarce attention (Reusch, 2014), notwithstanding these researches would be indispensable for achieving a full-understanding of population dynamics and for setting distribution (Robinson *et al.,* 2017) or dispersal (Giangrande *et al.,* 1994; 2017) species models.

Thermal and phenology shifts are faster in marine ecosystems than in terrestrial ones (Burrows *et al.,* 2011). It has been observed that spring and summer growth/reproductive phases of marine benthos often advance especially in high latitude, whereas, autumn events may delay (Poloczanska *et al.,* 2016). Climate-induced changes are leading to a mismatch between optimal levels of irradiance and temperature altering trophic interactions among species (Thackeray *et al.,* 2010) and reproductive performance (de Bettignies *et al.,* 2018).

Climate changes might have more evident effects in coastal zones and relatively small basins such as the North Adriatic Sea (Mediterranean Sea), which is sensitive to fluctuations of abiotic factors due to its peculiar hydrologic features (Di Camillo & Cerrano, 2015; Stachowitsch, 1991). In the last ten years, the Italian side of the Adriatic coasts have been characterized by milder winters and false springs respect to the past; positive anomalies of the superficial water temperature were recorded; moreover, rainfalls have decreased in frequency and have increased in intensity (Desiato *et al.,* 2017) triggering peaks of intense and sudden turbidity and low salinity.

The aim of this study is to assess population dynamics of two fast-growing species – the sea anemones *Anemonia viridis* (Forsskål, 1775) and *Exaiptasia diaphana* (Rapp, 1829) – from the North Adriatic Sea. We hypothesized that anemone abundance and gamete production varied over time in relation to environmental factors such as sea temperature, irradiance and height waves.

Due to their high densities and their unmistakable morphological characteristics, the two sea anemones may represent a good model to monitor eventual alteration in the phenology of marine sessile species in shallow waters. Our results may lay the basis for further and future studies as well. By comparing such data with surveys conducted in the long term, the detection of variations in the anemone life cycles can contribute to clarify the responses of benthic communities to changes in environmental parameters.

Materials and Methods

Sampling site

Sea anemones belonging to the species *Anemonia viridis* and *Exaiptasia diaphana* were collected in the North Adriatic Sea (Passetto, Ancona, Italy, Fig. 1) which is characterized by shallow waters (up to 4 m depth), high sedimentation rates, wide temperature excursion from winter (7-10°C) to summer (25-27°C, http://www.mareografico.it). Here, rocky substrates are mainly colonized by the mussel *Mytilus galloprovincialis* (Lamarck, 1819), sponges and cnidarians (Ciabatti *et al.,* 1986). The presence in this area of rocky bottoms (Ancona and Conero Promontory) and artificial breakwaters (Afghan *et al.,* 2020) leads to the interruption of the typical sandy coastline of the western Adriatic Sea, thus representing a key steppingstone for benthic species dispersal at basin level. In particular, the sampling site was chosen as a consequence of numerous pre-surveys conducted in about twenty-year time interval along the entire rocky coast (Di Camillo *et al.,* 2012; 2014; 2015).

Population dynamics of two zooxanthellate sea anemones, *Anemonia viridis* and *Exaiptasia diaphana*, were studied in two sites, Site A (43.618639° N, 13.532489°W) and Site B (43.618069°N, 13.533586°W; see Fig. 1). Site A harbors a dense population of *A. viridis* from 1.5 m to 4 m depth, while Site B hosts the species *E. diaphana* from 3 m to 4 m depth. From July 2013 to June 2014, every month, samples were taken by SCUBA diving. Survey and sampling were not feasible during the month of November 2013 due to unfavorable weather conditions

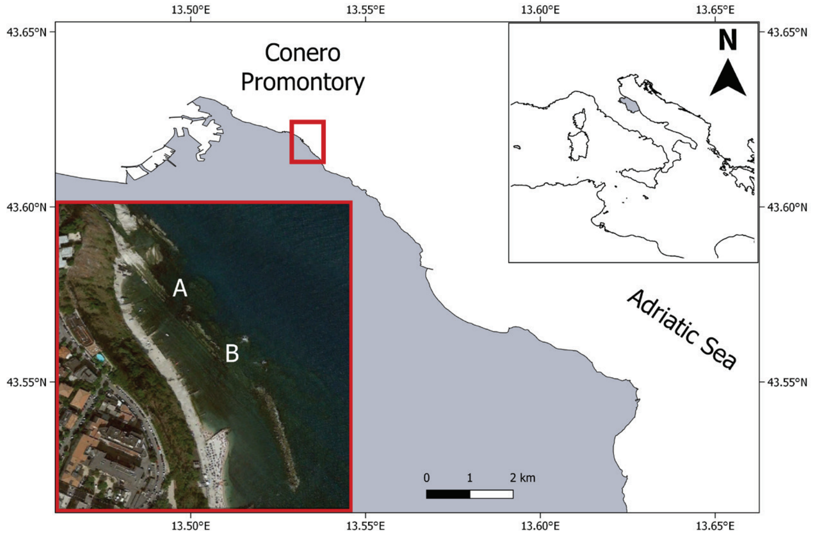

Fig. 1: Sampling area (Passetto, red frame) at Conero Promontory (Italy, Adriatic Sea). *Anemonia viridis* and *Exaiptasia diaphana* were collected from July 2013 to June 2014 at Site A (43.618639° N, 13.532489° W) and Site B (43.618069° N, 13.533586° W), respectively. QGIS elaboration (QGIS Development Team 2017).

throughout the entire month.

A dataset containing information about sampling sites and collected species (following Di Camillo *et al.,* 2018) is supplied as Supplementary Material S1.

Environmental data

Monthly average values of wave heights (HW, cm) were downloaded from the website of The Italian Institute for Environmental Protection and Research (ISPRA), from www.ancona.telemisura.it and www.ilmeteo.it; superficial water temperature (T, °C) was obtained from the National Tidegauge Network website (www.mareografico.it), while irradiance data (IR, Wm^{-2}) were taken from Byun & Pinardi (2007).

Temporal variations in abundance of sea anemones

In order to estimate temporal variations in abundance (indicated as percentage coverage ± SD) of the two anthozoans, photographic surveys were monthly conducted on rocks through SCUBA diving from July 2013 to June 2014, using frames of 25 x 25 cm haphazardly placed along a horizontal transect in the sites A and B. Replicates were separated at least 50 cm to avoid overlapping among sampling areas. Every month, 30 pictures were taken for each site with a total of 330 photo-samples. PhotoQuad Software was used to perform photo analysis (Trygonis & Sini, 2012) and to estimate the monthly average percentage coverage ± *SD* of the two sea anemones.

