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# Oxygen consumption in Mediterranean octocorals under different temperatures

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ABSTRACT

Ecosystem resilience to climate anomalies is related to the physiological plasticity of organisms. To characterize the physiological response of some common Mediterranean gorgonians to fluctuations in temperature, four species (*Paramuricea clavata*, *Eunicella singularis*, *Eunicella cavolinii* and *Corallium rubrum*) were maintained in aquaria, in which the temperature was increased every ten days with increments of 2–3 °C, starting at 14 °C, ending at 25 °C. Oxygen consumption, number of open/closed polyps and percentage of necrotic tissue were monitored. All species showed similar activity patterns with increasing temperature. *P. clavata* and *E. singularis* showed the highest respiration rate at 18 °C, *E. cavolinii* and *C. rubrum* at 20 °C. Above these temperatures, both oxygen consumption and polyp reactivity decreased in all species. The present data confirm a reduction of the metabolic activity in Mediterranean gorgonians during periods of high temperature. At temperatures above 18 °C, the percentage of open polyps (considered as a parameter to evaluate polyps reactivity) decreased, thus mirroring the trend of oxygen consumption. The average values of $Q_{10}$ indicated that gorgonians have a definite temperature limit over which the metabolism (oxygen consumption) stop to follow the temperature increase. After three days at 25 °C, metabolic activity in *E. cavolinii*, *C. rubrum* and *P. clavata* further decreased and the first signs of necrosis were observed. At this temperature, activity remained unchanged in *E. singularis*. This species seems to more resistant to thermal stress. The symbiotic zooxanthellae present in this species are likely to provide an alternative source of energy when polyps reduce their feeding activity.



## 1. Introduction

In the Mediterranean Sea, many abiotic factors fluctuate throughout the year. Due to seasonal patterns, annual changes in temperature in the Mediterranean have a range of up to 15 °C (Founda et al., 2004). Such strong fluctuations in temperature have profound impact on animal metabolism. Temperature and metabolism have been related since the work of Arrhenius (1889; 1915) and Van't Hoff (1896), who proposed a mechanistic approach. However, due to the complexity of cellular life and the metabolic feedback mechanisms by which cells respond to temperature fluctuations, the thermodynamic response to temperature cannot be predicted from physical principles only (Clarke and Fraser, 2004). Thermo-tolerance is likely to be settled by the adjustment of the oxygen demand and influences the metabolic activities and energy-demanding processes in cells. This is particularly the case for benthic sessile organisms (Arillo et al., 1989; Frederich and Pörtner, 2000; Hochachka and Somero, 2002; Clarke, 2003; Hadas et al., 2008), which cannot easily migrate to other, more suitable habitats.

Tolerance to naturally occurring fluctuations is likely to be challenged by global warming (Gambiani et al., 2009). Due to global change, the upper threshold for temperature tolerance may be exceeded, which will have profound impact on marine life, in particular on benthic communities. As reviewed by Hughes (2000), recent climatic and atmospheric trends are already affecting species physiology, distribution and phenology. In the NW Mediterranean Sea, massive mortality events are occurring nearly every summer/autumn season, involving mainly benthic invertebrates between 0 and 50 m depth (Cerrano et al., 2000; Perez et al., 2000; Garrabou et al., 2001; Cerrano et al., 2005; Linares et al., 2005; Garrabou et al., 2009). These episodes may be due to synergistic effects of thermal anomalies, summer energy shortage, and pathogens (Romano et al., 2000; Garrabou et al., 2001; Lesser et al., 2007; Cerrano and Bavestrello, 2008; Coma et al., 2009; Ferrier-Pagès et al., 2009; Vezzulli et al., 2010). Moreover, high temperatures reduce the oxygen solubility (Carpenter, 1966; Truesdale et al., 1955), thus limiting its availability. The importance of oxygen availability for marine invertebrates has recently been summarized by Riedel et al. (2008), evidencing both direct (physiological) and indirect (behavioural) effects. A reduced availability of oxygen coinciding with an increased metabolic oxygen demand may cause severe oxygen limitation in marine invertebrates under high temperatures (Legovic and Justic, 1997). In this context, it is important to describe patterns in

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respiration activity of marine invertebrates under increasing temperature, in particular for those species that are most vulnerable to climate anomalies. Such knowledge is needed to draw a more complete scenario of the parameters possibly involved in mass mortalities.

Gorgonians (Cnidaria, Alcyonaria) are among the most endangered groups by mass mortality events (Cerrano and Bavestrello, 2008; Garrabou et al., 2009). Sea-fans, a typical component of the benthic Mediterranean communities, are considered important foundation species of coralligenous habitats, amplifying the ecosystem complexity from both a physical and biological point of view (Sarà, 1969; True, 1970; Gili and Ros, 1985; Sebens, 1991; Ballesteros, 2006; Scinto et al., 2009). Since the last ten years, these ecosystem engineer species suffered from mass mortality with a biomass decrease of more than 50% in the case of *Paramuricea clavata* (Linares et al., 2005).

Despite the large number of studies about mass mortality events (see Cerrano and Bavestrello, 2008 for a review), gorgonian physiology is still poorly known. Some data are available for the symbiotic with zooxanthellae *Eunicella singularis* where oxygen consumption is clearly affected by light exposure (Brafield et al., 1965; Chapman and Theodor, 1969; Ferrier-Pagès et al., 2009). Concerning the oxygen consumption of *P. clavata*, the only data available were collected *in situ* in the Mediterranean Sea by Coma et al. (2002), who found that low respiration rates during summer correlated with a low availability of planktonic food.

