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New data on the seasonality of *Flabellina affinis* (Gmelin, 1791) and *Cratena peregrina* (Gmelin, 1791) (Gastropoda Nudibranchia) in the Ionian Sea, Central Mediterranean

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ABSTRACT *Flabellina affinis* (Gmelin, 1791) and *Cratena peregrina* (Gmelin, 1791) are two common nudibranchs in the Mediterranean Sea. However, there are only a few studies on their seasonality which reported these species principally in summer and in well-lit shallow areas. Instead, through the present study carried out throughout three years (from 2017 to 2019) in three areas sited along the Ionian coast of Sicily (Italy), it has been observed that: 1) both species may be present in any season of the year with a high number of specimens; 2) *F. affinis* in the study areas is more competitive than *C. peregrina*; 3) both species showed a less photophilous lifestyle than that usually reported in literature, since in this study both species were found in a deeper bathymetric range; 4) *F. affinis* and *C. peregrina* could be considered warm-water species and their strong presence in cold seasons might be used as an indicator of the increase in the seawater temperature of the Mediterranean Sea.

KEY WORDS *Cratena peregrina*; *Flabellina affinis*; Ionian Sea; Nudibranchia; seasonality.

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INTRODUCTION

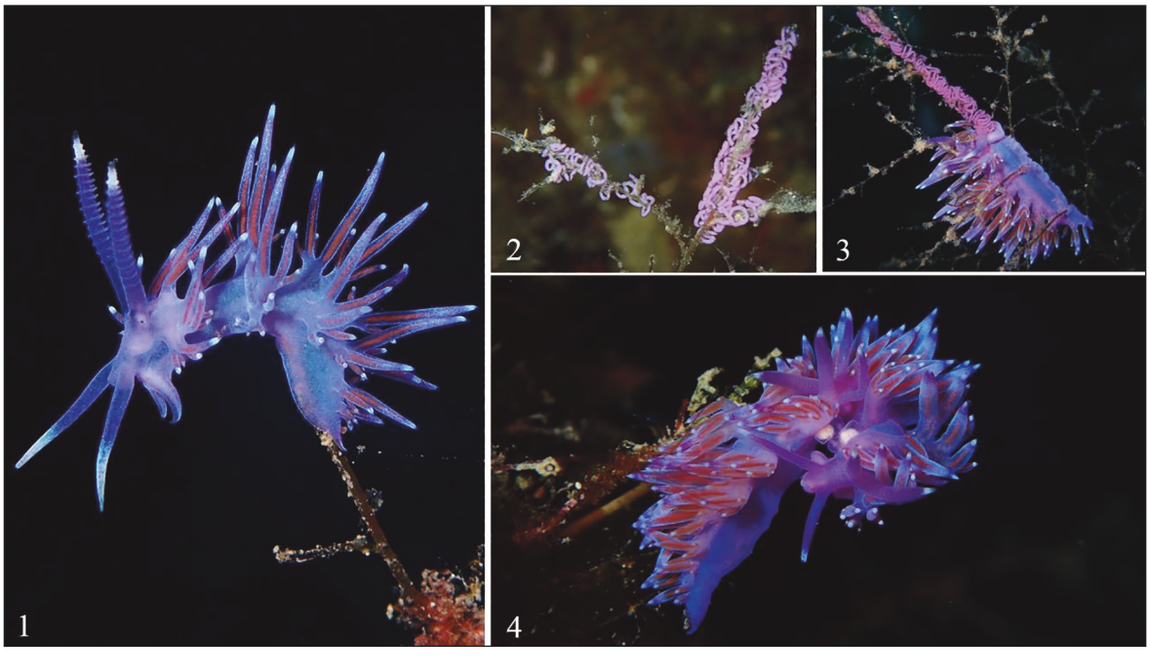
Flabellina affinis (Gmelin, 1791) and *Cratena peregrina* (Gmelin, 1791) are two nudibranchs of the family Flabellinidae and Facelinidae, respectively. *Flabellina affinis* (Fig. 1) is a common species, distributed in the Mediterranean Sea, along the Atlantic African coasts and Canary Islands (Trainito & Doneddu, 2014; Ballesteros et al., 2012–2020). This species is irregularly pink coloured with from six to nine ceratal clusters on each side of the back. Each ceratal cluster originates from a peduncle, which divides in several branches. Rhinophores have from 17 to 36 annulations and oral tentacles are shorter than cerata and almost long as rhinophores (Schulze & Wägele, 1998). The eggs of this species usually are produced in a tangle with a pink-violet colouring (Figs. 2, 3) (Trainito & Doneddu, 2014).

