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Observations on the dorid *Peltodoris atromaculata* Bergh, 1880 (Gastropoda Nudibranchia) along the central-eastern coast of Sicily, Ionian Sea

Andrea Lombardo[1]* & Giuliana Marletta[1]

[1]Department of Biological, Geological and Environmental Sciences, University of Catania, 95124 Catania, Italy
*Corresponding author, e-mail: andylombardo94@gmail.com

ABSTRACT In the present study the seasonality and some biological aspects of the dorid nudibranch *Peltodoris atromaculata* Bergh, 1880 (Gastropoda Nudibranchia) have been investigated. In fact, in literature there is no accordance among authors regarding its life span: annual for some authors or biennial for others. However, there is a general agreement concerning its wide breeding-spawning period. Throughout four years of study (from 2017 to 2020), through underwater visual census in three areas located along the central-eastern coasts of Sicily (Italy), data on *P. atromaculata*'s populations were collected. The only site that allowed to develop a hypothesis on seasonality of this species was Catania. In fact, in this site it was observed that *P. atromaculata* is present all year round with a life span of a year and few months, with different generations which live simultaneously throughout the year. The reproductive period of this species is from February to August and population peaks are from May to September and from September to February. Moreover, it was demonstrated that this species, despite showing different defensive strategies, can be frequently attacked by predators. Therefore, the most typical scars and injuries encountered, have been described.

KEY WORDS Doridina; Dotted sea slug; *Peltodoris atromaculata*; Sea cow; Seasonality; Sicily.

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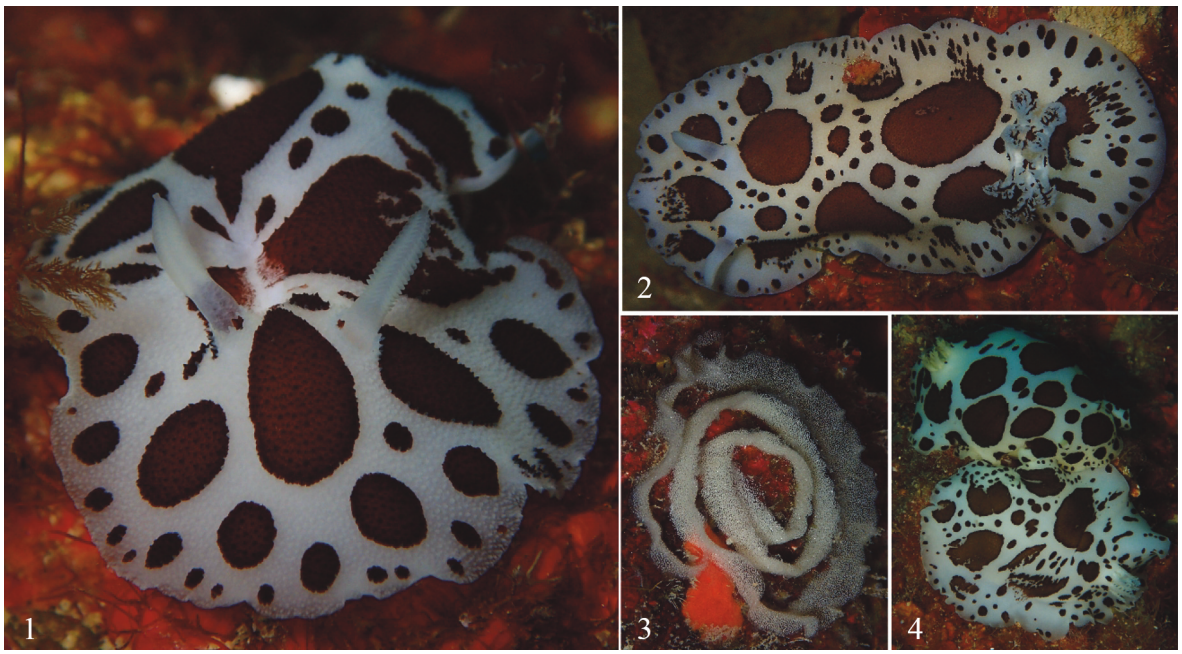
INTRODUCTION

Peltodoris atromaculata Bergh, 1880 is a nudibranch of the family Discodorididae Bergh, 1891, distributed in the Mediterranean Sea and in the eastern Atlantic Ocean (Portugal, Canary Islands and the Azores) (Dayrat, 2010; Trainito & Doneddu, 2014). This species has a typical aspect, characterized by a flattened, oval-shaped body with a mantle covered by conical papillae (Schmekel & Portmann, 1982) (Figs. 1–4). On the notum, which usually is white beige, there are dark patches ranging from brown to black. Normally, these patches are greater in the central part on the notum than the edges of the body (Trainito & Doneddu, 2014). Recently, Dayrat (2010) confirmed that there is a darkening of the notum along a western-eastern Mediterranean gradient, as already observed by Thompson (1985), who stated that specimens from the western Mediterranean Sea exhibit widely dispersed, smaller blotches, which tend to enlarge and coalesce in animals from eastern Mediterranean. On each rhinophore there are about 25 lamellae, the gills are 8 and tripinnate, and both rhinophores and gills can be retracted into the sheaths (Schmekel & Portmann, 1982). The maximum size reported for this species is 120 mm



Figures 1–4: *Peltodoris atromaculata* from the central-eastern coasts of Sicily. Fig. 1: anterior view of a *P. atromaculata* specimen. Fig. 2: dorsal view of another specimen. Fig. 3: *P. atromaculata*'s egg mass. Fig. 4: two specimens during breeding (photos by A. Lombardo).

