Life-cycle traits in the demosponge *Hymeniacidon perlevis* living in a land-based fish farm (#74587)

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Life-cycle traits in the demosponge *Hymeniacidon perlevis* living in a land-based fish farm

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Background. The demosponge *Hymenaicidon perlevis,* characterized by wide geographic distribution, shows great ability to adapt its life cycle to high variable climatic and hydrological conditions. The extreme adaptive capacity allows it to colonize even artificial environments such as drainage channels, as happened in a marine land-based fish farming plant where the sponge grows spontaneously using the farm waste water as the only form of sustenance. This population offered the opportunity to investigate for the first time, both growth and reproductive cycle within a whole year in relation to some controlled environmental conditions.

Methods. The growth rate and the reproduction cycle were put in relation with the availability of trophic resources, the water temperature and the flux of waste water. Biological traits of the farmed sponges were compared with those of other wild populations, allowing the evaluation of the impact of these ecological drivers in the life cycle of the sponge.

Results. The sponge showed marked growth variations along the study period, with the greatest increase in sponge volume mainly determined by the reduction of the fish reared biomass, the increase of pellet amount available and the increase of the waste water flow in the drainage conduit. Sponge reared specimens exhibited a vital physiological state during the whole year. Anyway, it has been not possible to recognise in the farmed specimens of *H. perlevis* a true sexual cycle, being the occurrence of sexual elements sporadic throughout almost all the year. No asexual elements were observed.

Discussion. Being an intertidal marine sponge, *H. perlevis* possess excellent flexibility and adaptability to the variations of the environmental conditions. The sponge living in the drainage conduit, is constantly in dark conditions, with a constant water temperature of 18 °C and subjected to a continuous nutritional supply, exhibited a vital physiological state during the whole year, avoiding the stages of decline and long dormancy observed in wild populations. It seems quite plausible that for *H. perlevis* living in the drainage conduit characterized by constant values of some basic ecological parameters, the sexual phase was an almost continuous process under the control of endogenous factors. No asexual elements were observed, even though we cannot exclude the occurrence of asexual phenomena in the origin of the newly settled sponges, repeatedly detected thought the study period. The growth variations during the study period are linked to some specific fish farming conditions, providing usefull indications on the best rearing conditions for *H. perlevis* potentially usable in integrated multitrophic farming systems for the waste water treatment.

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Abstract

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Introduction

Porifera are primitive pluricellular invertebrates characterized by a high plasticity, being able to display special adaptive strategies at cellular, structural, and reproductive levels, so adapting their life cycle to a wide range of environmental variations (Gaino, Manconi & Pronzato, 1995; Hill & Hill, 2002; Manconi & Pronzato, 2015).

The effects of environmental factors on sponges' life cycles have been extensively studied (Witte, 1996; Whalan et al., 2007; Gaino et al., 2010; Wahab et al., 2014); in particular, the water temperature is known to be one of the most important environmental drivers, being able to affect gametogenesis, sex ratios, and sexual reproductive output (Reiswig, 1973; Fell, 1976; Maldonado & Young, 1996; Usher et al., 2004; Ettinger-Epstein et al., 2007; Riesgo et al., 2007; Whalan et al., 2007; Maldonado & Riesgo, 2008; Ereskovsky et al., 2013; Wahab et al., 2014, 2017; Lanna et al., 2014, 2018; Shaffer et al., 2020).

The ability to adapt their life cycle to high variable climatic and hydrological conditions is particularly enhanced in sponges with wide geographic distribution, such as the demosponge *Hymeniacidon perlevis* (Montagu, 1818), one of the most common species of European waters (*Erpenbeck & Van Soest, 2002*) so much that can be considered cosmopolitan (Pansini et al., 1987; Xue et al., 2004; Gaino et al., 2010; de Voogd et al., 2022). It can colonise both hard and soft bottoms in shallow subtidal and intertidal zones (Cabioch, 1968; Stone, 1970; Erpenbeck et al., 2002; Corriero, 1990). *H. perlevis* is also one of the most common benthic species inhabiting the central Mediterranean lagoons (Longo et al., 2016; 2017), where it is also able to survive prolonged periods of air exposure (Gaino et al., 2010) and high anthropogenic impacts (Gaino et al. 2010; Longo et al., 2016; 2017), showing peculiar adaptive strategies (Juniper & Steel, 1969; Stone, 1970; Gaino et al., 2010; Cao et al., 2007). In particular, it exhibits the life cycle that lasts one year and that consists of four stages, linked to the seasonal variations: dormancy, resuscitation, bloom, and decline. During these stages the sponge biomass increases or decreases according to the environmental conditions (Stone, 1970; Cao et al., 2007; Gaino et al., 2010; Zhang et al., 2010), with an optimal water temperature survival ~~range~~ ranging between 10-20°C, corresponding to the intermediate seasons (Stone, 1970; Cao et al., 2007; Gaino et al., 2010; Zhang et al., 2010).

H. perlevis is viviparous, gonochoric and/or hermaphroditic non-simultaneous in which oocytes preceded spermatogenesis (Sarà, 1961; Stone, 1970; Diaz, 1973; Gaino et al., 2010). Ovocites are fertilised in the sponge body, where they give rise to ciliated parenchymella larvae (Stone, 1970; Diaz, 1973; Xue et al., 2009; Gaino et al., 2010). In general, sexual reproduction starts in late spring and ends in summer, even if it is subjected to a wide influence of hydrological parameters (Stone, 1970; Gaino et al., 2010). Asexual reproduction by means of fragmentation is also important, especially under adverse environmental conditions (Stone, 1970; Gaino et al., 2010), a process supported by the high regenerative properties of the sponge.

The extreme adaptive capacity allows it to colonize even artificial environments such as drainage channels, as happened in a marine land-based fish farming plant where the sponge grows spontaneously using the farm waste water as the only form of sustenance, as the incoming water is taken from a deep, nutrient-free aquifer. This population offered the opportunity to investigate ~~for the first time,~~ both growth and reproductive cycle within a whole year in relation to some controlled environmental conditions, ~~such as~~ the availability of trophic resources, the water temperature and the waste water flux, in conditions like breeding rather than natural growth. The biological traits of the farmed sponges were compared with those from which the propagules that colonized this drainage channel come (Gaino et al., 2010) together with other wild populations (Sarà, 1961; Stone, 1970; Diaz, 1973), allowing us to evaluate the impact of these ecological drivers in the life cycle phases of the sponge.

Moreover, the present study adds information about the life cycle and the best environmental conditions for the survival of *H. perlevis*, since in the last years it has become very important as a model organism in bioremediation researches thanks to its ability to remove potential pathogenic bacteria and vibrions from the cultured media (Fu et al., 2006, Longo et al., 2010, 2016; Zhang et al., 2010).