Also *C. peregrina* (Fig. 5) is a common species, distributed in the Mediterranean and along the Atlantic coasts of Iberian Peninsula and in Canary Islands (Trainito & Doneddu, 2014). *C. peregrina* has a white transparent body with cerata arranged in 9 groups on each side of the back. The cerata can be long as the rhinophores, which are smooth and cylindrical. The oral tentacles are twice as long as the rhinophores. From the base of oral tentacles to the base of the rhinophores, on each side of the head there is an evident orange spot (Schmekel & Portmann, 1982). The eggs of *C. peregrina* are produced in a tangle which can

be whitish-pink coloured (Figs. 6, 7) (Trainito & Doneddu, 2014).

Flabellina affinis is a photophilous species, reported on different habitats: among photophilous seaweeds in rocky coasts, in the coralligenous biocenosis, in *Zostera* meadows, on sandy bottoms, on encrusting sponges, rarely on *Posidonia oceanica* (Linnaeus) Delile, 1813 and frequently on hydrozoans [particularly on *Eudendrium ramosum* (Linnaeus, 1758) and *Eudendrium racemosum* (Cavolini, 1785)]. Sometimes, *F. affinis* can be also found in dark cavities and on deep bottoms, even though this species was reported in lighted areas near the surface (Ros, 1975; Barletta & Melone, 1976). Concerning its seasonality, according to Ros (1975), *F. affinis* is abundant during summer along with other species such as *C. peregrina* and *Dondice banyulensis* Portmann & Sandmeier, 1960. Moreover, Ballesteros (1980), in his faunistic study on nudibranchs and sacoglossan of Catalan coasts, reported that *F. affinis* is a prevalently summer species and its principal bathymetric range is between 5–10 m. According to Betti (2011), in the area of Conero (Adriatic Sea), *F. affinis*, with *C. peregrina*, is a species very frequent during summer, which disappears in autumn and reappears the next summer. *F. affinis* usually feeds on some species of hydrozoans such as *Eudendrium glomeratum* Picard, 1952, *E. racemosum*, *E. ramosum* (Haefelfinger, 1960; Ros, 1975; Barletta & Melone, 1976; Schmekel & Portmann, 1982; Hirano & Thompson, 1990; McDonald & Nybakken, 1997; Betti, 2011, Trainito & Doneddu, 2014).

Similarly, *C. peregrina* is a photophilous species which was reported on different types of substrates: on rocky coasts, among seaweeds at 20 m depth, in *P. oceanica* and *Zostera* meadows, at caves' entrance, on sandy bottoms, rarely in coralligenous biocenosis and frequently on *Eudendrium* spp. (particularly on *E. ramosum* and *E. racemosum* colonies in well-lit vertical walls) (Ros, 1975; Barletta & Melone, 1976). Ballesteros (1980) reported that *C. peregrina* is particularly distributed in a range of depth of 0–10 m. According to Betti (2011), in the area of Conero this species is very abundant and particularly frequent on well-lit rocky bottoms, also in shallower waters, on *Eudendrium* spp. colonies and it spawns at the beginning of summer. Furthermore, in this area, *C. peregrina* is



Figures 1–4: *Flabellina affinis* from the central-eastern coasts of Sicily. Fig. 1: lateral view of a *F. affinis* specimen. Fig. 2: *F. affinis* eggs on *Eudendrium* sp. Fig. 3: a *F. affinis* specimen during spawning on *Eudendrium* sp. Fig. 4: two specimens during breeding (photos A. Lombardo).