(Schmekel & Portmann, 1982). Egg masses are laid anticlockwise forming a spiral. The eggs are white yellow with a rounded irregular shape (Haefelfinger, 1961; Schmekel & Portmann, 1982).

Peltodoris atromaculata is a common species in pre-coralligenous and coralligenous assemblages (Haefelfinger, 1961; Avila, 1996, Cattaneo-Vietti et al., 2001). Indeed, as reported by Ros (1975) and Barletta and Melone (1976), this species is abundant from 10 to 40 m of depth, particularly on sciaphilous and deep bottoms, at the entrance of caves, on *Halimeda* J.V. Lamouroux bottoms and on sand-silty bottoms. Nearly always, this species shows a sedentary behaviour on or nearby the sponge Petrosia ficiformis (Poiret, 1789) or the species it feeds on and it is strictly dependant on. This Mediterranean demosponge hosts in its cortex a population of cyanobacteria, with which it realizes a symbiotic relationship (Cattaneo-Vietti et al., 2001). Moreover, it has been demonstrated that the ability of *P. ficiformis* to regenerate its symbiocortex, avoiding necrosis, is not a constraint for *P. atromaculata* population (Cattaneo-Vietti et al., 2001), thus, this latter species has a constant availability of food throughout its life cycle.

According to Ros (1976), the colour of *P. atromaculata*'s patches is similar to that of the sponge, especially from about 10 m of depth, when the red colour of the sponge confounds with the brown blotches. The result of this behaviour is that the nudibranch becomes cryptic to a possible predator, which sees a dark sponge with white blotches, also due to the flattened and sedentary character of *P. atromaculata*. This is an example of disruptive coloration, in which the shape of the animal breaks, confounding with the habitat where it lives. Furthermore, Perrone (1989) hypothesized that the colour pattern of *P. atromaculata* has an aposematic or semaphoric effect, which adverts possible predators of its dangerousness, due to metabolites coming from the diet, in this case consisting of *P. ficiformis* (Castiello et al., 1979). In addition, according to Perrone (1988), the chromatic ornamentation of *P. atromaculata* can have a double purpose: aposematism pattern and a disruptive mimicry, depending if the animal is isolated or in numerous individuals continuously disposed. For this reason, he proposed the definition "destructive aposematism" to surmount an apparent antinomy existing in the literature (Perrone, 1988).

Another defensive strategy of *P. atromaculata* is autotomy: when parts of mantle of this animal,

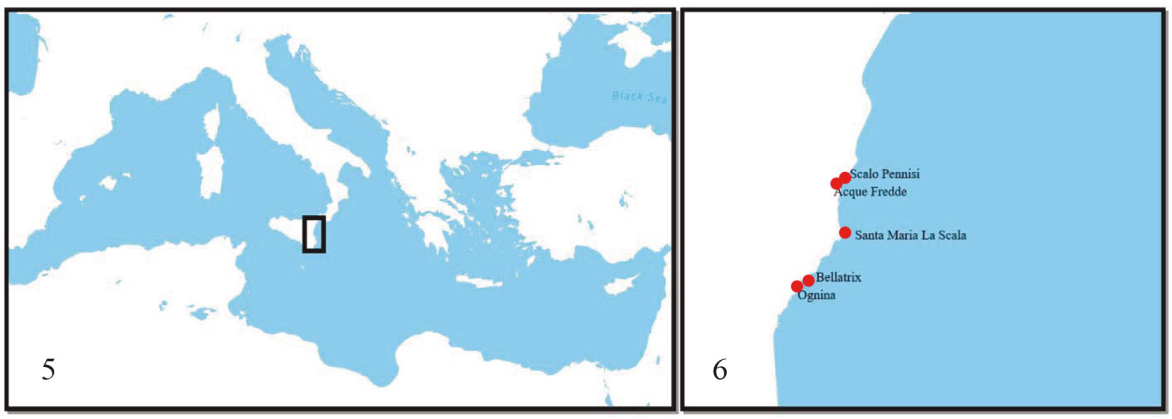
that contains a high number of calcareous spicules, detach from the body. According to Avila (1996), this process is rather related to the natural senescence and, thus, it is not correlated to the defensive systems against predators. Moreover, as reported by Haefelfinger (1961), this phenomenon could also be linked to bad environmental conditions (e.g. change in the level of oxygen and variation in salinity). Furthermore, he observed that several of the collected specimens of *P. atromaculata* presented scars and a high mantle regenerative potential. Finally, another behaviour observed in *P. atromaculata* is the anachoresis: younger specimens hide inside the cavities of *P. ficiformis*. Nevertheless, the adults do not show this behaviour since they are yet protected by their thick tegument (Ros, 1976).