We provide a list of species sharing the same habitat with *A. viridis* and *E. diaphana* and their temporal variations in Supplementary Material S2.

Reproductive biology

20 individuals of *A. viridis* and 20 individuals of *E. diaphana* were monthly collected by gently detaching them from the substrate with a knife. All the collected specimens were stored into a cooling bag, previously filled with fresh seawater, and immediately transported to the laboratory. All individuals were firstly anesthetized using a $MgCl_2$ solution (Sigma-Aldrich), in a concentration chosen according to the size of the animal and to Perez's protocol (Oregon State University, 2006) to avoid muscle contractions during fixation. Then, each sample was longitudinally sectioned and observed at stereomicroscope in order to determine the *sex ratio* and to count the total number of mesenteries. When visible, male and female gonads were easily recognizable since oocytes were dark (reddish in living specimens) and opaque, while spermatocytes were whitish and more transparent, as confirmed by histological analyses.

To estimate the number of gonads, five fertile mesenteries were haphazardly collected from each fertile anemone and preserved in 4% formalin for 48 hours (Williams, 2007). Then, samples were dehydrated using ethyl alcohol solutions with increasing concentrations and finally mounted onto a microscope slide using Eukitt® *glue (Sigma-Aldrich)*. Each fertile mesentery was characterized by clusters of oocytes or sperm cysts, hereinafter referred to as 'gonads'. Sex ratio was reported as % of a specific gender (e.g. infertile, female, or male) out of the total number (n=20) and also as a females/males ratio. The average number of 'gonads' per mesentery in each individual was then multiplied for the total number of fertile mesenteries of the considered anemone. Similarly, the number of fertile mesenteries and of the number of gonads per fertile mesentery were converted to number per individual ± SD.

Finally, histological inclusions were prepared to study the ultrastructure of gonads: to determine the monthly average number of oocytes/spermatic cysts per 'gonad' ± SD, further five fertile mesenteries were selected for each species and preserved in 2.5% glutaraldehyde (buffered in filtered seawater 7.8 pH) for three hours. Then, washed in filtered water and dehydrated in ethyl alcohol solutions as described above and finally embedded in resin (cold-curing resin Technovit 8100). The samples were subsequently sectioned on a sliding microtome (Histo-Line Laboratories MRS3500) at 25 μm, stained with toluidine blue polychrome and mounted on slides. These sections were finally observed under a light microscope and photographed. Size of gametes was measurable only for oocytes. Data about diameter of sex cells allowed us to determine frequency of size classes (%) and to define a size range in each quarter. Size classes were chosen in relation to the size of the anemone species and to the difficulty to examine gonads in the two considered species (Size classes for *Anemonia viridis*: 20-60 μm, 61-100 μm, 101-140 μm, 141-180 μm, 181-220 μm, 221-260 μm, 261-300 μm, 301-340 μm, 341-380 μm, 381-420 μm, 421-460 μm; size classes for *Exiptasia diapahana*: 60-100 μm, 101-140, 141-180 μm, 181-220 μm). Complete histograms of size frequency are shown in Supplementary Material S3.

The above described methods are simple and applicable to many organisms and samplings sites. Such versatility simplifies the reproducibility of the methods for future studies. Additionally, they have been used multiple times by researchers to understand the phenology of different species (Goffredo *et al.,* 2002; Bautista-Guerrero *et al.,* 2010).

Statistical analysis

Data about abundance and fertility were tested for normal distribution using the Shapiro test in R Studio (Version 1.1.463). Where normal distribution was found One-way ANOVA was performed followed by the parametric Tuckey HSD to test the differences between months and quarters. In order to verify whether there was a correlation with an environmental parameter (e.g. temperature, waves, and irradiance) the parameter Pearson's correlation was performed. On the other side, when the data was not normally distributed the non-parametric Kruskall-Wallis and

Mann-Whitney pairwise tests were used. In order to verify the eventual correlation with environmental parameters the Spearman's correlation was performed.

Results

Environmental data

Irradiance values peaked in July (IR=362 Wm^{-2}) and dropped in January (IR=80 Wm^{-2}) while water temperature reached the highest average value in August (26.2° C) and the minimum in January (10.2° C). Wave heights (HW) data showed that the period of more extreme conditions was from January to February (HW=128-229 cm), while the period from March to December was characterized by calm waters, reaching a minimum value in June 2014 equal to 10.1 cm. Trends of irradiance, temperature and wave heights are shown in Figure 2.

Anemonia viridis

The species *A. viridis* was observed in sheltered areas, likely characterized by a low water exchange. Its upper limit of distribution was ~1.5 m depth. Individuals of this species were found on substrates with significant exposure to light, both on vertical and horizontal surfaces. They are usually weakly attached onto the substrate. Table 1 summarises main features of *A. viridis*.

Temporal variations in abundance

Anemonia viridis specimens were observed all year round in the studied area, however its cover varied monthly as shown in Figure 2A. The anemone was particularly abundant from July to September 2013 with a peak in August (percentage cover: 45.7% ± 26.8% SD), then it decreased steadily from October to December, reaching a

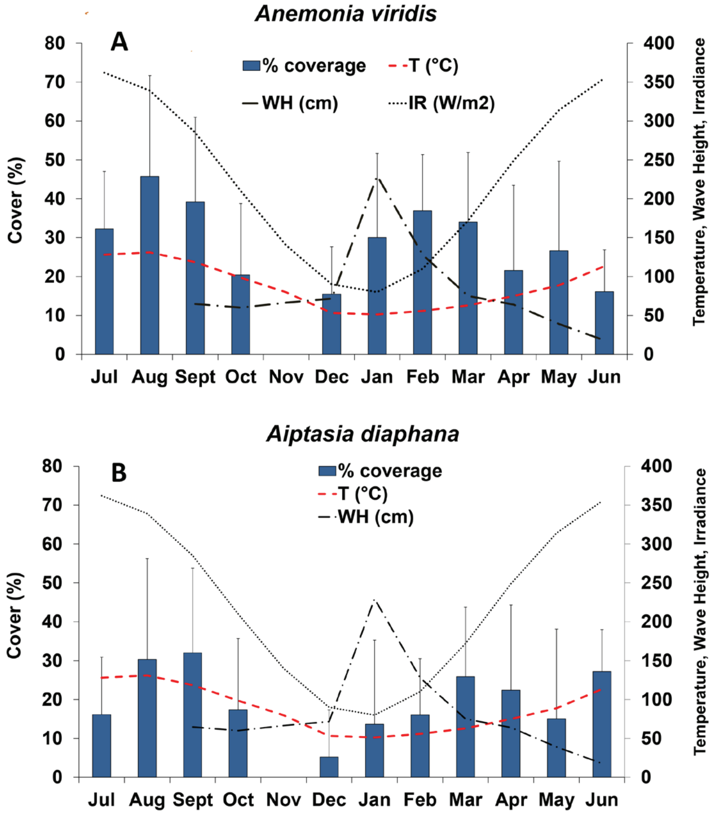

Fig. 2: Temporal variations in abundance of *Anemonia viridis* (A) and *Exaiptasia diaphana* (B) expressed as cover percentage in relation to monthly fluctuations of surface water temperature (°C), irradiance (W/m^2) and wave heights (m).