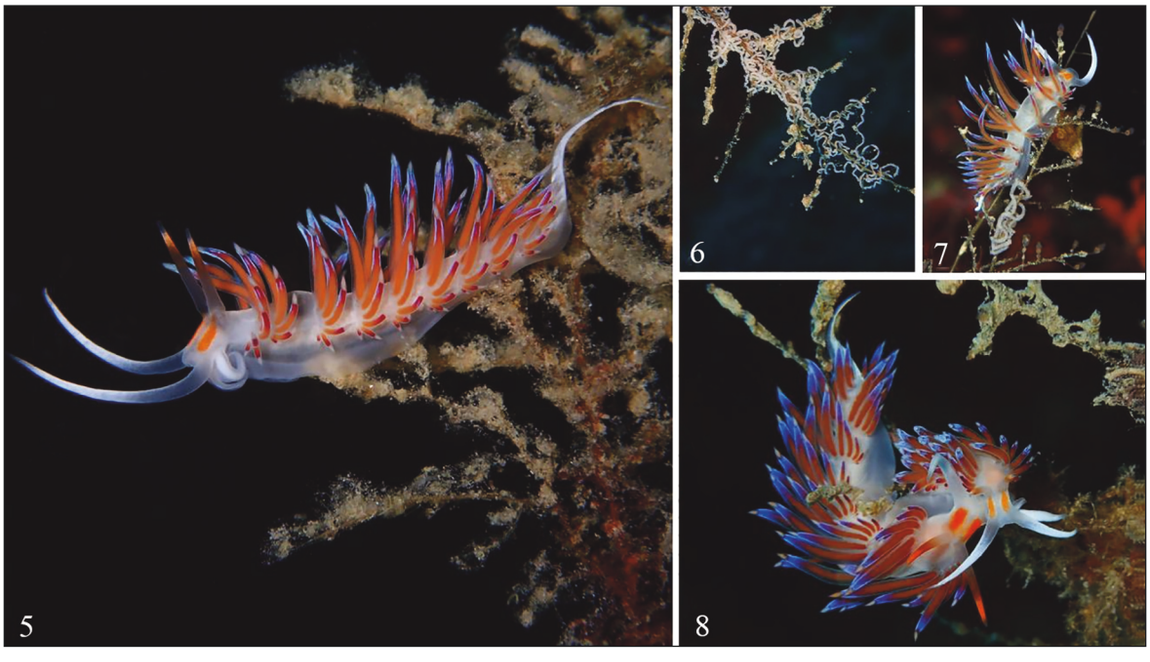
present from the late spring to the beginning of autumn (Betti, 2011).

Cratena peregrina usually feeds on different hydrozoans: *Eudendrium rameum* (Pallas, 1766), *E. racemosum*, *E. ramosum*, *Pennaria disticha* Goldfuss, 1820, *Pennaria* spp. e *Tubularia* spp. (Haefelfinger, 1961; Ros, 1975; Barletta & Melone, 1976; Schmekel & Portmann, 1982; McDonald & Nybakken, 1997; Betti, 2011; Trainito & Doneddu, 2014).