Regarding the seasonality and the life cycle of this species, Ros (1973) reported that *P. atromaculata* has an annual life cycle, while Avila (1996), through laboratory and field observations, stated that this dorid can live more than a year. Moreover, Cattaneo-Vietti et al. (1993) suggest that *P. atromaculata*'s life history could be biennal. Also in Cattaneo-Vietti et al. (2001) it is reported that the whole life cycle of this species is about 24 months. In fact, population with both juveniles and adults have been observed. This occurrence, according to Cattaneo-Vietti et al. (1993), could be explained because the larval metamorphosis happens during a wide period of the year.

Although there is not accordance among authors about the duration of *P. atromaculata*'s life history, most of them reported a wide spawning period: from April to September (Avila, 1996), from July to September (Ros, 1973) and from April to October (Cattaneo-Vietti et al., 1993). Therefore, the aim of this study is to report the seasonality and life history of *P. atromaculata* in the central-eastern coast of Sicily, hitherto an area poorly explored regarding marine Heterobranchia fauna (Lombardo & Marletta, 2020a). Moreover, some specimens with scars and missing parts of the mantle have been observed and here described.

MATERIAL AND METHODS

This study has been conducted throughout four years (2017-2020) in different sites located along the central-eastern coast of Sicily (Italy) (Figs. 5–6). These locations were selected considering the different environmental conditions which characterize each of them. Two sites, Ognina (37°31'50.4"N – 15°07'10.8"E) and Bellatrix (37°32'03.2"N – 15°07'35.2"E), both located in the municipality of Catania, are affected by a heavy human impact. In fact, in these areas, there are several apartment buildings and bathing establishments. In addition, nearby to these sites, there are a harbour and a collector. Therefore, due to proximity and environmental similarities, Ognina and Bellatrix were considered as a single site listed from now as "Catania". Two other sites, Acque Fredde (37°38'15.7"N – 15°10'52.1"E) and Scalo Pennisi (37°38'23.2"N – 15°11'04.6"E), both located in the hamlet of Santa Tecla (in the municipality of Acireale), presented the most



Figures 5, 6: Study area. Fig. 5: Ionian coast of Sicily. Fig. 6: Study sites within the central-eastern coast of Sicily.

natural conditions among the other sites examined in this work. Therefore, these two locations have been considered as a single site listed from now as "Santa Tecla". The last area considered in this study is Santa Maria La Scala (37°36'46.5" N – 15°10'31.4" E) located in the municipality of Acireale. This site presented intermediate conditions between those of Catania and Santa Tecla. Data were collected through underwater visual census with scuba diving. A total of 305 dives (98 in Santa Maria La Scala, 117 in Catania and 90 in Santa Tecla) has been conducted all year round (marine-weather conditions permitting), twice a week, during daylight, between 9–11:30 am. In each scuba dive (in a range of depth between 0-45 m, according to the seabed geomorphology) the same path was conducted and all *P. atromaculata* specimens and their egg masses were photographed with an Olympus TG–4 underwater camera and counted in situ. Moreover, for each specimen, also information on depth and substrate have been annotated. Through Excel, the average number of specimens per month for each site (for every year of study) has been calculated, and graphics have been produced (Figs. 7–8). In addition, for each study area, the minimum and maximum mean depth range were calculated. Because of the lockdown due to Covid-19, it has not been possible to conduct the diving activities in April 2020, so the mean number of *P. atromaculata* specimens for this month has been calculated through the average number of specimens between March 2020 and May 2020.

RESULTS

Through data collection, throughout four years (from 2017 to 2020), a different total average number of *P. atromaculata* specimens has observed in each site. In particular, the location which showed the highest average number of observed specimens was Catania, with a total average number of specimens equal to 4.07. Instead, in Santa Tecla and Santa Maria La Scala, a total average number of specimens, equivalent to 0.20 and 1.11, respectively, has been found.