Table 1. Summary of the characteristics of *Anemonia viridis* and *Exaiptasia diaphana.*

	Anemonia viridis	*Exaiptasia diaphana*
Colour	Column brownish-green; tentacles opaque often with purple tips	Column brownish-green; tentacles transparent
Number of tentacles	40-160 (118.1 ± 15.7 SE)	20-50 (35.2 ± 3.6)
Location of achroragi	On margin, under tentacles	Absent
Presence of acontiae	No	Yes
Number of mesenteries	56-220 (105.8 ± 5.0 SE)	12-64 (27.4 ±1.7)

minimum value in December (13.8% ± 12.1% SD). The abundance resulted higher in the quarter July-September (average in the quarter: 35.1% ± 22.3% SD) and minimal in the quarter October-December (16.9% ± 15.4% SD). Abundance of *A. viridis* was not correlated to temperature, waves height and irradiance values (Kruskal-Wallis, respectively $r = 0.29$, $r = 0.38$, $r = 0.14$, $N = 11$, $p > 0.05$). Moreover, there was no significant variation among all the considered quarters (Kruskal-Wallis, $H = 5.38$, $p > 0.05$).

Reproductive biology

Monthly variations in *sex ratio* are shown in Figure 3A. Female individuals occurred all year round except for July. The maximum number of females was reached in February 2014 (87.5%); then, females decreased in the period from March 2014 and the minimum value was observed in June 2014 (21.7%). Males were observed from January 2014 to June 2014 with a maximum value in May 2014 (42.1%). July 2013 was the only month in which all the specimens collected were infertile. The *sex ratio* (F/M) varied from 14:1 in February 2014 to 1:1 in June 2014. Males were more abundant than females only in May 2014 (42.1% male and 26.3% female). The average F/M ratio was 6:1. Hermaphrodites were never found.

In Figure 4A average monthly variations in the number of female gonads per individual is shown. Female gonads were visible starting from August 2013 (85.8 gonads individual^{-1} ± 40.4 SD); the number of gonads was higher from October to April, reaching a maximum during April 2014 (322.0 gonads individual^{-1} ± 85 SD). From May, their abundance decreased and dropped in June 2014 (99.8 gonads individual^{-1} ± 45.5 SD). Number of gonads individual^{-1} were inversely correlated to temperature (Pearson's correlation, $r = 0.70$, $df = 9$, $p < 0.05$). Considering the quarterly variations of gonads number individual^{-1}, the highest values were observed from October to June, with a maximum in winter (265.3 ± 68.6 SD; when the mean temperature was equal to T_{av}=11.3 ± 0.02°C) and lower in July-September (154.9 ± 80.2 SD; T_{av}=21.7 ± 0.07°C). Statistical analysis showed a significant difference in the number of gonads during different quarters in females (One-way ANOVA, $F = 3.92$, $p < 0.05$). Values observed in the period from July to September were significantly different from those of periods January-March and April-June (One-way ANOVA, $F = 3.92$, $p < 0.05$, followed by Tukey HSD, $p < 0.05$), but not from October-December (Tukey HSD, $p < 0.05$). October-December values showed no significant difference with January-March and April-June and the same occurred between values occurred in January-March and April-June (Tukey HSD, $p > 0.05$).

Oocytes (Fig. 4B) were observed from August 2013 to June 2014; however, it was not possible to count cells from August to October due to their small size (and in November no samples were collected due to unfavorable

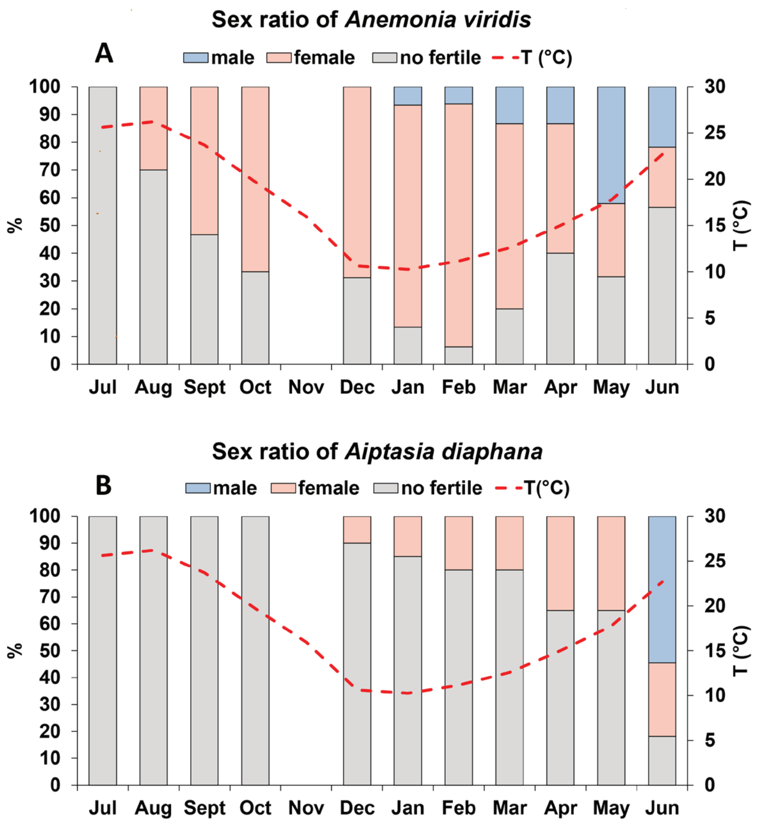

Fig. 3: Sex ratio of *Anemonia viridis* (A) and *Exaiptasia diaphana* (B) throughout the period July 2013-July 2014 with relative percentages of male, female and infertile individuals.