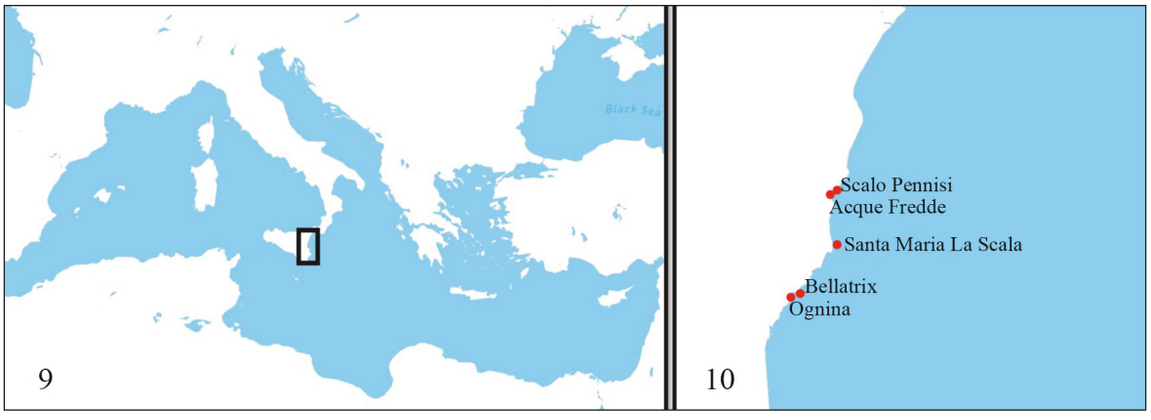
Although *F. affinis* and *C. peregrina* are very common and easily observable species in the Mediterranean Sea, data on their seasonality are scarce, particularly in the Ionian Sea. Indeed, information on the seasonality of these two species came only from the Catalan coasts of Spain (Ros, 1975) and the Adriatic Sea (Betti, 2011). The aim of the present research is to gain knowledge on seasonality of *F. affinis* and *C. peregrina* along the central-eastern coast of Sicily (Ionian Sea).

MATERIAL AND METHODS

This study has been carried out throughout three years (from 2017 to 2019) in different sites located along the central-eastern coast of Sicily (Italy) (Figs. 9, 10). The sites were selected basing on different environmental conditions. 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) are both located in the municipality of Catania and are the most anthrophized sites among the study areas. Since they have the same environmental conditions and are situated close to each other, were considered as a single site, listed from now on as "Catania". Two sites, "Scalo Pennisi" (37°38'23.2"N – 15°11'04.6"E) and "Acque Fredde" (37°38'15.7"N – 15°10'52.1"E), are both situated in the hamlet of Santa Tecla, in the municipality of Acireale. These sites are the least polluted among the study areas. Therefore, since they have similar environmental conditions and are close to each other, they have been considered as a single site and listed from now on as "Santa Tecla". Finally, the last study station was Santa Maria La Scala (37°36'46.5" N – 15°10'31.4"E), in the municipality of Acireale, which has intermediate conditions between those of Catania and Santa Tecla and, thus, was considered



Figures 5–8: *Cratena peregrina* from central-eastern coasts of Sicily. Fig. 5: lateral view of a *C. peregrina* specimen. Fig. 6: *C. peregrina* eggs on *Eudendrium* sp. Fig. 7: a *C. peregrina* specimen with eggs on *Eudendrium* sp. Fig. 8: two specimens before breeding (photos A. Lombardo).



Figures 9, 10: Study area. Fig. 9: eastern coast of Sicily. Fig. 10: study sites within the central-eastern coast of Sicily.

individually. Both Santa Maria La Scala and Santa Tecla present several springs due to the flow of freshwater from the Etna to the sea (Ferrara, 1977; Catra et al., 2006). Data were collected through underwater visual census with scuba diving. In particular, a total of 224 dives has been realized: 74 in Santa Maria La Scala, 83 in Catania (Bellatrix and Ognina), 67 in Santa Tecla (Scalo Pennisi and Acque Fredde). Each scuba dive (in a range of depth of about 0–45 m, according to the seabed geomorphology) was conducted all year round (marine-weather conditions permitting), twice a week, during daylight, between 9–11:30 am. For each site, the same path was followed and *F. affinis* and *C. peregrina* specimens were photographed with an Olympus TG–4 underwater camera and counted in situ. For each station and for each species, the average minimum depths and the average maximum depths, in which the specimens were encountered, have been obtained.

The seasons were considered as follows: winter (December, January, February), spring (March, April, May), summer (June, July, August) and autumn (September, October, November). Through data collection, the demographic trend of both species was assessed. In addition, for each station, specific density was evaluated as mean number of individuals/diving number per site. The experimental design adopted was completely randomized and replicated four times. Data were subjected to analysis of variance (ANOVA). Mean comparisons were performed according to Tukey Minimal Difference (MDS).

