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Reproductive ecology of *Epizoanthus arenaceus* (Delle Chiaje, 1823) (Cnidaria: Anthozoa) from the North Adriatic Sea

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A B S T R A C T

The North Adriatic Sea is a shallow basin characterized by peculiar trophic and hydrologic conditions affecting the composition, species abundance and seasonal cycles of the benthic assemblages. Due to high productivity rates comparable to those of the north seas, here the benthic organisms reach sizes or densities significantly higher than the same species observed in other Mediterranean localities and show wider periods of reproduction. Concerning the dynamics of zoanthids, there are very few studies about these Mediterranean species, and this research is the first on the abundance and reproduction of *Epizoanthus arenaceus*. The zoanthid from shallow waters of the NW Adriatic Sea has a maximum abundance of 25,000 polyps m^{-2} (about 40 g C m^{-2}). *E. arenaceus* shows a growth phase during winter and a regression period from summer to autumn coinciding with gametogenesis. During summer, the decreases in current intensity and prey concentration combined with the increase in the reproductive effort may cause shrinkage and fission/fragmentation of the colonies. On the contrary, the maturation and release of gametes are positively correlated with the temperature trend. A temporal and spatial separation of the production of male and female gametes was observed, leading us to hypothesize that temperature could affect sex determination.

The comparison of patterns in abundance and reproduction at 3–4 m and 6–7 m shows that the shallower colonies seem to be more sensitive to extreme values of temperature. The NW sector of the Adriatic Sea, vulnerable to severe anthropogenic pressure and subject to frequent thermal anomalies, is particularly prone to the mortality episodes of benthic organisms. For these reasons, the study of the dynamics of invertebrates from this area is crucial to forecast the effects of environmental changes in the entire Mediterranean basin.

1. Introduction

Zoanthids are benthic suspension feeders belonging to the order Zoantharia. From an ecological point of view, passive suspension feeders play a significant role in plankton–benthos coupling in the shallow waters of the Mediterranean Sea (Cocito et al., 2013). Most of the papers dealing with the ecology of zoanthids focus on their symbiosis with other organisms such as zooxanthellae (Reimer et al., 2013), sponges (Crocker and Reiswig, 1981), hermit crabs (Ates, 2003; Schejter and Mantelatto, 2011) and hydrozoans (Di Camillo et al., 2010 and references herein) or on their sexual reproduction (Muirhead et al., 1986; Previati et al., 2010; Ryland, 1997, 2000), production of natural products (Genji et al., 2010; Kuramoto et al., 2004), geographical or bathymetric distribution (Goreau, 1959; Reimer et al., 2008) and their feeding behaviour (Reimer, 1971; Sorokin, 1991). Notwithstanding cnidarians are clonal invertebrates able to reach high biomass and cover wide surfaces (Garrabou, 1999), studies on population dynamics (Acosta et al., 2001; Bastidas and Bone, 1996; Karlson, 1986, 1988; Muko et al., 2001; Shiroma and Reimer, 2010; Tanner, 1997, 1999) or trophic ecology (Sebens, 1977) mainly pertain to tropical zoanthids and there are very few works concerning the Mediterranean species. Garrabou (1999) described several key aspects of the life history of *Parazoanthus axinellae* (Schmidt, 1862) from the Medes Islands (NW Mediterranean) highlighting it is a dynamic species, which is able to spread rapidly over the substrate mainly by asexual reproduction processes. Previati et al. (2010) investigated the seasonal reproductive cycle of *P. axinellae* and *Savalia savaglia* (Bertoloni, 1819) from the Ligurian Sea elucidating that both species reproduce at the end of autumn when seawater temperature starts decreasing. Cerrano et al. (2006) reported episodic mortality events of *P. axinellae* while Cerrano et al. (2010) emphasized that colonies of *S. savaglia* create a complex tertiary structure enhancing benthic biodiversity and ecosystem functioning in the mesophotic zone.

In the Mediterranean Sea, zoanthids are represented by four genera only—*Savalia*, *Parazoanthus*, *Epizoanthus* and a deep sample tentatively ascribed to *Isozoanthus* (Bo et al., 2013; Gili et al., 1987). *Epizoanthus arenaceus* (Delle Chiaje, 1823) is a temperate species (Herberts, 1972a, b) frequently recorded in the Mediterranean basin (Gili et al., 1987; Pax and Müller, 1962). In the Adriatic Sea the species spreads both on

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natural and artificial substrates suggesting that the zoanthids represent one of the most common benthic cnidarians of the western Adriatic Sea; nonetheless, there are no studies concerning its actual abundance and reproductive biology.

The NW sector of the Adriatic Sea is a peculiar shallow, semi-closed basin with high productivity rates comparable to those of the north seas (Gilmartin and Revelante, 1983) and exposed to severe anthropogenic pressure. Because of its trophy, hydrology and orography, the NW Adriatic sea is subject to frequent thermal anomalies (Desiato et al., 2012), long periods of calm sea conditions alternating with extremely rough waters (Arpa Bulletin, 2012; ISPRA, 2013), and it is particularly prone to mortality episodes of benthic organisms. For these reasons, the study of the dynamics of invertebrates from this area is crucial to forecast the effects of climate changes at Mediterranean scale.

The main objective of this work is to study the population dynamics of *E. arenaceus* from shallow waters of the Conero Promontory (North Adriatic Sea), testing the hypothesis that its abundance as well as sizes and the density of gametes vary in time (seasons) and space (depths).