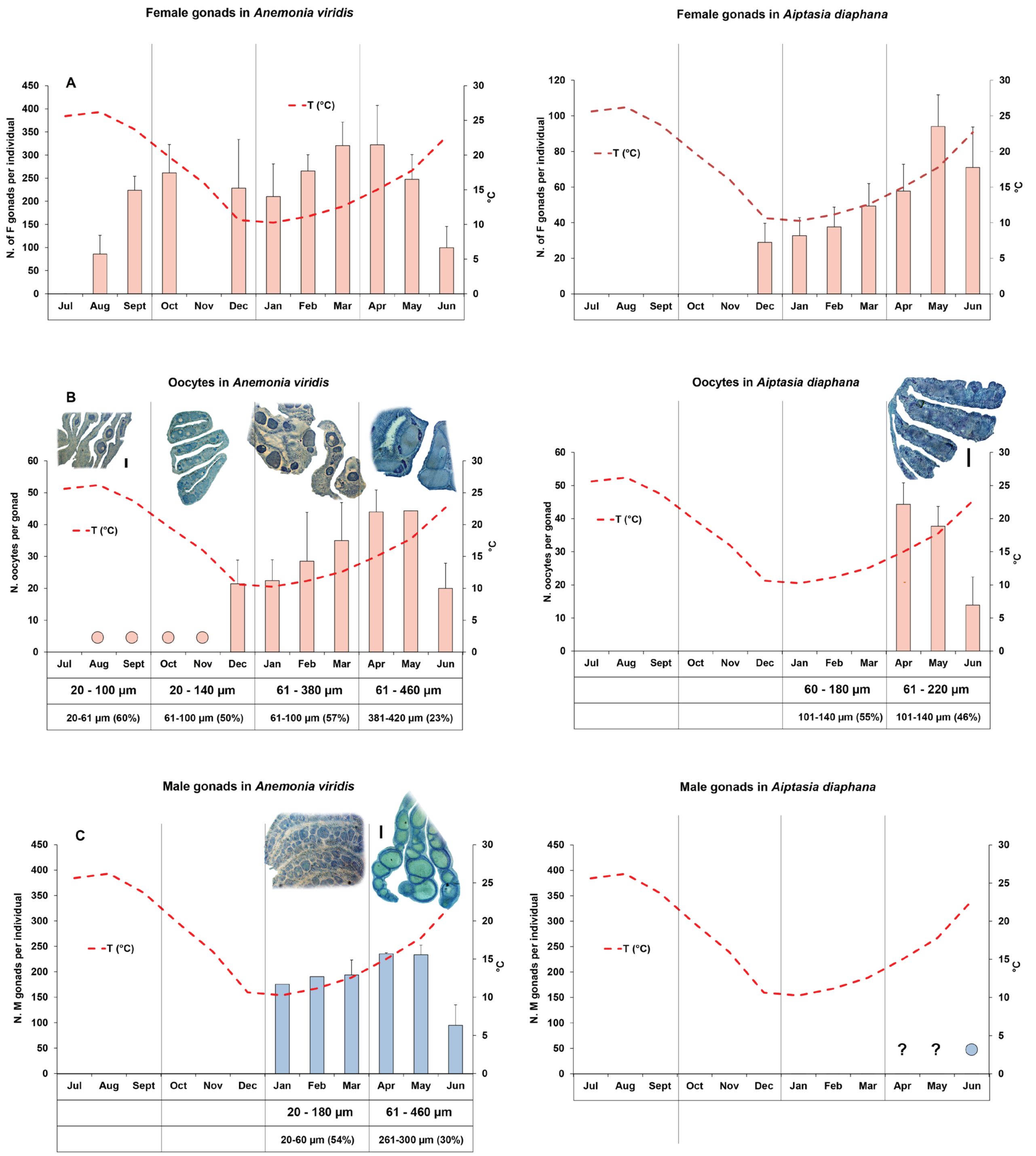

Fig. 4: Reproductive biology of *Anemonia viridis* (A-C) and *Exaiptasia diaphana* (D-F). The histograms show variations in number of female (A, D) and male (C, F) gonads and number of oocytes per gonad (B, E) in each species throughout the period July 2013- July 2014.

weather conditions, as already mentioned). The number of female sex cells increased from December 2013 to May 2014 reaching a peak in April (44.0 oocytes per gonad ± 6.9 SD), followed by a sharp decrease in June (20 ± 7.9 SD). Variations in the number of oocytes per gonad in *A. viridis* were inversely correlated to temperature (Spearman's correlation, $r = 0.59$, $N = 11$, $p < 0.05$). The investigation of variations in the oocyte number per gonad in the considered quarters highlighted that the higher reproductive effort occurred from April to June (in average 31.6 oocytes per gonad ± 13.1 SD). Statistical analysis showed no significant difference between sample medians in all considered quarters (Kruskal-Wallis, $H = 4.26$, $p > 0.05$), but Mann-Whitney pairwise test showed a significant difference only between quarters April-June and October-December ($p < 0.05$).

Concerning the size of oocytes (Fig. 4B, Supplementary Material S3), it was observed that gametocytes are smaller from July to September (size range: 20-100 µm; size class more frequent (60%): 20-61 µm), while they reach the maximal sizes in April-June (size range: 61-460 µm; size class more frequent (23%): 381-420 µm) with the highest value in May 2014 (421-460 µm).

The number of male gonads individual^{-1} is shown in Fig. 4C. No gonads were observed from July 2013 to December 2013. Male gonads were detectable from January 2014; then, their number started increasing, peaking in April 2014 (235.4 gonads per ind. ± 1.9 SD) and dropping in June 2014 (94.8 ± 40.3 SD). Monthly Variations in the number of gonads per male was not correlated to temperature (Pearson's correlation, $r = 0.55$, $df = 10$, $p > 0.05$). Statistical analysis showed no significant difference between sample medians in quarters January-March and April-June (188.4 ± 181.9 SD and 181.9 ± 75.9 SD, respectively; One Way ANOVA, $F = 0.027$, $p > 0.05$). It was not possible to count the number of sperm cysts for each gonad due to their small size. However, we measured the diameter of the gonads containing sperms (spermatocysts). Biggest spermatocysts (Fig. 4C, Supplementary Material S3) were observed in the quarter April-June (size range: 61-460 µm; size class more frequent (30%): 261-300 µm); on the contrary, in the period January-March gametocysts ranged from 20 to 180 µm (class more frequent (54%): 20-60 µm).

Exaiptasia diaphana

Individuals of *E. diaphana* were mostly retrieved in sheltered substrates, with high water exchange and current exposure. The maximum depth that limits their distribution at Passetto was 3 m. Contrary to *A. viridis*, *E. diaphana* was usually observed on shady and vertical/subvertical surfaces, where it tends to strongly attach and to colonize the micro asperities found on the substrate.