Materials & Methods

The Demospongiae *Hymeniacidon perlevis*, family Halichondriidae Gray, 1867 has a very variable external morphology, being able to assume the shape of small and thin crusts up to large size massive aspect (more than 30 cm in maximum diameter). Usually, short papillae and small digitations emerge from the sponge surface. Its colour varies from yellow-orange to red or pale green. Spicules and spicule bundles are made up of slightly curved styles, often with a faint subterminal swelling tylote.

Under the name *Hymeniacidon perlevis,* thanks to a wide systematic and molecular revision, are now accepted species that in the past were named in a different way, such as *H. perleve* (Erpenbeck & Van Soest 2002; Sun et al. 2007; Mahaut et al. 2013), *H. sanguinea*, *H. caruncula* (de Voogd et al. 2021), *H. sinapium* and *H. heliophila* (Turner, 2020). Genetic and morphological data referred to Europe, the Atlantic coasts of North and South America, the Pacific coast of North America and Asia, support the occurrence of only one species. The literature data also reported some records from New Zealand, Southwest Africa, and the Pacific coast of South America, but these records are not confirmed by genetic analysis.

Over all, *H. perlevis* can be effectively considered a cosmopolitan sponge species (Juniper & Steele, 1969; Stone, 1970; Erpenbeck & Van Soest, 2002; Xue et al., 2004; Picton et al., 2007; Mahaut et al. 2013; Turner, 2020). In Mediterranean Sea its range comprises eastern and western basins (Erpenbeck & Van Soest, 2002).

The population here investigated lives on the bottom of a concrete waste channel which conveys the waste water of a marine land-based fish farming (COISPA experimental station fish farm, Torre a Mare, southern Italy; www.coispa.it) to the sea. The sponge specimens have been detected for the first time at the end of an experience in which the rearing performances of this sponge, collected from the Mar Piccolo of Taranto (Italy) (Gaino et al., 2010), have been assesses within the COISPA plant. One year later, some specimens of *H. perlevis* were found growning spontaneously on the bottom of the drainage conduits. No specimens of this species had ever been observed before this experience in any area of the fish farm, so that the origin of these specimens is to be attributed to the reared sponges also because the circulating sea water into the fish farm is a marine nutrient-free ground water, without macroscopic organisms of the benthos.

Environmental parameters

The portion of the drainage conduit where the sponge grows here investigated consists of a concrete canal 4.0-meter-long, 0.57-meter-wide and 0.15 m deep, closed by a walkable inspection cover that makes this part of the conduit completely dark (Figure 1). The wastewater from the fish tanks flows out through the drainage conduit, passing on the area colonized by the sponges. Each tank has an overflow system and a tap at the bottom. To eliminate residues of feed and faces, the tap of each tank is daily opened and approximately one third of the entire water volume is discharged.

The ground sea-water entering the fish farm has temperature and salinity values constant throughout the year (18°C and 34‰, respectively).

Growth performance

Along the drainage conduit, 9 pvc frames of 20x20 cm were randomly positioned on sponges (Figure 2A); three sampling frames were located in the area furthest from the wastewater inlet (frames A, B and C), three sampling frames in the intermediate area (frames D, E and F), and three in the area closest to the wastewater inlet (frames G, H and I) (Figure 1). Only the frame D was placed in an area free from the presence of the sponge, to test the colonization process and the growth rate of the new recruits. Each frame in turn was divided into 4 squares 10x10 cm (Figure 2B) for a more detailed assessment of growth patterns.

Growth performance was monthly registered from March 2018 to March 2019 for each sponge frame and expressed as volume (cm^3). The estimate of the volume of the sponge was calculated as the product of the total area occupied by the sponge in each square by the average thickness in the center of each 10x10 cm sub-square, calculated by means of a millimeter bar fixed in the sponge. Photographic sampling of the nine pvc frames was monthly performed and the area occupied by the sponge in each sub-square was detected thanks to ImageJ software and expressed in cm^2.

Reproductive cycle

With the aim to estimate the relevance of sexual reproduction in the sponge life-cycle, from March 2018 to April 2019, ten sponge fragments of *H. perlevis* of about 3 cm^3 were monthly taken along the drainage conduit, avoiding the sponge biomass present in the frames to not invalidate the growth pattern estimation; fragments were fixed for 24 hours in 4% formaldehyde in filtered sea-water used as a buffer, repeatedly washed in the same buffer, dehydrated in the crescent alcohol series and paraffin-embedded. For histological observations, 7 µm sections were cut from the paraffin blocks by means of a Rotary One microtome and then processed according to the routinely used methodology for setting up stable preparations. Sections were stained with toluidine blue and observed under a light microscope (Olympus BH2).

In order to compare the biological traits of the farmed sponges with those of other wild populations (Sarà, 1961; Stone, 1970; Diaz, 1973; Cao et al. 2007; Gaino et al., 2010), an accurate bibliographic research was performed.

GAM analysis

With the perspective to better describe the growth performances of the species, a generalized additive model (GAM) analysis was used to describe the most effective variables influencing the increase in the volume of the sponge in the specific environmental conditions of the aquaculture facilities. The analysis was accomplished using the sponge total volume at any sampling time as the response variable. Different trials were done using either volume data from all the sampling stations or volume data from each sampling position (frames furthest from the wastewater inlet: A, B and C; the intermediate frames: D, E and F; and frames closest to the wastewater inlet: G, H and I). The best modelling performances were obtained from modelling data from frames G, H and I together.

The GAM analysis was performed using the mgcv library (Wood, 2017) in the R environment considering eight factors influencing the sponge growth pattern. The first variable that was thought to be likely responsible for the growth of sponges was the amount of feed provided to the fish reared in the aquaculture facilities. In particular, due to the different feeding regimes of the fish reared in the facilities, three feed categories were considered: monthly amount of pellet, fresh feed and total feed provided. To include in the model variable useful to describe the animals load of the facilities, other four variables were initially considered: number of active tanks, total number of animals hosted in the facilities, the corresponding biomass and the monthly water volumes provided. Also, month effect was initially tested in the model aiming to describe a temporal trend in the model.

The variance inflation factor analysis (VIF) was used to assess the presence of multicollinearity. Only covariates showing VIF values lower than 5 were retained in the analysis (Zuur et al.

2010), rejecting the others. Furthermore, a subsequent selection of the analysis was accomplished using a backwards stepwise approach. The covariates firstly included in the model were rejected following the following three criteria: (i) if the estimated degrees of freedom of the variable was close to 1; (ii) if the interval of confidence was zero; and (iii) if the generalized cross-validation (GCV) score (Gu & Wahba, 1991) decreased when the covariate was removed from the model. Among the different family distributions tested in the final analysis the Poisson family was chosen using the default logarithmic link function.

Results

Growth performance

Figure 3 reports the variations of sponge biomass within each sampling frame from March 2018 to March 2019. In general, the examined specimens showed an initial biomass decrement during the spring months (April-May), followed by a period of growth stasis during the summer (June-August); from August/September to January/March we assist to a general but non constant growth increase (Figure 3).