The aim of this work is to measure the effects of temperature increase on the physiology of four of the most abundant octocorals species of the Mediterranean pre-coralligenous and coralligenous communities by, analyzing oxygen consumption, polyps activity and necrosis signs of these octocorals under controlled aquarium conditions.

## 2. Materials and methods

### 2.1. The species studied and their habitats

To facilitate the interpretation of results it is important to take in account some ecological peculiarities of the four studied species and their habitat.

The coralligenous communities are among the most important hot-spot of biodiversity in the Mediterranean Sea. In terms of biomass, Cnidaria are the main constituent of these communities (see Ballesteros, 2006 for a review). True (1970) described three different coralligenous assemblages: one dominated by *Eunicella cavolinii* (Von Koch, 1887), one dominated by *P. clavata* (Risso, 1826) and one dominated by *Corallium rubrum* (Linnaeus, 1758).

*E. cavolinii* has a bathymetric distribution that ranges from 10 to 150 m, with the optimum between 15 and 70 m depth. It preferably grows on vertical substrates, orienting itself to the main currents. As such, it is regarded an indicator of the prevailing water flow (Russo, 1985). Although *E. cavolinii* is a common species, there is little information regarding its ecology. It can live sympatrically with *P. clavata* suggesting similar ecological needs.

The red gorgonian (*P. clavata*) is considered a true ecosystem engineering species, because its presence strongly affects community structure, both in terms of biomass and in terms of biodiversity (Scinto et al., 2009). Its depth distribution ranges from 5 to 110 m (Weinberg, 1991) with an optimum between 35 and 80 m depth. This species has an evident skiophilous habitus and needs high water movement (True, 1970; Weinberg, 1978; Mistri, 1994; Linares et al., 2007).

*C. rubrum* has the widest bathymetric distribution, ranging from 7 to 200 m, with the optimum between 30 and 100 m depth (Giannini et al., 2003; Tsounis et al., 2006). The lack of sedimentation, dim-light or dark conditions, and a continuous but not intense water movement are the main ecological requirements for this species. Among the four species studied, *C. rubrum* is the only one with dimorphic polyps (autozooids and siphonozooids).

*E. singularis* (Esper, 1794), is the only Mediterranean gorgonian that lives symbiotically with unicellular algae (zooxanthellae), shows a bathymetric range comprised between 5 and 70 m depth (Weinberg and Weinberg, 1979; Weinberg, 1991). It is predominantly present on horizontal substrata (both hard and detritic) and it is a common species in pre-coralligenous assemblages (Linares et al., 2008).

### 2.2. Sampling and aquarium maintenance

During winter 2008 nine apical fragments (about 5 cm long) of each of the four species studied were randomly collected between 20 and 30 m depth at the Marine Protected Area of Portofino (Ligurian Sea, Italy). Fragments were cut from the primary branch of each specimen sampled (one fragment per specimen).

Directly underwater, each fragment was placed in plastic 50 ml tubes that were then transferred within 24 h to the facilities of the Aquaculture and Fisheries Group at Wageningen University, The Netherlands. During transfer, fragments were kept at 14 °C, the same temperature of the natural environment during collection.

In order to acclimate the fragments, they were kept in an aquarium (70 cm long × 34 cm deep × 25 cm wide) for two weeks under stable conditions (temperature: 14 °C, salinity: 37.7–38‰, irradiance by artificial light: 5.8–5.5 μE $m^{-2}$ $s^{-1}$ for 12 h following a day/night rhythm) before starting the temperature experiments.

Water flow in the aquarium was generated by two pumps: the first pump re-circulated the water through a temperature controller (1/5HP TECO SeaChill Chiller TR15 ± 0.5 °C) to maintain a constant temperature, while the second pump (located in the tank) created a constant, turbulent water movement (0.9 ± 0.1 cm/s) around the colonies. The aquarium water was continuously filtered through activated carbon filters and oxygenated with an air stone, which was positioned at the top of the aquarium in order to minimize its influence on the polyp activity. The tank was cleaned regularly to remove any microalgal build up.

The gorgonians were fed every day with 1 ml of concentrated baby brine shrimp (*Artemia nauplii*) suspension.

### 2.3. Temperature regimes

After two weeks of acclimation, the temperature in the aquarium was raised every ten days with 2 °C–3 °C increments: 14 °C (this initial temperature was maintained for another 10 days consecutive to the 14-day acclimation period), 16 °C, 18 °C, 20 °C, 22 °C and 25 °C. The temperatures between 14 °C and 22 °C match the average seasonal temperatures measured between 15 and 30 m depth in the studied area during sampling dives using a UWATEC underwater thermometer (± 0.5 °C). According to a previous study (Bally and Garrabou, 2007), the 25 °C value represents the upper thermal tolerance limit, especially for *P. clavata*.

After each temperature increase, the gorgonians were allowed to acclimate for five days, while the other five days were used to perform oxygen consumption measurements. The highest temperature regime (25 °C) was maintained only for one week and the oxygen consumption was measured on the first and third day.

### 2.4. Oxygen consumption

To analyze the oxygen consumption under different temperature regimes, three fragments of each species were separately put in hermetic glass chambers. The cap of the chamber bears a hole where a luminescence sensor (HACH LDO® Process Dissolved Oxygen Probe) is inserted to monitor the oxygen concentration (HQ40d Dual-Input Multi-Parameter Digital Meter) in the chamber. The measured