Temporal variations in abundance

Its variations *Exaiptasia diaphana* were always present during the study period, but its abundance fluctuated as shown in Figure 2B, reaching a maximum value in September 2013 (31.6% ± 18.1% SD) and a minimum value in December 2013 (5.0% ± 7.6% SD). Table 1 summarises main features of *E. diaphana*. Their abundance was positively correlated with temperature patterns ($r = 0.59$, $N = 11$, $p < 0.05$), but not with wave heights and irradiance (Kruskal- Wallis, respectively with $r = 0.35$, $N = 11$ and $r = 0.57$, $N = 11$, $p > 0.05$). *A. diphana's* abundance peaked during the quarter July-September (on average: 24.4% ± 17.8% SD), while the minimal value occurred between October and December (15.3% ± 20.4% SD). Statistical analysis showed significant variations in *E. diaphana* coverage among quarters (Kruskal- Wallis, $H = 22.6$, $p < 0.001$). Table 1 summarises main features of *E. diaphana*.

Reproductive biology

Figure 3B summarizes data about *sex ratio* in the species *E. diaphana*. The first fertile individuals (females) were recorded in December 2013 (10%). The following months showed an increasing in the number of females (Fig. 4d-f), reaching a peak in April and May 2014 (35%), while males were observed only in June 2014 (54.5 %), with a sex ratio (F/M) 1:2.

Monthly variations in the number of female gonads individual^{-1} were shown in Figure 4D. The minimal value was recorded in December 2013 (29 gonads individual^{-1} ± 4.2 SD) reaching a maximum in May 2014 (94.0 ± 12.4 SD), then, their abundance dropped in June (71 ± 21.2 SD). Considering the quarterly variations, the number of gonads peaked in April-June (76.8 ± 24.3 SD) and decreased in October-December (29.0 ± 2.1 SD). Variations in the number of gonads individual^{-1} in *E. diaphana* were negatively correlated to temperature (Pearson's correlation, $r = 0.71$, $df = 28$, $p < 0.001$). Differences among quarters were statistically significant (One Way ANOVA, $p < 0.01$, followed by Tuckey HSD, $p < 0.05$).

The number of oocytes gonad^{-1} was counted only from April to June 2014, as the increase of size of the oocytes allowed us to count them. In April the number was 44.3 ± 36.5 SD, in May it was 37.7 ± 6.0 SD and in June it was equal to 13.9 ± 8.5 SD. It showed a decreasing pattern in time.

Sizes of female gametes are shown in Figure 4E and Supplementary Material S3. Their size ranged from 60 to 180 µm in January-March, with the 55% of the measured cells within the class 101-140 µm; while sizes varied between 60 and 220 µm in April-June (size class more frequent (46%): 101-140 µm). Finally, Male individuals were not found in the sampling site; however, we observed males in June 2013 in some specimens collected nearby for other purposes.

Discussion

This work is the first quantitative study on the variations in abundance and reproductive biology of the common anthozoans *Anemonia viridis* and *Exaiptasia diaphana f*rom the North Adriatic Sea. Anthozoans may have species-specific strategies to persist and to colonize new substrates based on growth, shrinking, fission and fusion (Garrabou, 1999). The dynamics of the two anemones considered in this study are summarized in Table 2 and Figure 5. *Anemonia viridis* and *Exaiptasia diaphana* were observed all year round at the study area but their abundances varied along the considered period, alternating phases of expansion (January-March and July-September) with phases of regression (October-December and April-May). The variations in cover of the two species were not directly correlated to temperature, wave height and irradiance values, suggesting that other factors influence the dynamics of the two species.

Table 2. Comparison of gametogenetic periods of *A. viridis* and *E. diaphana* from temperate waters.

References	Locality	Species	J	F	M	A	M	J	J	A	S	O	N	D
Present work	North Adriatic Sea	*Anemonia viridis*	- - - ———	- - - ———	- - - ———	- - - ———	- - - ———	- - -		- - -	- - -	- - -	- - -	- - -
Chintiroglou & Koukouras, 1992	North Aegean Sea	*Anemonia viridis*	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •			
Schäfer, 1984*	France, Mediterranean Sea	*Anemonia viridis*	- - -	- - -	- - -	- - -		- - -	- - -	- - -	- - -	- - -	- - -	- - -
Shmidt, 1972	Gulf of Naples and Trieste	*Anemonia viridis*							• • •	• • •				
Present work	North Adriatic Sea	*Exaiptasia diaphana*	- - -	- - -	- - -	- - -	- - -	- - - ———						- - -
Schlesinger *et al.,* 2010	Israel	*Exaiptasia diaphana*				- - - ———	- - - ———	- - - ———						

- - - - - Oocytes ——— Sperm cysts • • • • • • • Sex not specified

*males not analyzed in this study.

The number of gonads started decreasing from June; therefore, the spawning and fertilization likely occurred during this month. Sea anemones grow fast (Suggett *et al.,* 2012), and quickly divide by fission (Horton, 2000; Shick, 1991); moreover, the increase of the temperature occurring in July-September could promote asexual reproduction (Louis, 1960). The peak in cover occurring in July-September is probably due to both asexual reproduction and planulae settling.

Furthermore, a nutrient enrichment due to the massive presence of tourists in summertime (Accoroni *et al.,* 2015) could increase the food availability in the area.

The decrease in abundance observed in October-December is probably driven by other abiotic factors. The peculiar morphology and orientation of the Conero Promontory, together with its low bathymetry, affect the hydrodynamics of the area causing prolonged stay of low-salinity waters of riverine origin in the northern area, especially in spring and autumn (Poulain *et al.,* 2004, ARPAM bulletin, 2011). In particular, in November 2013, exceptional rainfall and flood events occurred along the entire region (www.protezionecivile.regione.marche.it) permitting to hypothesize that the life cycle of the two sea anemones could be strongly affected by salinity; however, further analysis should be done to confirm this hypothesis.

Furthermore, with intense water movement, the anemones can be easily dislodged and carried away (Chintiroglou & Koukouras, 1992). This could happen also during October-December: when the sea conditions become unfavourable, the anemones could lose the adhesion of their pedal disc and be drifted away. The exceptional rough sea conditions registered in November 2013 (www.ilmeteo.it), may have caused the detachment of the anemones. The effects of wave motion were probably more evident in *Anemonia viridis* since *Exaiptasia diaphana* lives inside asperities and holes in the rock and has less possibility to be dislodged. Indeed, it has been suggested that individual locomotion may represent an essential strategy to survive to adverse conditions or stochastic environments (Bedgood *et al.,* 2020). It is unlikely that the observed regression could be due to low food availability since the basin has a high productivity (Giordani *et al.,* 2002).