At the end of 13 months of observations, the sponges with the greatest growth, located in the frames G and I, showed an increase in their biomass of about a third compared to the initial volume. In particular, their volume increased from 1550 cm^3 and 1150 cm^3 to 2230 cm^3 and 2040 cm^3 respectively (Figures 4 A,B,C). At the beginning of the survey, their average thicknesses were 3.875 cm and 2.875 cm respectively; after one year the average thickness observed was 5.575 cm and 5.10 cm, respectively. Also, the sponge in the frame H showed a fair increase in biomass, its volume ranging from 840 cm^3 to 1033 cm^3 and its thickness from 2.10 cm to 3.275 cm. In the central part of the channel the frame D, positioned on the bare substrate of the dredging channel, was colonized by *H. perlevis* after two months, reaching the final volume of 256 cm^3. The sponge in frame F showed a slow biomass decrease between March and September, its volume ranging from 580 cm^3 to 311 cm^3; the thickness ranged from 1.45 cm to 0.85 cm. In October the sponge died probably for the accumulation of dry food in the central part of the channel that caused severe phenomena of necrosis (Figures 5A,B). The sponge in frame E showed no significant variation in volume during the first 11 months of observation, but at the same way of the sponge in frame F, showed some areas affected by necrosis until its total disappearance in the last month of observation.

Sponges in frames B and C showed no significative biomass and thickness variations, ranging their volume from 520 cm^3 to 362 cm^3 and from 950 cm^3 to 900 cm^3 respectively; thickness ranged from 1.3 cm to 1.475 cm and from 2.375 cm to 2.975 cm respectively. Finally, the sponge in frame A had an initial biomass and thickness of 450 cm^3 and 1.125 cm respectively, but died in August; it recolonized the frame in October and quickly grew until it reached the final volume of 600 cm^3 and the thickness of 1.5 cm in March 2015.

Sexual cycle

~~The monthly presence of the reproductive elements in *H. perlevis* is reported in Figure 6~~.
Reproductive elements were discontinuously detected over the thirteen months of observations. In particular: oocytes were found in July and January; spermatic cysts (January, March, May,

and September) and embryos (January, April, July, and October) were both found in four months. No larvae have been observed.
The sexual reproductive effort was different in female and male specimens: oocytes were detected only in two fragments, with very low densities (2 elements for each examined section), while the production of spermatic cyst involved up to 100% of the examined sponge fragments in March and September, but with very low densities (7-10 spermatic cysts for each examined section). Embryos were rarely found (four sponge fragments, in total) with variable density values (6-10 embryos for each examined section). None of the fragments showed the coexistence of oocytes and sperm cysts according to the more widespread gonochoric condition of the species (Gaino et al., 2010).
~~In Table 1 are reported the results of the previous studies about the sexual cycle of *H. perlevis*; where available, literature information on life stages is also reported.~~
Gaino et al. (2010) reported that the reproductive timing in the population of *H. perlevis* from the Mar Piccolo of Taranto, lasts five months from April to August; the *sex ratio* was unbalanced, with a female overabundance; two hermaphroditic specimens were also observed. A group of specimens living close to the soft bottom and influenced by severe anoxic crises showed a significantly lower density of sexual elements than those belonging to the intertidal specimens, subjected to moderate water movement. This population shows four vital stages, corresponding to the seasonal variations: dormancy in late autumn and winter, resuscitation in spring, bloom in summer, and decline in late summer and early autumn.
Stone (1970), studying an England population living in Langstone Harbour, reported that even if the percentage of specimens containing embryos was higher in July and August, ranging from 45% to 100% of the sampled specimens, no trace of larvae settlement was found at any time. This population shows only two vital stages: bloom in five months (May-September) and decline in seven months (October-April).
The sexual cycle of *H. perlevis*, under the name of *H. caruncula*, was also investigated by Diaz (1973) in the Thau pond. In this environment this species showed successive hermaphroditism in which oogenesis preceded spermatogenesis: the rise in water temperature in spring triggers the onset of oogenesis, which lasts from March to May; spermatogenesis begins a month later and runs from April to June. Sarà (1961) reported for a population of *H. perlevis* of the Gulf of Napoli, under the name of *H. sanguinea,* that the presence of oocytes and spermatic cysts was concentrated in June, and few sexual elements were also observed in July, August and September; a certain degree of subsequent hermaphroditism was also observed. The *sex ratio* was unbalanced, with a male overabundance.
Finaly Cao et al. (2007) reported for a popultaion of *H. perlevis* living in the Lingshui Bay (Dalian, China), the presence of the four vital stages, with a seasonality very similar to that observed in the population of *H. perlevis* from the Mar Piccolo of Taranto.

GAM analysis
The VIF analysis showed the presence of collinearity (VIF > 5) among variables and some of them were hence rejected from the GAM analysis: month, total feed, number of active tanks, and number of animals in tanks.
The best model describing the increase in volume of sponges uses the Poisson family distribution with the default logarithmic link function and fish biomass, amount of pellet and water volume as covariates, following the formula here reported:

$$\text{proximal.frame.vol} \sim \alpha + f_i\,(\text{biomass}_i, \text{pellet}_i) + \text{water.vol}_i + \varepsilon_i$$

The final model explains up to 87.9% of deviance and has an R^2 of 0.61. The estimated degrees of freedom of the estimated smoother and of the linear relationship used in the model were significantly different from zero (p-value<0.05).

The residuals distribution is quite normal around the zero, giving also a good dispersion of fitted values against response in the plots reported in Figure 7.

Thanks to the partial effect of explanatory variables reported in Figure 8, the model shows that the sponge volume increase is mainly determined by the reduction of fish biomass and consequent increase of pellet amount provided to fish, together with the increase of the water flow in the drainage conduit.

Discussion

The life cycle dephasing in metapopulations of sponges, and related consequences at the level of genetic isolation, has been scarcely discussed and needs further investigation, particularly focusing on the problem of differentiation among specimens inside a population up to specific level. Even the pluriannual persistence of timing in the life cycle (Manconi & Pronzato, 1991) and its relationships with environmental factors, such as light and temperature, suggest that both endogenous and exogenous factors contribute to drive the life cycle in Porifera (Simpson & Fell, 1974; Lanna et al. 2014, 2018).

Being an intertidal marine sponge, *Hymeniacidon perlevis* has to face extreme conditions, such as air exposure, excess rainwater, anoxic conditions and so on. Therefore it should possess excellent flexibility and adaptability to such variations in environmental conditions. Literature data report that wild European populations of this species exhibit dormancy in winter months; however, they are able to change their life phases according to the seasonal variations. Atlantic populations exhibit dormancy in winter, when the species greatly reduces its biomass (Stone, 1970; Cao et al., 2007), sometimes completely disappearing (Zhang et al., 2010); restarting in spring, blooming in summer and declining in autumn, according to the optimal temperature survival range encompassed between 10–20°C, corresponding to the intermediate seasons. South Mediterranean intertidal populations shift their active phase of rapid growth in spring, whereas they apparently disappeared from late summer to winter months (Gaino et al., 2010).