Consequently, considering the scarce average number of seen specimens in these last two sites, only Catania has been deemed for the data elaboration. In particular, in Fig. 7, the different trends of the average number of specimens for each year are described. In 2017 (blue line) from January to March a decrease of the average number of specimens has been observed. Then, a slight increase has occurred between April and May. After this month, in June, no specimens have been found. Subsequently, there was a peak in July and then, a constant decrease in the average number of specimens has been observed. From November there was a strong rise in the average number of specimens which continues until January of 2018 (red line). During that year, a decrease in the average number of specimens has been observed between January and February and, then, from this month to June there was a strong increase of the population. From June until the end of autumn, a sharp decrease of population happened and

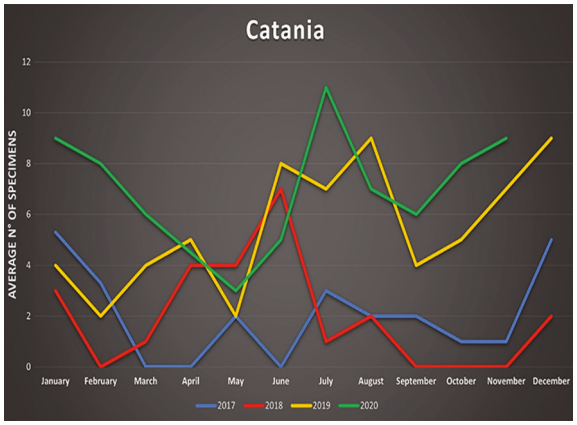


Figure 7. Seasonal trends of the average number of specimens from 2017 to 2020, in the study area of Catania.

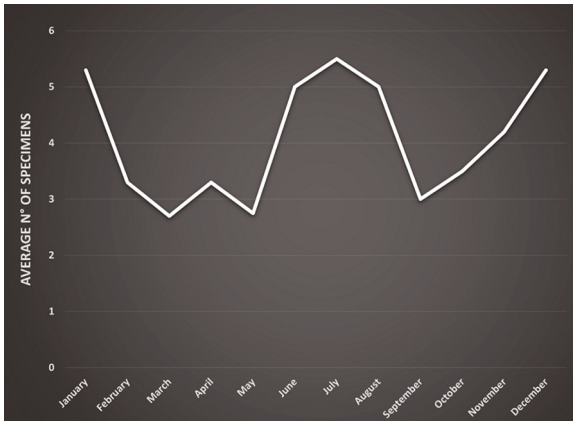


Figure 8. Seasonality of *P. atromaculata* in Catania, obtained by averaging the seasonal trends from 2017 to 2020.

subsequently, from November there was a new increase of the average number of specimens. In 2019 (yellow line), between January and February, a downfall of the average number of specimens occurred, and from this last month to April there was an increase in the population. After a slight decrease between April and May, there was an increase of the average number of specimens until August. Then a strong decline of the population occurred until September, after which there was a strong rise of the average number of specimens until December. In the last year (green line), a constant decrease of the population has been observed from January to May. From this last month until July, a strong increase in the average number of specimens has been notated. Then, until September, a marked decrease of population occurred. Finally, from this last month to November a constant rise of population has been seen. Generally, from 2017 to 2020, in Catania, an increase of the average number of *P. atromaculata* specimens has been notated, passing from an average number of specimens equal to 2.05 in 2017 to 6.95 in 2020 (with a percentage increase of + 245.5%). Moreover, in the site of Catania also the breeding activity and the egg masses of *P. atromaculata* have been observed throughout this study. In particular, during 2017, the egg masses were documented only in July, while no breeding activity has been seen. During 2018, the egg masses were found in May and July, while the breeding activity has been registered in April, June and August. In 2019, the egg masses have been only found in July and reproduction has been observed only in April. Finally, during 2020, the egg masses have been seen in June and the breeding activity has been observed in February, March and July. The egg masses were found on the following substrates: on *Codium bursa* (Olivi) C. Agardh and, more frequently, on encrusting calcareous Rhodophyta.

In Catania, the average depth range in which *P. atromaculata* specimens have been encountered, throughout this study, was 22.6–30.3 m. Instead, the minimum and maximum depth range were 15.8 and 38.5 m, respectively. In Santa Maria La Scala, the average depth range was 20.7–32.2 m and the minimum and maximum depth range were 11.7 and 38.3 m. Finally, in Santa Tecla, an average depth range of 22.2–23.7 m has been observed, while minimum and maximum depth range were 9.6 and 29.2 m.