As in other corals (Caroselli *et al.,* 2016 and references therein), the gametogenesis of the Adriatic specimens of *A. viridis* and *E. diaphana* from the North Adriatic is clearly driven by temperature variations (Chintiroglou & Koukouras, 1992).

In *A. viridis* the oocyte production was continuous throughout the year except for July, while the male gametes were produced for a shorter period (January-June). Sperm cysts were visible from January when water temperature reaches about 10° C, suggesting that this could be the critical value triggering the sperm production. The highest number of male gonads and the maximal size of the cysts occurred in May, while these values dropped in June, leading us to think that i) the maturation of spermatozoans is favoured by the temperature increase and ii) most of gametes are liberated in June. The highest percentage of female individuals occurred in February,

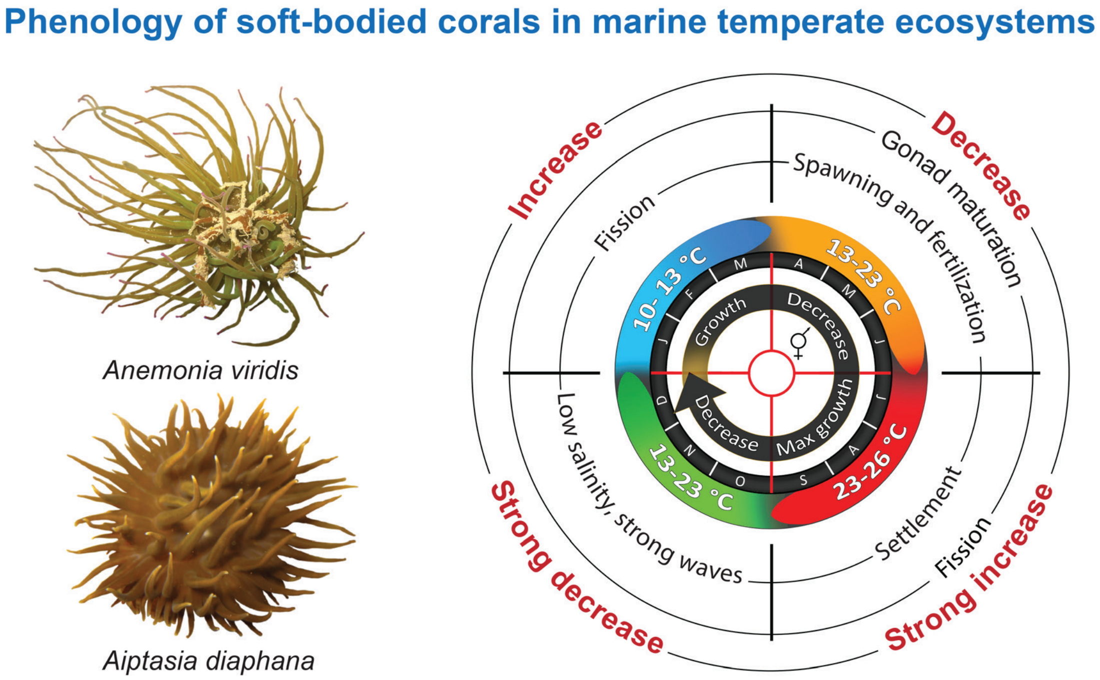

Fig. 5: Scheme summarizing the life history traits of *Anemonia viridis* and *Exaiptasia diaphana.* Quadrants of the circle correspond to quarters; each quadrants shows phases of expansion (January-March and July-September) and regression (October-December and April-May) together with traits of the reproductive cycle (settlement, fission, gonad maturation, spawning etc.) and sea temperature ranges.

and then the abundance of females decreased, suggesting that the spawning of the oocytes began from March with a temperature of 12.5° C. However, the majority of oocytes were shed in June, since the number of gonads per individual dropped during this month and the maximal oocyte size was observed in May. In July all the gametes were liberated since no fertile individuals were found.

Competition for space (Coma *et al.,* 2000) and energy shortage due to a higher investment in sexual reproduction (Di Camillo *et al.,* 2014) may explain the seasonal alternance between high abundance/regression in sea anemones and macroalgae occurring in April-June (Herbets, 1972).

In *E. diaphana* the reproductive time was reduced with respect to *A. viridis*; indeed, the first female individuals occurred in December, with the highest number of gonads observed in May.

Both the number of female individuals and the number of gonads decreased in June, suggesting that the spawning mainly occurred during this period. This is also confirmed by the fact the oocytes reach the maximal size in May and June. No male individuals are found in the sampling area; however, male individuals were present in the surrounding zone. Additive studies should be carried out to highlight this point and understand how the two sexes segregate. The factors driving the sex determination in anemones are unknown (Schlesinger *et al.,* 2010) and actually, we could only raise these hypotheses: i) the anemones of a site are genets (i. e. they derive all from the division of the same individual); ii) the sex determination is driven by environmental factors; iii) the sex determination is genetic and planulae of the same sex aggregate on the same substrate.

Table 2 shows the comparison with the gametogenesis of the two studied species from other localities. Hermaphrodites were observed in cultured specimens of *E. diaphana* (Schlesinger *et al.,* 2010; Armoza-Zvuloni *et al.,* 2014), while this peculiar strategy was never observed in wild anemones, neither in the study area nor in the rest of the Mediterranean (see references in Table 2). The period of gamete production differs depending on the locality and the anemones from the North Adriatic Sea show the longest gametogenesis. This evidence suggested that the production of male and female gametes require different threshold values. However, the duration of the gametogenesis is probably affected by other factors, such as food availability. The dynamics of *A. viridis* and *E. diaphana* reflect those of several lower metazoans of the area (Di Camillo *et al.,* 2012; 2014). In the North Adriatic Sea, indeed, most of the benthic species of hard substrates show a summer growth phase while the gametogenesis has a peak in spring. Since the high productivity rates (Giordani *et al.,* 2002), the benthic organisms from the North Adriatic basin reach sizes or densities significantly higher than the same species observed in other Mediterranean localities and the duration of the gametogenesis is generally longer. It is evident that only long-term studies can completely clarify the complexity of the life cycle of species, especially in case of iteroparous organisms. Anyway, the contribution of this research represents an important baseline for further comparisons and assessment on the studied species, not only on local scale. In particular, the peculiar effect of food availability of the Adriatic Sea has been evidenced also during this study. Gametes production is here present during a wider fraction of the year highlighting once again that the typical effects of physical environmental features and competitive interactions dynamics can be overwhelmed by trophic availability.

References

Afghan, A., Cerrano, C., Luzi, G., Calcinai, B., Puce, S. *et al.,* 2020. Main Anthropogenic Impacts on Benthic Macrofauna of Sandy Beaches: A Review. *Journal of Marine Science and Engineering,* 8 (6), 405.