2. Materials and methods

2.1. Samplings

Specimens of *E. arenaceus* were photographed every month and collected directly underwater by SCUBA from April 2010 to March 2011 at the Conero Promontory (North Adriatic Sea), along a natural rocky pier called Scoglio del Trave (43°34′54.05″N, 13°34′15.15″E). Since the zoanthid distribution in the area is highly patchy, a 100 m long horizontal transect was considered at two bathymetric ranges: 3–4 and 6–7 m. In order to avoid altering the natural trend of the abundance of polyps studied by photos, the biological samplings were carried out in separate areas, at intervals of 10 m (Fig. 1).

The sea-water surface temperatures of the Conero Promontory were downloaded from the National Tidegauge Network website (http://www.mareografico.it). Data were collected daily from the water temperature transducer T020 TTA placed on the sea surface in the Ancona's NT station (43° 37′ 29.16″; 13° 30′ 23.46″) every 10 min. The data were elaborated to calculate the average monthly temperature.

2.2. Abundance

In order to estimate the abundance of *E. arenaceus*, we used a Canon Ixus 960 IS digital underwater camera. Each month, from April 2010 to March 2011, 20 pictures of a standard quadrat of 15 × 10 cm were taken haphazardly along the vertical rocky wall for a total of 60 pictures per season. Ten photographic samplings were carried out monthly at 3–4 and another ten at 6–7 m depth, along a 100 m long transect at intervals of 10 m (Fig. 1).

Photographs were analysed in order to determine the polyp number m^{-2}, the zoanthid cover (%), and the number of patches; hence, we calculated the monthly average values ± SD. The area recovered was expressed as the percentage of the substrate covered by *E. arenaceus* in the standard quadrats; the areas of each colony were measured by ImageJ software.

In order to show the seasonal variations of the polyp number m^{-2}, the datasets of spring (from April to June), summer (from July to September), autumn (from October to the first half of December) and winter (from the second half of December to March) were averaged, and the standard deviation (SD) was calculated.

In order to estimate the polyp's biomass (μg C), 100 polyps (five replicates of 20 polyps each) were collected randomly along the transect inside the 10 m intervals designated for the biological samplings (Fig. 1). They were dried at 110 °C for 24 h (Zintzen et al., 2007); thus, the dry weight (DW) of each replicate was determined at the analytical scale. Since zoanthids may incorporate a great amount of foreign materials in their body (Haywick and Mueller, 1997), the dried polyps were immersed in hypochlorite 5% in order to dissolve the organic matter and obtain the engulfed particles. The samples were washed in distilled water and in a graded series of ethylic alcohol; then, the debris was dried and weighted. Lastly, the weight of the inorganic fraction was taken away from the total weight and the average net DW ± SD was determined. The polyp biomass was calculated as the 50% of the net dry weight (Biswas and Biswas, 1979). Polyp sizes (average diameter and length) were determined analysing the pictures of completed expanded polyps by ImageJ software.

2.3. Reproductive biology

The monthly underwater samplings were conducted inside the 10 m intervals without photosampling, in order to avoid affecting population trend in the same area of the photographic analysis.

About 30 portions of colonies of *E. arenaceus* were randomly collected with a blade at both 3–4 m and 6–7 m depth. In laboratory, 30 polyps for each depth were dissected using a cutter, and their mesenteric septa were observed under a light microscope in order to verify the presence of gametes and establish the sex ratio.

Other ten polyps for each bathymetric range were fixed in 2.5% glutaraldehyde (buffered in filtered sea water 7.8 pH) for a histological analysis. Three hours later, samples were washed in filtered sea waters and then dehydrated in a graded ethanol series. Subsequently, polyps were included in a cold-curing resin (Technovit 8100) and finally mounted on plastic supports. Longitudinal sections of the polyps were obtained by microtome Histo-Line MRS3500; then the slices were coloured with Toluidine blue and mounted with Eukitt.

For each polyp, the central sections were analysed under a compound microscope, and three entire mesenteric septa were chosen. Hence, the number of gametes in each septum was counted, and the monthly abundance of oocytes and spermatic cysts—expressed as the average number of gametes per mesenteric septum ± SD—was calculated.

Moreover, the diameter of all the reproductive elements contained in the septa was determined with the micrometric lens; subsequently, the average values of sizes ± SD were calculated.

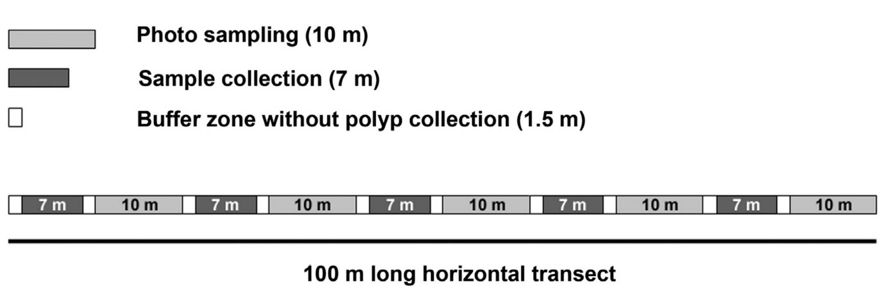

Fig. 1. The scheme illustrates the sampling modality along the horizontal transect. To avoid altering the natural trend of the abundance of polyps, the sample collection and photo surveys were carried out in separate areas, alternating intervals of 10 m.