RESULTS

Catania (Figs. 11, 12)

Catania was the site with the highest average number of *F. affinis* specimens, throughout the three years of study. During 2017, winter represented the season with the maximum average number of found specimens (27.83). The other seasons presented the same trend (f), which was different than the winter trend. Indeed, between winter and the other seasons, there was a decrease of -78.87% ($p<0.01$) in the average number of specimens. During 2018, each season presented a different trend. In fact, winter had the highest average number of specimens (36), while autumn was the season with the lowest average number of specimens (5). Between these seasons there was a reduction of -86.11% ($p<0.01$). In spring, there was a reduction in the average number of specimens, while in summer there was a slightly increase (21.66). During 2019, there was the most stable seasonal trend than the previous years. During this year, winter was the season with the highest average number of specimens (19.66), while autumn was the season with the lowest average number of specimens (8.66). Between winter and autumn, there was a decrease of -55.95% ($p<0.01$). Spring and summer presented the same trend (e). In Catania, *F. affinis* specimens were found at an average depth-range of 16.5–32.1 m. Concerning the breeding and spawning activities (Figs. 3, 4), during 2017 they were observed in any season of the year. In 2018, the eggs were found all year round, while the breeding activity was

documented in winter, spring and summer. In 2019, the eggs were seen throughout all year, while the breeding activity was observed in summer and autumn.

Regarding *C. peregrina*, in this site the seasonal trends were all different in each year of study, with an almost stable presence of a low average number of individuals. During both 2017 and 2018, a rather steady and similar population trend has been observed, with the exception of spring 2017 in which the lowest average number of specimens has been found (1.00). Indeed, between winter and spring 2017, there was a decrease of -75.96% ($p<0.01$) of the average number of specimens. During 2019, contrary to the previous years, winter presented the least average number of individuals. In this year, spring and summer showed a similar trend with a slightly higher average number of specimens. In 2019, autumn had the highest average number of individuals among all year of study (9.66). Between summer and autumn, there was an increase of 190% ($p<0.01$). In the site of Catania, *C. peregrina* specimens were averagely found in the bathymetric range of 9.7–25.8 m. With regard to *C. peregrina* eggs (Fig. 6–7), they were found during 2017 in summer and autumn, in 2018 they were observed in winter, summer and autumn, in 2019 they were documented in all seasons. For this species, the breeding activity (Fig. 8) was seen only in the winter 2018.

Santa Maria La Scala (Figs. 13, 14)

Santa Maria La Scala was the site with an average number of *F. affinis* specimens which was intermediate between the values observed for Catania and Santa Tecla. During 2017, winter was the season with the highest average number of

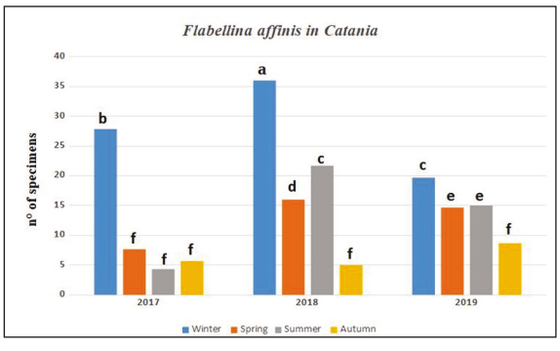


Figure 11. Oscillations in the average number of *F. affinis* specimens found in Catania during the three years' trial. Different letters indicate differences at $p<0.01$.

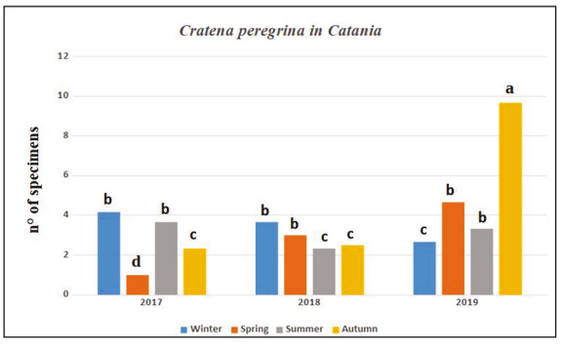


Figure 12. Oscillations in the average number of *C. peregrina* specimens found in Catania during the three years' trial. Different letters indicate differences at $p<0.01$.