ARPAM Bullettin, 2011. Relazione annuale sulla sorveglianza dei fenomeni eutrofici dell'ambiente marino-costiero. Agenzia Regionale per la Protezione Ambientale - Regione Marche pp. 1-50.

Accoroni, A., Glibert, P.M., Pichierri, S., Romagnoli, T., Marini, M. *et al.,* 2015. A conceptual model of annual *Ostreopsis* cf. *ovata* blooms in the northern Adriatic Sea based on the synergic effects of hydrodynamics, temperature, and the N:P ratio of water column nutrients. *Harmful Algae,* 45, 14-25.

Armoza-Zvuloni, R., Kramarsky-Winter, E., Loya, Y., Schlesinger, A., Rosenfeld, H. 2014. Trioecy, a unique breeding strategy in the sea anemone *Exaiptasia diaphana* and its association with sex steroids. *Biology of reproduction*, 90 (6), 122, 1-8.

Basile, G., Cerrano, C., Radjasa, O., Povero, P., Zocchi, E. 2009. ADP-ribosyl cyclase and abscisic acid are involved in the seasonal growth and in post-traumatic tissue regeneration of Mediterranean sponges. *Journal of experimental marine biology and ecology*, 381 (1), 10-17.

Bautista-Guerrero, E., Carballo, J.L., Maldonado, M., 2010. Reproductive cycle of the coral-excavating sponge *Thoosa mismalol*li (Clionaidae) from Mexican Pacific coral reefs. *Invertebrate Biology*, 129 (4), 285-296.

Bedgood, S.A., Bracken, M.E., Ryan, W.H., Levell, S.T., Wulff, J. 2020. Nutritional drivers of adult locomotion and asexual reproduction in a symbiont-hosting sea anemone *Exaiptasia diaphana. Marine Biology,* 167 (4), 1-12.

Birchenough, S. N., Degraer, S., Reiss, H., Borja, A., Braeckman, U. *et al.,* 2011. Responses of marine benthos to climate change. ICES *Status Report on Climate Change in the North Atlantic*. ICES Cooperative Research Report, 310, 123-146.

Birchenough, S.N., Reiss, H., Degraer, S., Mieszkowska, N., Borja, Á. *et al.*, 2015. Climate change and marine benthos: a review of existing research and future directions in the North Atlantic. Wiley interdisciplinary reviews: climate change, 6 (2), 203-223.

Burrows, M.T., Schoeman, D.S., Buckley, L.B., Moore, P., Poloczanska, E.S. *et al.,* 2011. The pace of shifting climate in marine and terrestrial ecosystems. *Science,* 334, 652-655.

Byun, D.S., Pinardi, N. 2007. Comparison of marine insolation estimating methods in the Adriatic Sea. *Ocean Science Journal,* 42, 211-222.

Caroselli, E., Goffredo, S. 2016. Population dynamics of temperate corals in a changing climate. In *The Cnidaria, Past, Present and Future*. Springer, Cham, 317-328.

Cheng, L., Abraham, J., Hausfather, Z., Trenberth, K.E. 2019. How fast are the oceans warming? *Science,* 363, 128-129.

Chintiroglou, C.C., Koukouras, A. 1992. A population of the Sea Anemone *Anemonia viridis* (Fòrskal, 1775) and its associated flora and fauna, in the North Aegean Sea. *International Review of Hydrobiology,* 77 (3), 483-495.

Ciabatti, M., Curzi, P.V., Ricci Lucchi, F. 1986. Sedimentazione quaternaria nell'Adriatico centrale. Atti della Riunione del Gruppo di Sedimentologia del C.N.R, Ancona, 125-139.

Cohen, J.M., Lajeunesse, M.J., Rohr, J.R. 2018. A global synthesis of animal phenological responses to climate change. *Nature Climate Change*, 8 (3), 224-228.

Coma, R., Ribes, M., Gili, J.M., Zabala, M. 2000. Seasonality in coastal benthic ecosystems. *Trends in ecology & evolution*, 15 (11), 448-453.

Corriero, G., Pierri, C., Accoroni, S., Alabiso, G., Bavestrello *et al.,* 2015. Ecosystem vulnerability to alien and invasive species: a case study on marine habitats along the Italian coast. *Aquatic Conservation: Marine and Freshwater Ecosystems,* 26, 392-409.

De Bettignies, T., Wernberg, T., Gurgel, C.F.D. 2018. Exploring the influence of temperature on aspects of the reproductive phenology of temperate seaweeds. *Frontiers in Marine Science,* 5, 218.

Desiato, F., Fioravanti, G., Fraschetti, P., Perconti, W., Piervitali, E. *et al.,* 2017. Gli indicatori del clima in Italia nel 2016. ISPRA - Istituto Superiore per la Protezione e la Ricerca Ambientale. *Stato dell'Ambiente*, 72, 2017.

Di Camillo, C.G., Cerrano, C. 2015. Mass mortality events in the NW Adriatic Sea: phase shift from slow- to fast-growing organisms. PLoS ONE, 10, (5), e0126689.

Di Camillo, C.G., Bosato, S., Cerrano, C. 2014. Reproductive ecology of *Epizoanthus arenaceus* Delle Chiaje, 1823 (Cnidaria: Anthozoa) from the North Adriatic Sea. *Journal of Experimental Marine Biology and Ecology,* 461, 144-153.

Di Camillo, C.G., Coppari, M., Bartolucci, I., Bo, M, Betti, F. *et al.,* 2012. Temporal variations in growth and reproduction of *Tedania anhelans* and *Chondrosia reniformis* in the North Adriatic Sea. *Hydrobiologia,* 687, 299-313.

Di Camillo, C.G., Gravili C., De Vito D., Pica D., Piraino S. *et al.,* 2018 The importance of applying Standardized Integrative Taxonomy when describing marine benthic organisms and collecting ecological data. *Invertebrate Systematics,* 32, 794-802.

Dulvy, N.K., Baum, J.K., Clarke, S., Compagno, L.J., Cortés, E. *et al.,* 2008. You can swim but you can't hide: the global status and conservation of oceanic pelagic sharks and rays. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 18 (5), 459-482.

Garrabou, J. 1999. Life-history traits of *Alcyonium acaule* and *Parazoanthus axinellae* (Cnidaria, Anthozoa), with emphasis on growth. *Marine Ecology Progress Series*, 178, 193-204.

Giangrande, A., Geraci, S., Belmonte, G. 1994. Life-cycle and life-history diversity in marine invertebrates and the implications in community dynamics. *Oceanography and Marine Biology. An Annual Review*, 32, 305-333.