In the present study we observed that, even if monthly variations in sponge biomass were detected, *H. perlevis* was not subjected to the dormancy phase which typically occurs in the wild populations. The reared specimens, constantly living in dark conditions, with a constant water temperature of 18 °C and subjected to a continuous nutritional supply, exhibited a vital physiological state during the whole year, avoiding the stages of decline and long dormancy. The reared specimens, however, showed marked growth variations during the study period. The applied model shows that the increase in sponge volume was mainly determined by the reduction of fish biomass and at the same time an increase of pellet amount available, together with the increase of the water flow in the drainage conduit. The synergic effects of these factors would lead to an increase in the volume of the sponges. Indeed, the decrease in fish biomass would lead to a greater amount of waste feed which in turn would produce a greater amount of organic matter and bacteria in the wastewater. These factors with an increase in water flow, which would consist in a greater nutritional supply, could explain the growth of the sponge under constant environmental factors (e.g. temperature, salinity). Indeed, taking into account the effective microbiological filtering capability of *H. perlevis* (Longo et al., 2010), it is plausible to hypothesize that food would be the determining factor in a constant environmental conditions to explain the growth variation of this species.

Zhang et al. (2010) reported that *H. perlevis* reared in an intensive mariculture water system, showed growth, stationary, and decline stages during the test period. At the end of the experiment, the sponge biomass was less than that at the beginning. This trend has also been observed in a previous laboratory study (Zhang et al., 2005). In an applicative research performed in Mar Piccolo of Taranto (Bioremediation in aquaculture activities through the use of bentic invertebrates - FEP Project 39/OPI/010), the biomass of *H. perlevis* reared in vertical structures along the water column increased in the first months after implantation, to undergo to a significant decrease due to the substrate competition with the other filter-feeder organisms.

Data collected in the present research showed that the total sponge biomass of *H. perlevis* was slightly increased compared to the beginning of the observations. On the whole, the sponge in the frames located in the area closest to the water inlet showed the greatest growth performance throughout the survey year. The sponges positioned in the central area showed evident signs of necrosis until their total disappearance, especially in correspondence with the intake of large quantities of solid food and a decrease in flow water rate, causing the food stagnation in the central part of the drainage conduit, suffocating the sponges. The sponges located farthest from the water inlet area showed a generally low growth rate, probably due to the lower food availability mostly captured by the sponges placed upstream.

The reproductive strategy is mainly determined by phylogeny (Riesgo et al., 2014), but there is a high degree of plasticity correlated to the abiotic conditions of the environment; among these the water temperature is the most studied environmental driver (Reiswig, 1973; Fell, 1976; Maldonado & Young, 1996; Usher et al., 2004; Ettinger-Epstein et al., 2007; Riesgo et al., 2007; Whalan et al., 2007; Mercurio et al., 2007; 2013; Maldonado & Riesgo, 2008; Ereskovsky et al., 2013; Wahab et al., 2014; 2017; Lanna et al., 2014; 2018). In wild populations of *H. perlevis,* the onset of gametogenesis seems to be triggered by the rapid seasonal changes in water temperature. At the Mar Piccolo of Taranto, Gaino et al. (2010) reported that the increase in water temperature promoted female gamete differentiation, which preceded the presence of spermatocysts by one month; the reproductive timing lasted five months, from April to August, and the *sex ratio* was unbalanced, with a female overabundance. A similar trend was also observed in the Thau Pond, where gamete differentiation was triggered by the increase in water temperature and the species showed successive hermaphroditism in which oogenesis preceded spermatogenesis (Diaz, 1973).

In the present study, the course of the sexual cycle was very different from that observed in wild populations, being not possible to recognise in the farmed specimens of *H. perlevis* a true sexual cycle. The occurrence of sexual elements was sporadic throughout almost all the year with very low densities of gametes in the sponge tissue, with the exception of male elements that were subjected to short and fast peaks in frequency in two distinct months. No asexual elements were observed, even though we cannot exclude the occurrence of asexual phenomena, since the sexual or asexual origin of the newly settled sponges, repeatedly detected throught the study period, was not ascertained. It seems quite plausible that for *H. perlevis* living in the drainage channel characterized by constant values of some basic ecological parameters (water temperature, salinity, pH, dark conditions), the sexual phase was an almost continuous process under the control of endogenous factors. According to literature, in sponges, endogenous processes, using a biological clock, are involved in the control of an obligate set of life cycle phases, but the seasonality and length of phases are subject to exogenous factors (Simpson & Fell, 1974; Hill & Hill, 2002; Manconi & Pronzato, 2015).

Finally, the results of the present research not only add further information about the plastic and adaptable life-cycle of *H. perlevis*, but provides precise and usefull indications on the best rearing conditions for this species, that, thanks to its ability to remove pathogenic bacteria and vibrions from the cultured media (Fu et al., 2006; Longo et al., 2010; 2016; Zhang et al., 2010), is potentially usable in integrated multitrophic farming systems for the waste treatment, in contrast to usual technologies with high negative impacts on the whole ecosystems (Done et al., 2015; Chen et al., 2020).

Conclusions

H. perlevis is a cosmopolitan species showing peculiar adaptive strategies to extreme environmental conditions (Juniper & Steel, 1969; Stone, 1970; Cao et al., 2007; Gaino et al., 2010; Longo et al., 2016; 2017); its life cycle was extensively studied in wild populations demonstrating the presence of four stages, linked to the seasonal variations: dormancy, resuscitation, bloom, and decline (Stone, 1970; Cao et al., 2007; Gaino et al., 2010; Zhang et al., 2010). Moreover, several studies demonstrate that *H. perlevis* is viviparous, gonochoric and/or hermaphroditic non-simultaneous in which ovocites preceded spermatogenesis (Sarà, 1961; Stone, 1970; Diaz, 1973; Gaino et al., 2010); the onset of sexual reproduction is triggered by seasonal water temperature variation, starting in late spring and ending in late summer (Stone, 1970; Gaino et al., 2010). Asexual reproduction by means of fragmentation is also important, especially under adverse environmental conditions (Stone, 1970; Gaino et al., 2010). By contrast, very low informations are available about the life cycle of this species reared in mariculture water system (Zhang et al., 2010)