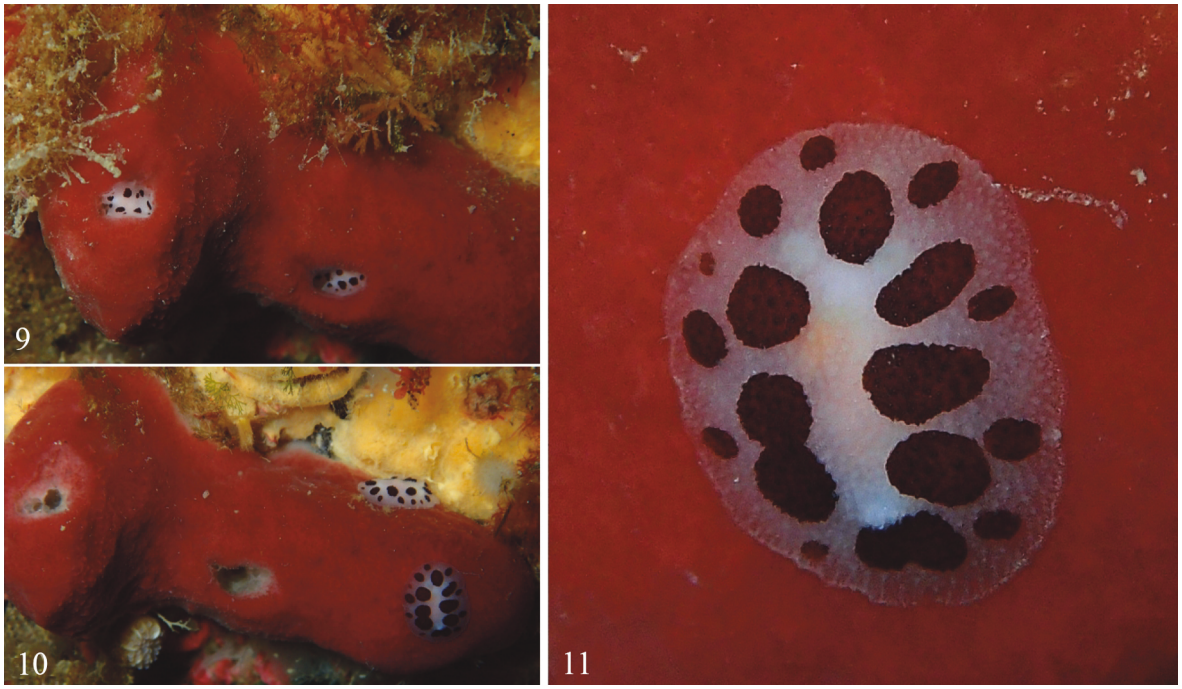
Through data collection, also the substrates, on which *P. atromaculata* specimens were found, have been annotated. The highest number of individuals were seen on *P. ficiformis*, but some specimens were also observed on*: Peyssonnelia* sp., mix of red and brown seaweeds, detritus, *Palmophyllum crassum* (Naccari) Rabenhorst, *Crambe crambe* (Schmidt, 1862), *Dictyota* sp., bare rock, *Bugula* sp., *Haliclona* sp., *Lithophyllum* sp., sand, *Halopteris scoparia* (Linnaeus) Sauvageau, *C. bursa*, *Chondrilla nucula* Schmidt, 1862.

DISCUSSION AND CONCLUSIONS

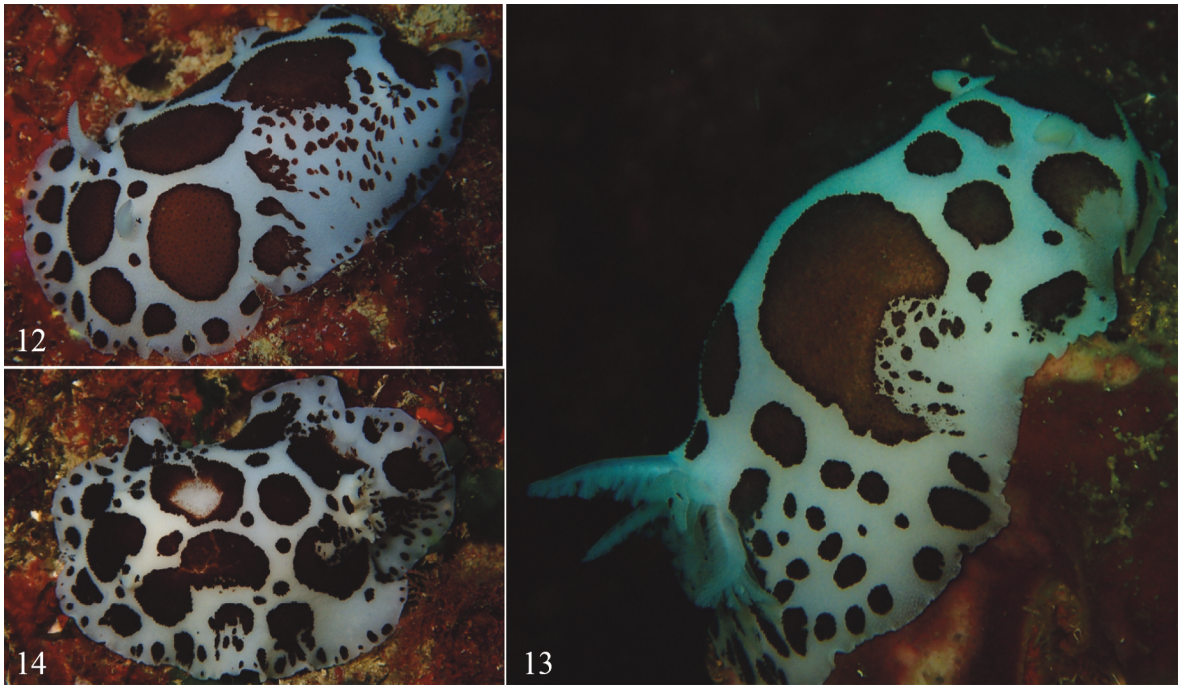
Through data collection, the seasonality of *P. atromaculata* has been studied from 2017 to 2020. Among the study areas, the site with the highest number of specimens was Catania, while that with the lowest number of individuals was Santa Tecla. In literature there are no data on the development type [*sensu* Thompson (1976)] of *P. atromaculata*: planktotrophic, lecithotrophic or direct.