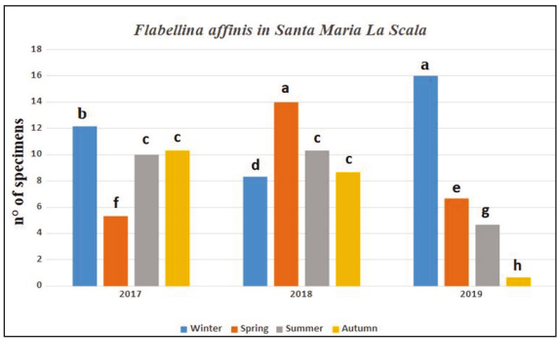


Figure 13. Oscillations in the average number of *F. affinis* specimens found in Santa Maria La Scala during the three years' trial. Different letters indicate differences at $p<0.01$.

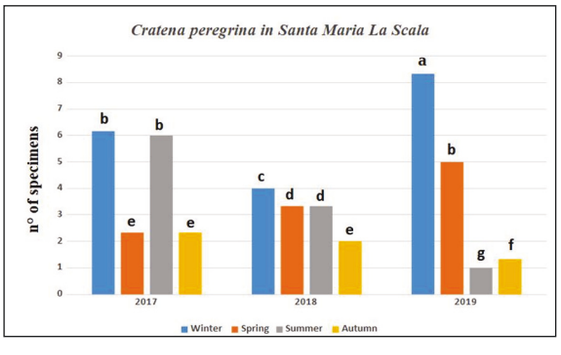


Figure 14. Oscillations in the average number of *C. peregrina* specimens found in Santa Maria La Scala during the three years' trial. Different letters indicate differences at $p<0.01$.

found individuals (12.17). Summer and autumn had the same trend, similar to the winter trend. Instead, spring was the season with the lowest average number of specimens (5.33). Between winter and spring there was a decrease of -56.20% ($p<0.01$). Oppositely, during 2018, the season with the greatest average number of individuals was spring (14.00), while that with the lowest average number of specimens was winter (8.33). Between winter and spring there was an increase of 68.06% ($p<0.01$). As in 2017, also in 2018, summer and autumn had the same trend. Instead, during 2019, a sharp decline of -95.81% in the average number of *F. affinis* specimens occurred between winter and autumn. In Santa Maria La Scala, *F. affinis* specimens were averagely found in a bathymetric range of 19.6–34.8 m. In this study area, the breeding and spawning activities were documented in all seasons during 2017. In 2018, the eggs were found all year round and the breeding activity was observed in winter and spring. During 2019, the eggs were found in spring, summer and autumn and no breeding activity was observed.

In Santa Maria La Scala, during each year of study, the seasonal trends of *C. peregrina* were different. In 2017, two main trends were observed: one identical for winter and summer (b) and another equal for spring and autumn (e). Each passage from trend (b) to trend (e) was signed by a decrease of -62.17% ($p<0.01$). During 2018, there was a more stable trend of the average number of *C. peregrina* specimens with a decrease from winter to autumn of -50% ($p<0.01$). During 2019, there was a winter peak (8.33) and sharp decline in summer (1.00) of the average number of specimens. From winter to summer, a decrease of -87.99 % ($p<0.01$) was observed. In this site, on average, *C. peregrina* specimens were observed in a depth-range of 12.4 –33.5 m. Furthermore, *C. peregrina* eggs were found in all seasons during both 2017 and 2018, while throughout 2019 they were seen in winter, spring and summer.