The results of this research demonstrate that the sponge present in the drainage conduit of a marine land-based fish farming plant, constantly living in dark conditions, with a constant water temperature of 18 °C and subjected to a continuous nutritional supply by means of waste water, exhibited a vital physiological state during the whole year, avoiding the stages of decline and long dormancy observed in wild populations. About the sexual reproduction, it has been not possible to recognise in the farmed specimens of *H. perlevis* a true sexual cycle, being the occurrence of sexual elements sporadic throughout almost all the year, with very low densities of gametes. It seems quite plausible that for *H. perlevis* living in the drainage channel characterized by constant values of some basic ecological parameters (water temperature, salinity, pH, dark conditions), the sexual phase was an almost continuous process under the control of endogenous factors, but the lack of the seasonal changes in water temperature does not allow the onset of a real sexual cycle, as happened instead in the wild populations in spring. No asexual elements were observed, even though we cannot exclude the occurrence of fragmentation in the origin of the newly settled sponges, repeatedly detected thought the study period. Moreover the sponge showed marked growth variations along the study period, with the largest biomass increase linked to the variations of some fish farming conditions (reduction of the fish reared biomass, increase of pellet amount available and increase of the waste water flow in the drainage conduit). Further research will be needed to implement knowledge on the best rearing conditions for this species in mariculture facilities in relation to its peculiar life-cycle characteristics, being this sponge excellent candidate in integrated multitrophic farming systems for the waste water treatment thanks to its ability to remove pathogenic bacteria and vibrions from the cultured media (Fu et al., 2006; Longo et al., 2010; 2016; Zhang et al., 2010).

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Figure 1

Plant Diagram of the drainage conduit and the sponge frames allocation; the arrow indicates the wastewater flow direction.

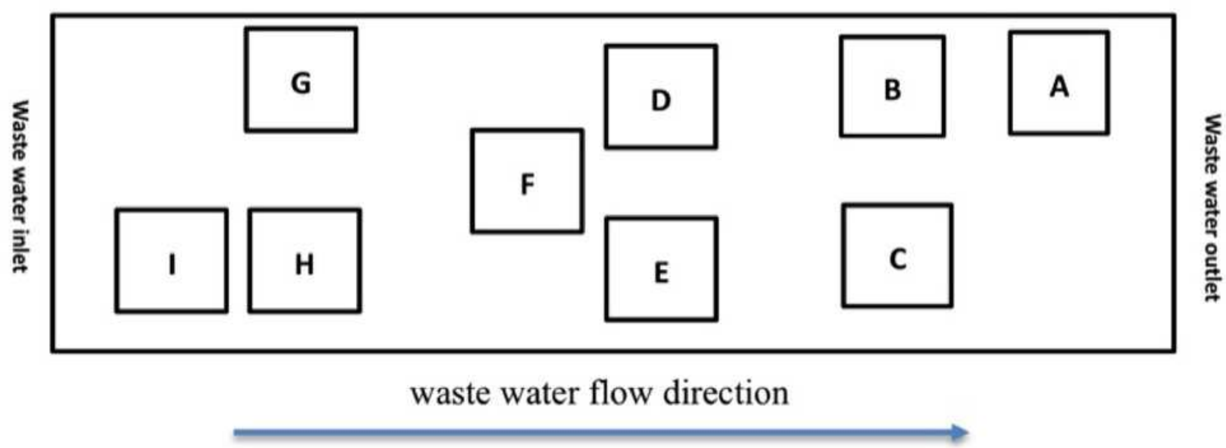

Figure 2

A: the sampling frames positioned along the drainage conduit; B: one sampling frames divided into 4 squares 10x10 cm each side; C: sample of *Hymeniacidon perlevis.*

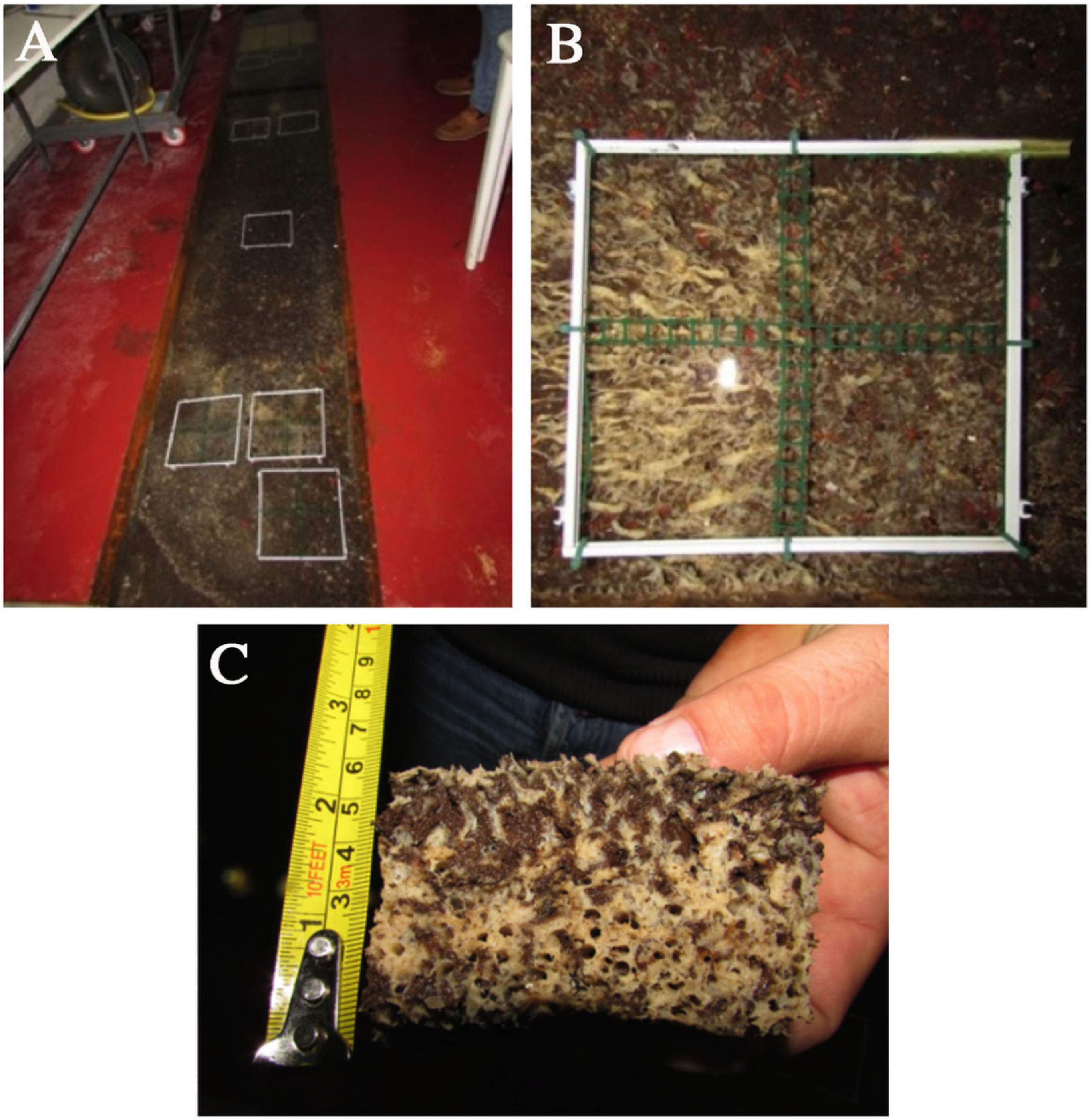

Figure 3

Growth performance (volume in cm^3) of the biomass of *H. perlevis* within the sampling frames from March 2018 to March 2019.