Consequently, the reason of this local difference in the study areas could be only hypothesized. If *P. atromaculata*'s larvae would be planktotrophic, the arrival of the veligers from the southern Mediterranean Sea, as in the case of *Flabellina affinis* (Gmelin, 1791) (Lombardo & Marletta, 2020b), could be possible thus, Catania, being the southernmost site among the studied areas, is that with the greatest recruitment. Consequently, the other areas, located northernmost, receive a lower number of larvae. Instead, if *P. atromaculata*'s larvae would be lecithotrophic, as suggested by Haefelfinger (1961), the veligers, once out of the eggs, spend little time in the water column and almost immediately settle on. Therefore, the population present in a site could self-supply and this could explain the highest number of specimens found in Catania. At the same time, some lecithotrophic larvae could be transferred away from the site of origin by currents in more distant sites (as Santa Maria La Scala and Santa Tecla). Finally, if *P. atromaculata* would be a direct-development species, the population of each site could be self-maintained.

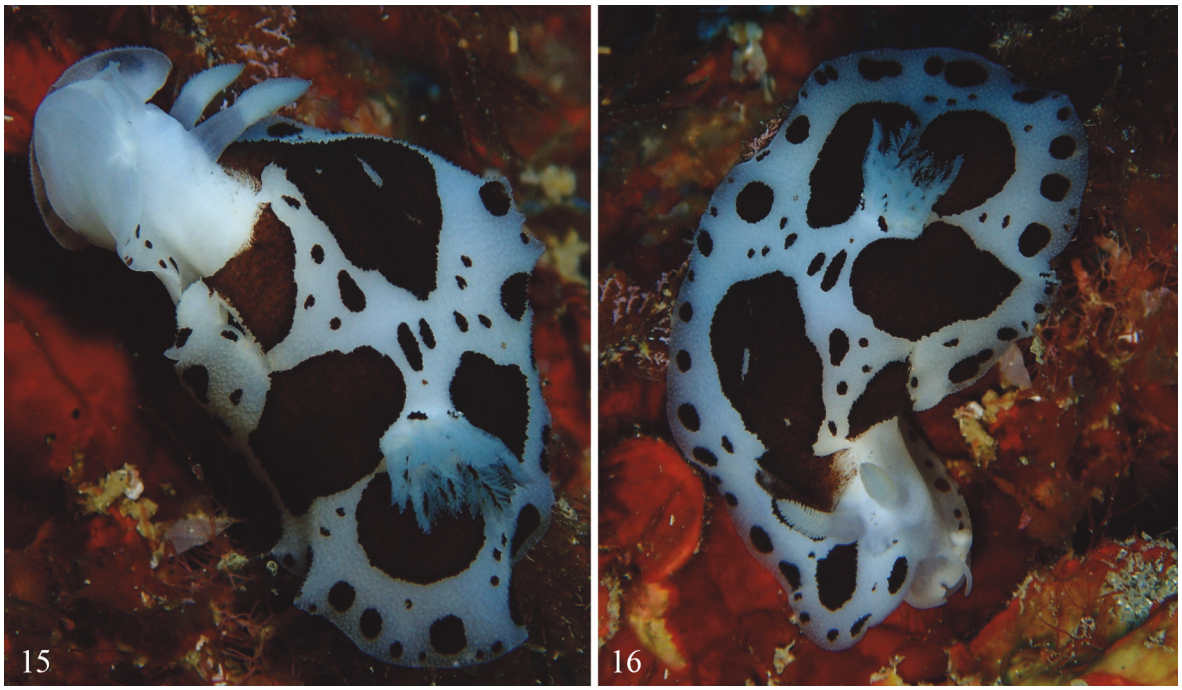
In literature, the life cycle of *P. atromaculata* has