Santa Tecla (Figs. 15, 16)

Santa Tecla was the study area with the lowest average number of *F. affinis* specimens (4.86). During 2017, the seasons with the highest values of the average number of specimens were summer and autumn (~7.40), while the season with the lowest average number of found specimens was spring (1.00). Between spring and summer-autumn there was an increase of 641% ($p<0.01$) in the average number of *F. affinis* specimens. During 2018, there was a more stable seasonal trend and only in spring there was a slight decrease. Instead, during 2019, almost all seasons, except for winter and autumn, presented different trends. Between winter and summer, the season with the greatest average number of specimens, there was an increase of 200.42% ($p<0.01$). In Santa Tecla, on average, *F. affinis* specimens were found in the bathymetric range of 16.6–26.1 m. In this site, during 2017, *F. affinis* eggs were found in spring and autumn. In 2018, the eggs were observed throughout all seasons, while the breeding activity was documented only in winter. During 2019, the eggs were found in spring, summer and autumn.

In Santa Tecla, during each year of study, a different seasonal trend of *C. peregrina* has been observed. During 2017, there was an identical trend for winter and summer (d), and two different trends for spring and autumn. Between winter and spring there was a decrease of -47.31% ($p<0.01$), while between summer and autumn there was a reduction of -66.66% ($p<0.01$) in the average number of specimens. During 2018, each season presented a different trend of the average number of *C. peregrina* individuals. After a decrease of -91.75% ($p<0.01$) occurred between winter and spring, the season with the lowest average number of found specimens in this site, there was an increase of 2830% ($p<0.01$) in autumn, the season with the highest average number of found specimens. Instead, during 2019, there were two distinct trends: one equal for winter and summer (e) and another identical for spring and autumn (b). Between these trends there was an increase of 154.62% ($p<0.01$) in the average number of specimens. In Santa Tecla, *C. peregrina* specimens were averagely found in the bathymetric range of 11.4–19.6 m. Regarding *C. peregrina* eggs, they were found during 2017 only in summer, during 2018 in summer and autumn and in all seasons of 2019.

DISCUSSION

In the present study the seasonality of two common eolidacean nudibranchs, *F. affinis* and *C.*

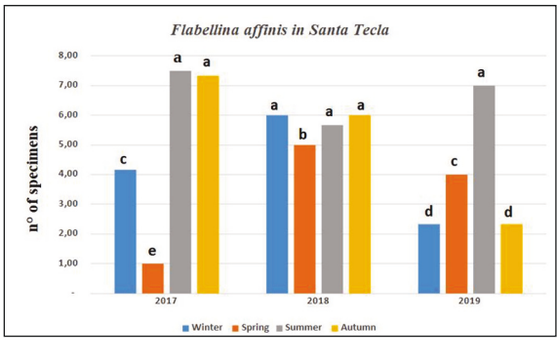


Figure 15. Oscillations in the average number of *F. affinis* specimens found in Santa Tecla during the three years' trial. Different letters indicate differences at p<0.01.

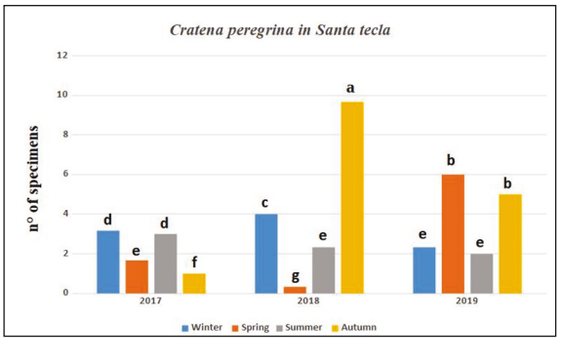


Figure 16. Oscillations in the average number of *C. peregrina* specimens found in Santa Tecla during the three years' trial. Different letters indicate differences at p<0.01.