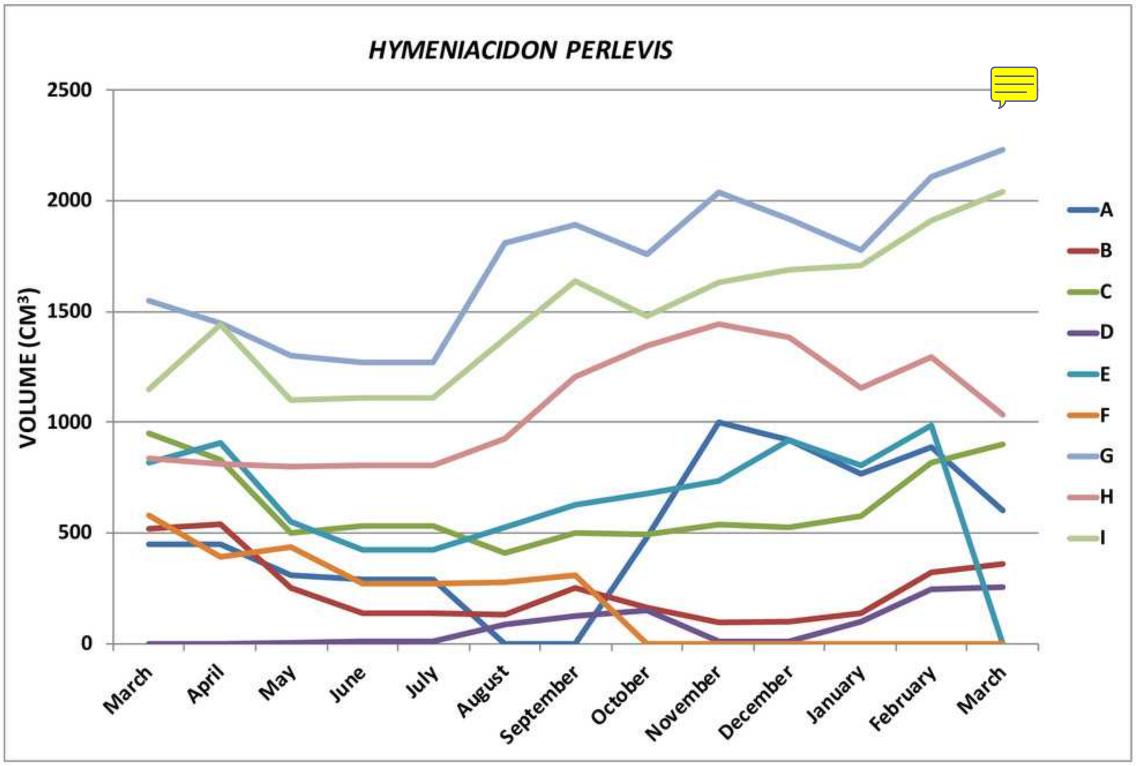
HYMENIACIDON PERLEVIS
2500
2000
1500
1000
500
0
VOLUME (CM³)
March
April
May
June
July
August
September
October
November
December
January
February
March
A
B
C
D
E
F
G
H
I

Figure 4

H. perlevis in frames G, H and I at the beginning (A) and at the end of the observation period (B); particular of the frame G at the end of the observation period (C).

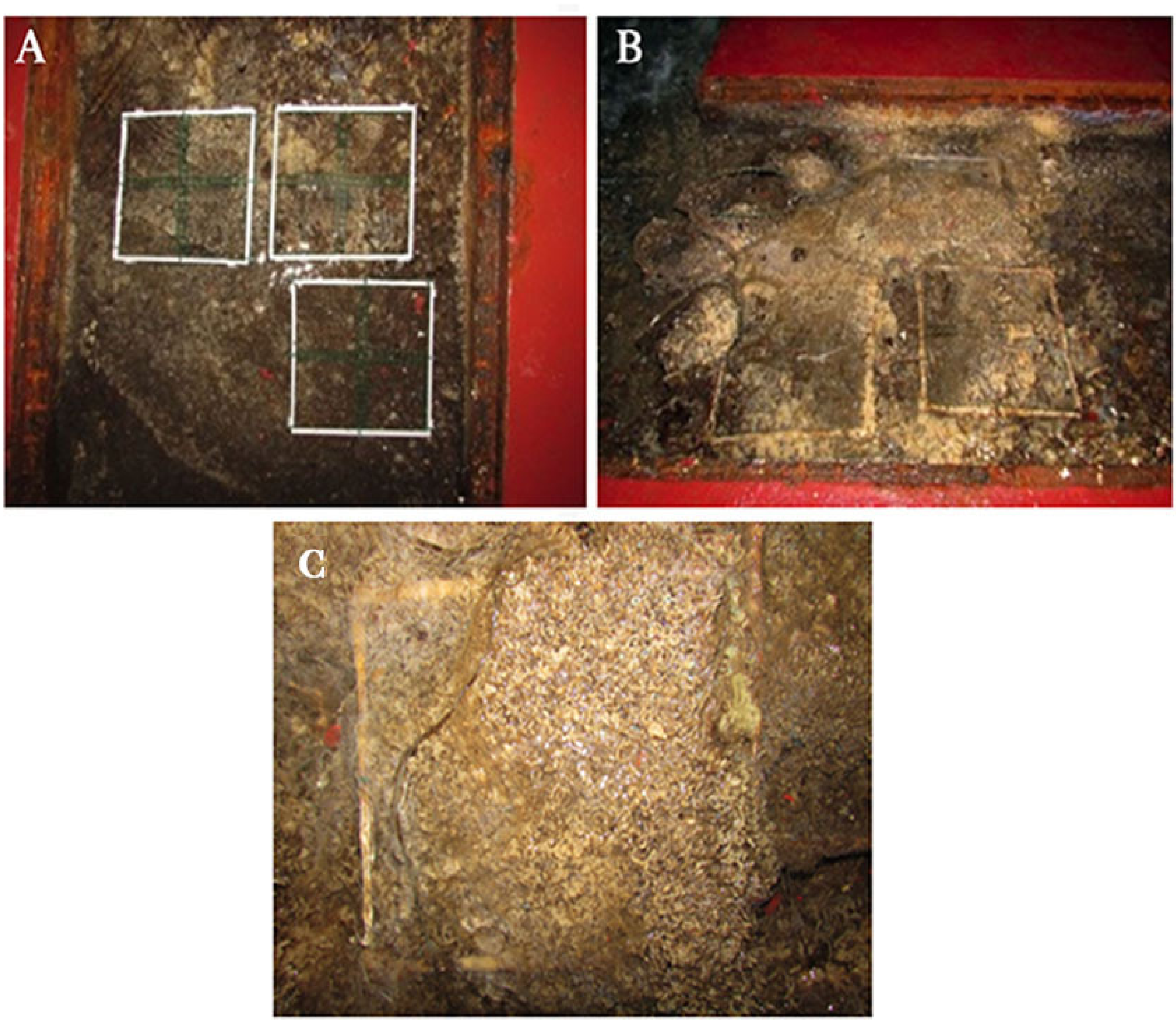

Figure 5

Frame F, where *H. perlevis* shows evident areas in necrosis (A); dry food residues (pellet) next to the necrosis areas (B).