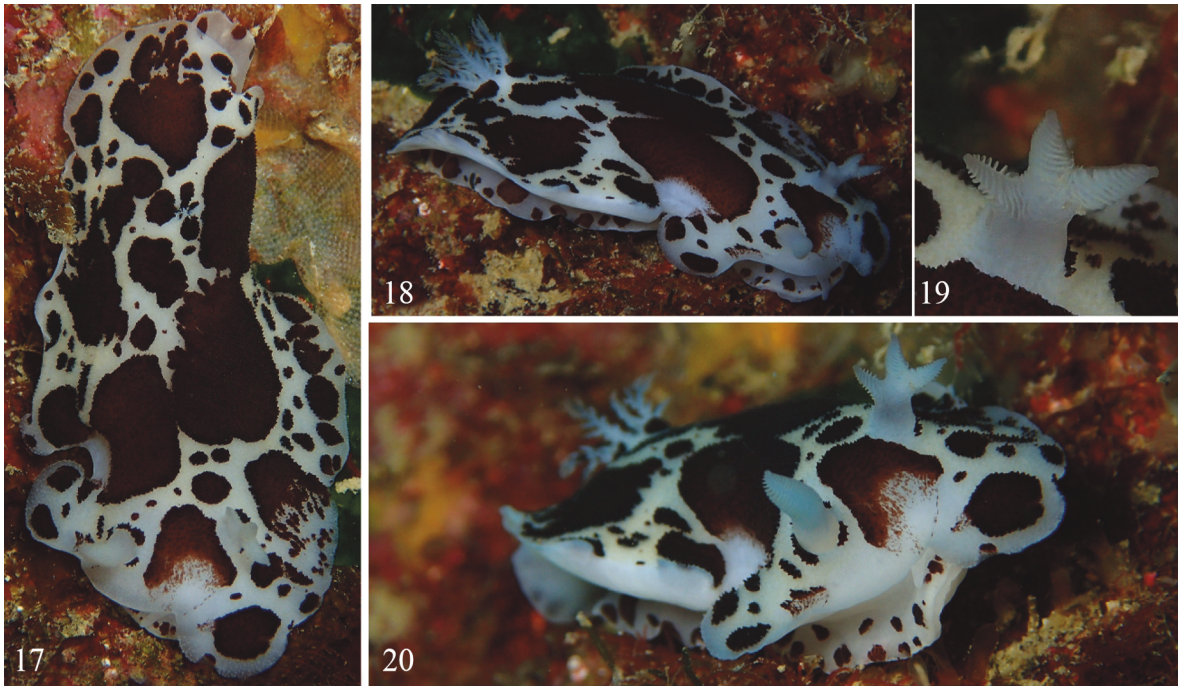
Figures 9–11. The two *Peltodoris atromaculata* followed from October to November 2020. Fig. 9: specimens embedded inside *P. ficiformis*' oscula. Fig. 10: the specimens found on the surface of the sponge with the two holes created by them. Fig. 11: detail of one of the two specimens presenting the red pigmentation in the centre of the notum (photos by A. Lombardo).



Figures 12–14. The most common *Peltodoris atromaculata*'s scars and injuries. Fig. 12: an example of notum restitutions. Fig. 13: two typical bite signs. Fig. 14: specimen presenting an evident sinking of the notum (photos by A. Lombardo).



Figures 15–16: *Peltodoris atromaculata*'s specimen with the left antero-lateral part of the notum completely missing. Fig. 15: lateral view of the injury. Fig. 16: dorsal view of the injury (photos by A. Lombardo).



Figures 17–20. An adult *Peltodoris atromaculata* specimen presenting a tripartite rhinophore, a foot protruded at the back and different signs of predations. Fig. 17: dorsal view of the specimen. Fig. 18: right latero-dorsal view. Fig. 19: detail of the tripartite rhinophore. Fig. 20: anterior view of the specimen (photos by A. Lombardo).

been considered annual (Ros, 1973; Avila, 1996) or biennial (Cattaneo-Vietti et al., 2001). Moreover, the reproductive activity has been reported from April to September (Avila, 1996) or from July to September (Ros, 1973). This long reproductive period is probably due to the constant presence, throughout the year, of the main source of food, *P. ficiformis* (Avila, 1996). According to Ros (1973; 1975) and Avila (1996) during *P. atromaculata*'s life cycle, several generations coexist in the same periods of the year and thus the possibility to encounter specimens with different sizes on the same *P. ficiformis* specimen, is likely. This occurrence could be possible because some adults, after the reproduction and oviposition, would be still alive when first juveniles are already developed (Avila, 1996).

Through the observation of the four annual trends (2017, 2018, 2019, 2020) (Fig. 7) in the site of Catania, a similar seasonal tendency has been noticed. Therefore, a single graph (Fig. 8) has been created by averaging the seasonal trends from 2017 to 2020, to simplify the representation of *P. atromaculata*'s seasonality. From this graph, it may be observed the presence of this species all year round. Moreover, throughout the year, there are two peaks in the average number of *P. atromaculata*'s specimens: the first one occurs between May and September, while the second is from September to February. Probably, the peaks are representative of the periods of the year in which adults coexist with juveniles. It should be highlighted that, through the comparison of different seasonal trends (Fig. 7), the reproductive period of *P. atromaculata* seems to start at the end of February and finish in August. Indeed, all reproductive activities and egg masses have been registered, throughout the years of study, in this period. Probably, since the breeding period is wide (spring-summer), it is likely that during this period there are several reproductive activities. For example, the adults, which reproduce in the first phase of the breeding period (February-April), probably spawn soon after. The individuals born from this first mating phase settle in the population from May-June, cohabitating with few old adults survived after the breeding period of February-April. This grouping of individuals of different ages generates the summer peak (May-September) and, at the end of summer, these old specimens will die. Other adults reproduce between May-June and August. The juveniles, born from this late mating phase, will settle in the population at the end of September. They will coexist with the medium sized individuals, born between February-April (generating the autumn-winter peak), and probably with some old adults, survived from the late mating phase of May-August and which will die between January and February. Therefore, a specimen conceived from a breeding at the beginning of the reproductive period (February-May) will mate in the same period, in which its parents did it. At the same way, an individual born from a breeding in the second mating phase (May-August), in turn will reproduce in the same period, in which its parents did. This hypothesis match to that reported from literature (see above). In the studied *P. atromaculata* population, an annual life cycle or a cycle of one year and a few months, could be very plausible. Moreover, two different generations, each with its breeding period, are present in the area at the same time. It is also probable that some specimens may be able to survive more than a year, generating a life span of about 24 months, as suggested by Cattaneo-Vietti et al. (2001).