Giangrande, A., Gambi, M.C., Gravina, M.F. 2017. Paradigm shifts in community ecology: Open versus closed units, challenges and limits of connectivity studies. *Marine Ecology,* 38.

Giordani, P., Helder, W., Koning, E., Miserocchi, S., Danovaro, R. *et al.,* 2002. Gradients of benthic-pelagic coupling and carbon budgets in the Adriatic and Northern Ionian Sea. *Journal of Marine Systems,* 33, 365-387.

Goffredo, S. Arnone, S., Zaccanti, F., 2002. Sexual reproduction in the Mediterranean solitary coral Balanophyllia europaea (Scleractinia, Dendrophylliidae). *Marine Ecology Progress Series*, 229, 83-94.

Herberts, C., 1972. Contribution a l'étude biologique de quelques zoantharies tempérés et tropicaux II. Relations entre la reproduction sexuée, la croissance somatique et le bourgeonnement. *Tethys,* 4, 961-968.

Horton, A., 2000. Snakelocks Anemome (On-line). Accessed April 9, 2003 at http://ourworld.compuserve.com/homepages/BMLSS/Snakelok.htm.

Kharouba, H.M., Ehrlén, J., Gelman, A., Bolmgren, K., Allen, J.M. *et al.,* 2018. Global shifts in the phenological synchrony of species interactions over recent decades. *PNAS* 115, 5211-5216.

Louis, C. 1960. Modalites et determinisme experimental de la scissiparite chez l'actinie *Anemonia-sulcata* pennant. *Comptes Rendus Hebdomadaires des Seances de l'Academie des Sciences*, 251 (1), 134-136.

Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics,* 37, 637-669.

Poloczanska, E.S., Brown, C.J., Sydeman, W.J, Kiessling, W., Schoeman, D.S. *et al.,* 2013. Global imprint of climate change on marine life. *Nature Climate Change*, 3, 919-925.

Poloczanska, E.S., Burrows, M.T., Brown, C.J., García Molinos, J., Halpern, B.S. *et al.,* 2016. Responses of marine organisms to climate change across oceans. *Frontiers in Marine Science*, 3, 62.

Puce, S., Bavestrello, G., Di Camillo, C.G., Boero, F. 2009. Long-term changes in hydroid (Cnidaria, Hydrozoa) assemblages: effect of Mediterranean warming? *Marine Ecology*, 30 (3), 313-326.

Poulain, P.M., Mauri, E., Ursella, L. 2004. Unusual upwelling event and current reversal off the Italian Adriatic coast in summer 2003. *Geophysical research letters*, 31 (5).

QGIS Development Team. 2017. QGIS geographic information system. Open Source Geospatial Foundation Project. URL http ://qgis.osge o.org

Reusch, T.B.H. 2014. Climate change in the oceans: Evolutionary versus phenotypically plastic responses of marine animals and plants. *Evolutionary Applications,* 7 (1), 104-122.

Rilov, G., Mazaris, A.D., Stelzenmüller, V., Helmuth, B., Wahl, M. *et al.,* 2019. Adaptive marine conservation planning in the face of climate change: What can we learn from physiological, ecological and genetic studies? *Global Ecology and Conservation,* 17.

Robinson, N.M., Nelson W.A., Costello M.J., Sutherland J.E., Lundquist C. 2017. A Systematic Review of Marine-Based Species Distribution Models (SDMs) with Recommendations for Best Practice. *Frontiers in Marine Science,* 4, 421.

Schäfer, W. G. 1984. Fortpflanzung und Entwicklung von Anemonia sulcata (Anthozoa, Actiniaria). I. Fortpflanzungszyklus und Struktur der Oocyten vor und nach der Besamung. *Helgoländer Meeresuntersuchungen*, 38 (2) 135-148.

Schlesinger, A., Kramarsky-Winter, E., Rosenfeld, H., Armoza-Zvoloni, R., Loya, Y. 2010. Sexual plasticity and self-fertilization in the sea anemone *Exaiptasia diaphana, PLoS One*, 5 (7).

Schmidt, H. (1972). Die Nesselkapseln der Anthozoen und ihre Bedeutung für die phylogenetische Systematik. *Helgoländer Wissenschaftliche Meeresuntersuchungen*, 23 (4), 422-458.

Shick, J. M. (Ed.). 2012. *A functional biology of sea anemones*. Springer Science & Business Media.

Stachowitsch, M. 1991. Anoxia in the Northern Adriatic Sea: Rapid death, slow recovery. In: Tyson RV, Pearson TH editors. Modern and Ancient Continental Shelf Anoxia. Geological Society, London, Special Publications pp. 119-129.

Suggett, D.J., Hall-Spencer, J.M., Rodolfo-Metalpa, R., Boatman, T. G., Payton, R. *et al.,* 2012. Sea anemones may thrive in a high CO2 world. *Global Change Biology*, 18 (10), 3015-3025.

Thackeray, S.J., Sparks,T.H., Frederiksen,M., Burthe,S., Bacon, P.J. *et al.,* 2010. Trophic level asynchrony in rates of phenological change for marine, freshwater and terrestrial environments. *Global Change Biology*, 16, 3304-3313.

Trygonis, V., Sini, M. 2012. photoQuad: a dedicated seabed image processing software, and a comparative error analysis of four photoquadrat methods. *Journal of Experimental Marine Biology and Ecology*, 424, 99-108.

Williams, G.C., Van Syoc, R.J. 2007. Methods of preservation and anesthetization of marine invertebrates. *The Light and Smith Manual: intertidal invertebrates from central California to Oregon.* University of California Press, Berkeley, 37-41.

www.ancona.telemisura.it

ilMeteo srl, www.ilmeteo.it (Accessed in 2013-2014)

ISPRA, www.mareografico.it (Accessed in 2013-2014)

www.protezionecivile.regione.marche.it (Accessed in 2013-2014)

Supplementary data

The following supplementary information is available online for the article:
Table S1. Table summarizing the associated benthic fauna and flora throughout the year (from July 2013 to June 2014). Information about phylum and species name are provided.
Fig. S2: Graphs summarizing the abundance of Female (pink) and Male (blue) gonads in the species *A. viridis* through the sampling year. X-axis indicates the gonad size (classified using size classes in (μM) and y-axis indicates the percentage of gonads belonging to each size class.
Fig. S3: Graphs summarizing the abundance of Female (pink) gonads in the species *E. diaphana* throughout the sampling year. X-axis indicates the gonad size (classified using size classes in μM) and y-axis indicates the percentage of gonads belonging to each size class.