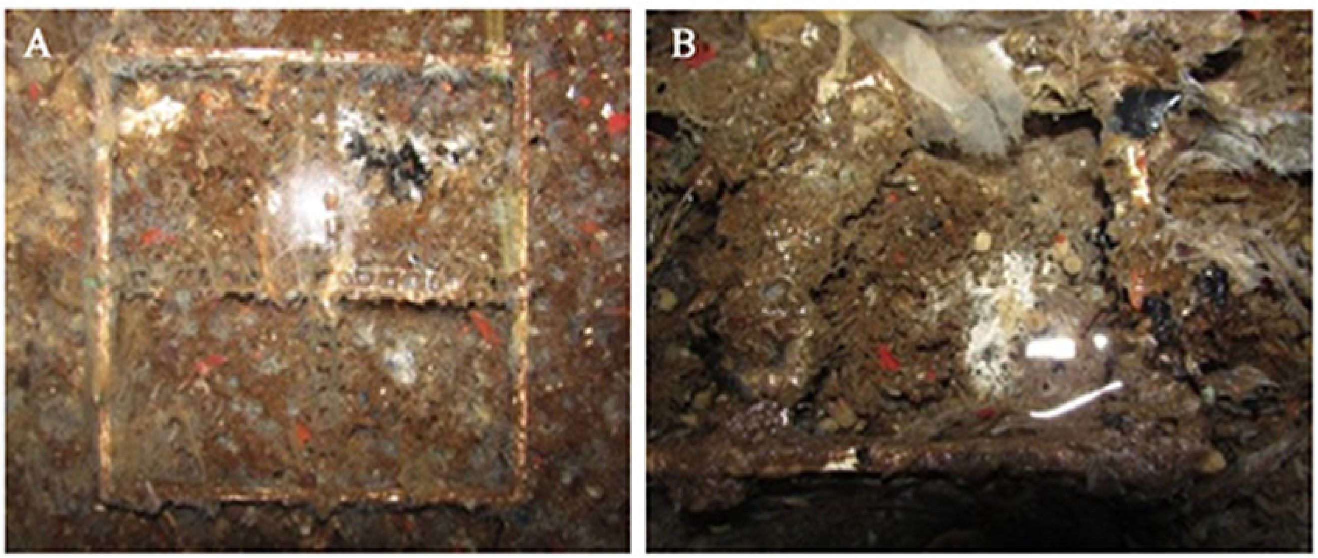

Figure 6

Monthly frequency of fragments of *H. perlevis* with oocytes, spermatic cysts and embryos from March 2018 to March 2019.

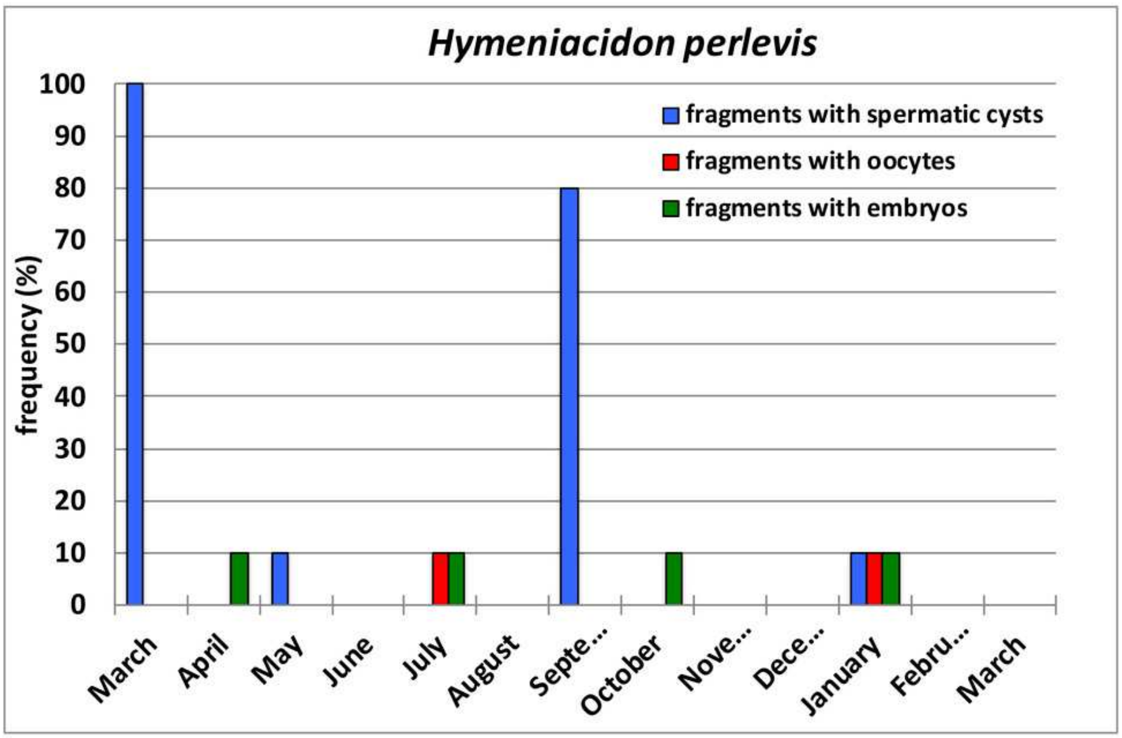
Hymeniacidon perlevis
frequency (%)
100
90
80
70
60
50
40
30
20
10
0
fragments with spermatic cysts
fragments with oocytes
fragments with embryos
March
April
May
June
July
August
Septe...
October
Nove...
Dece...
January
Febru...
March

Figure 7

Diagnostic plots for the GAM analysis: on the left is reported the dispersion of residuals around zero; on the right is reported the response vs. fitted values plot.