Throughout this study, small specimens have been seen inside *P. ficiformis*'s oscula several times. These juveniles, probably, spend the first time of their life within the sponge, then, once developed, remain on the sponge, grazing its surface. In particular, in the last time of this study, 2 small individuals (Figs. 9–11) perfectly embedded each in a P. *ficiformis*'s osculum, have been seen. Both specimens have been constantly observed by the authors from October to November 2020. For almost all this time, these small specimens stayed apparently motionless each in its osculum and, only at the end of November, they have been finally found on the surface of the sponge, having created a hole from which they probably had been exited. The specimens, once out, were about 6 mm long and it was possible to see through their tegument a red pigmentation in the centre of notum. Consequently, as reported by Ros (1975), probably *P. atromaculata* spend its first months of life inside *P. ficiformis*. Indeed, although the adults exhibit several defensive strategies (chemicals, mechanicals and behavioural) against possible predators, in the first time of its life, this species is vulnerable and, thus, it needs protection. Nevertheless, also during the adult phase, *P. atromaculata* is constantly in danger. In fact,

throughout this study, almost all the observed specimens presented various typology of scars and injuries (Figs. 12–20). The most common types found in this study were those defined by Perrone (1992) as "*restituzioni nottali*" (notum restitutions), phenomenon that describes the regrowth of mantle portions, previously lost, which cause the dysregulation of the original pattern of mantle's spots, creating a chaotic set of small points (Fig. 12). Another type of scar observed in several specimens presented a typical bite sign (Fig. 13). Moreover, a found specimen showed an evident sinking of the notum (Fig. 14). Finally, another specimen had the left antero-lateral part of the notum completely missing, revealing a great part of the head (Figs. 15–16). Therefore, although this species presents several defensive strategies, it is subjected by predation during all its life. Probably, these scars could be caused by curiosity of young and inexpert predators [e.g. *Serranus cabrilla* (Linnaeus, 1758), *S. scriba* (Linnaeus, 1758), *Epinephelus marginatus* (Lowe, 1834), *E. costae* (Steindachner, 1878), *Scorpaena notata* Rafinesque, 1810, *S. scrofa* Linnaeus, 1758, *Octopus vulgaris* Cuvier, 1797], which do not know that *P. atromaculata* is inedible. However, through *P. atromaculata*'s aposematic coloration, these predators learn soon to ignore this conspicuous nudibranch.

During the present study, an adult *P. atromaculata* specimen showed a particular aspect (Figs. 17–20): the left rhinophore, in the middle of its length, divided in three distinct parts, each of which presented the usual appearance of a rhinophore. Moreover, in this specimen, contrary to the other observed individuals, the foot protruded at the back and there were different signs of bites on the mantle. Perrone (1992), which reported the drawing of a *P. atromaculata* specimen with 2 left rhinophores, highlighted that this type of anomaly could be a genotypic variation or a consequence of the detachment and subsequent regrowth of a rhinophore. In the specimen found by us, the anomaly of tripartite rhinophore may be due to an incorrect regrowth. In fact, Perrone (1992), underlined that if this anomaly would be caused by a mutation, the animal should present different complete repetitions of the organ, while if the anomaly would be due to an incorrect regrowth, there would be a splitting of it. Another irregularity found by Perrone (1992), and also in our *P. atromaculata* specimen, is that the foot is longer than the mantle and, thus, the latter do not succeed to cover totally it. Therefore, except this last character, all other particular features of the specimen can be reconducted to repetitive attacks of predators.

In conclusion, in the area of Catania *P. atromaculata* is present all year round with a life span of a year and few months, with different generations which live simultaneously throughout the year. The reproductive period of this species is from February to August and population peaks are from May to September and from September to February. The first peak is due to the adults which breed in the first phase of the reproductive period, while the second is due to those which reproduce subsequently. Furthermore, although *P. atromaculata* is a species equipped with several defensive strategies, it is affected by an involuntary or casual predation by different possible predators. Regardless, this dorid nudibranch displays an elevated regenerative potential, which is evident from the different typology of reported scars and injuries.

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