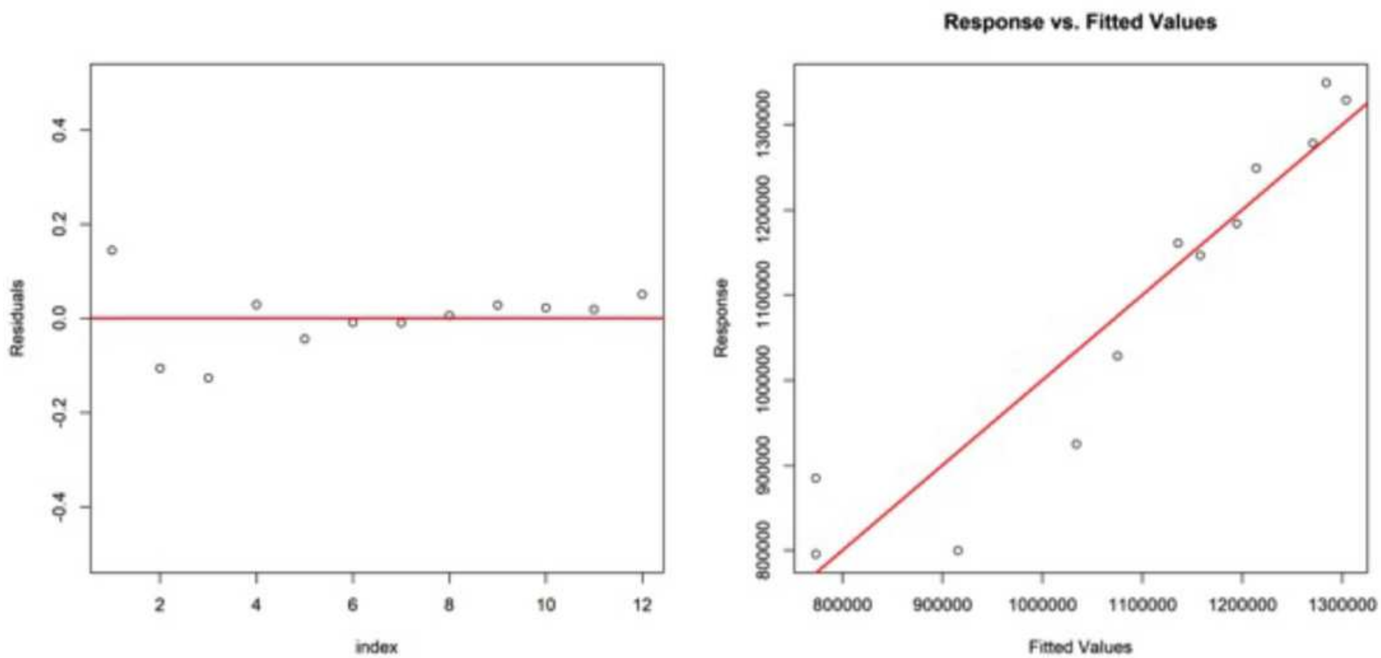

Figure 8

Smoother and linear partial effect for the explanatory variables estimated from GAM model for sponge volume growth.

Left: surface described by the bidimensional spline of biomass and pellet (edf = 6.99); Right: partial linear effect of the water volume variable (water.vol).

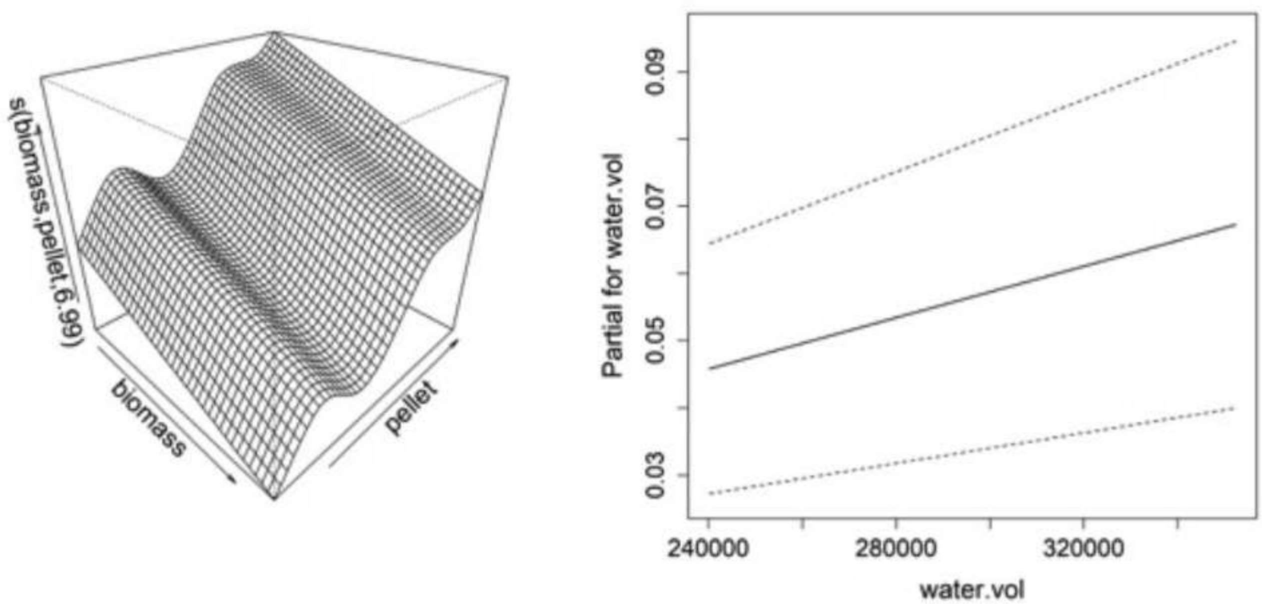

Table 1 (on next page)

Literature data: monthly frequency of *H. perlevis* with oocytes, spermatic cysts and embryos.

"X" is reported as qualitative observation when quantitative data are not available. Indication of vital stage: Do= Dormancy, R=Resuscitation, B=Bloom, De=Decline.

Reproduction	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Gen
Gaino et al. (2010): fragments with spermatic cysts				33%	33%	11%						
fragments with oocytes			22%	55%	55%	44%	33%					
fragments with embryos and larvae				33%	55%	44%	33%					
Diaz (1973): fragments with spermatic cysts				x	x							
fragments with oocytes			x	x								
fragments with embryos and larvae					x	x						
Sarà (1961): fragments with spermatic cysts				50%	85%		50%	25%				
fragments with oocytes					15%			50%				
fragments with embryos and larvae					7.5%							
Stone (1970): fragments with embryos						77%	82%	25%	3%			
Vital Stage												
Gaino et al.(2010)- Mar Piccolo of Taranto (Ionian Sea, Italy)	Do	R	R	B	B	B	De	De	Do	Do	Do	Do
Cao et al. (2007) - Lingshui Bay (Dalian, China)	Do	Do	R	R	B	B	B	De	De	De	Do	Do
Stone (1970) - Langstone Harbour (Hampschire-England)	De	De	De	B	B	B	B	B	De	De	De	De