peregrina, has been investigated throughout three years (from 2017 to 2019), in three areas along the central-eastern coast of Sicily. During the data collection, it has been observed that these species were present in all seasons and years. Probably, the presence of *F. affinis* and *C. peregrina* all year round could be related to the presence, in all study areas, of *E. racemosum* and *E. glomeratum*, which alternate during the year. In fact, according to Bavestrello et al. (2006), *E. racemosum* colonies are active from April and are fertile during the summer, degenerating in the autumn, while *E. glomeratum* is a winter species that appears in mid-October and decreases until April (Boero, 1984). Consequently, since these hydrozoans constitute the diet of both *F. affinis* and *C. peregrina*, these nudibranchs may have the food resources available all year round. Moreover, the presence in some seasons of a higher or lower number of specimens of these species, could depend on a major or minor larval recruitment. Indeed, according to Clark (1975), the sudden appearances of population often are due to the arrival of large numbers of allochtnous-produced larvae, that is related to critical temperatures that stimulate settling and metamorphosis. Furthermore, the decrease in the number of specimens of these species could also depend on the presence of predators. In particular, during 2019 in the site of Santa Maria La Scala, we observed a strong decrease in the populations of both *F. affinis* and *C. peregrina,* concomitantly with the increase in the number of specimens of another nudibranch species, *D. banyulensis* in the same bathymetric range of the examined species (personal observations). Indeed, *D. banyulensis* is a predator of other nudibranchs and hydrozoans (Betti, 2011). Consequently, *F. affinis* and *C. peregrina* populations in this station during 2019 may have been decimated by competition and predation caused by *D. banyulensis*.

Generally, in all study areas, the number of *F. affinis* specimens was greater than the number of *C. peregrina* specimens. There are two main hypotheses that could explicate this trend: in the study sites *F. affinis* might be a more competitive species than *C. peregrina,* otherwise the larval recruitment of *F. affinis* is higher than the recruitment of *C. peregrina.* Furthermore, it has been observed that the number of *F. affinis* specimens, increase southwards and, thus, Catania was the study area with the highest average number of individuals. This event could depend on the fact that allochtnous-produced larvae of *F. affinis* might be carried through the powerful Algerian Current (Di Silvestro et al., 2010), coming from the southern Mediterranean and thus, Catania, among the studied areas, is the site with the greatest recruitment. The same behaviour was not observed for *C. peregrina*, whose average number of specimens was almost identical for each site. However, we believe that generally the allochtnous-produced larvae of both species could be transported by the modified Atlantic water (MAW) flow from the western Mediterranean Sea otherwise through the Levantine intermediate water (LIW) flow form the eastern Mediterranean Sea (Pirkenseer, 2013). Through data collection, it has been seen that the *F. affinis* depth-range begins deeper than the bathymetric range of *C. peregrina*, which it has also been observed in shallower waters. These data are different than those reported in the

literature cited above (Ros, 1975; Barletta & Melone, 1976; Ballesteros, 1980; Betti, 2011), which stated that the presence of these nudibranchs is principally in shallower waters, in well-lit areas. Furthermore, differently from Betti (2011) who reported the presence of both species particularly in summer in the area of Conero (Adriatic Sea), we noticed that both species in the investigated areas can be present with peaks in any seasons of the year. This different trend could be explained by two hypotheses. On one hand, since Sicily belongs to the southern sectors of the Mediterranean Sea, the seawater temperature is usually warmer than the Adriatic Sea. Indeed, in the Ionian Sea the seawater temperature fluctuates between 14–23°C, while in the Adriatic Sea the seawater temperature ranges from 11°C to 23°C (Lejeusne et al., 2009). Therefore, since in the cited literature (Ros, 1975; Barletta & Melone, 1976; Ballesteros, 1980; Betti, 2011) *F. affinis* and *C. peregrina* are present principally during summer, they seem to be warm-water species. Consequently, in the Ionian coast of Sicily these species could settle in any period of the year, while in the Adriatic Sea the settlement might occur in summer, because of the colder winter seawater temperature. On the other hand, in the last decades, we are experiencing a general increase in the seawater temperature of the Mediterranean Sea, caused by the global warming (Bianchi et al., 2018). In fact, a 30-year data set (1974–2005) from the Spanish Catalan coast, revealed a clear warming trend at four different depths from the surface to 80 m, with an impressive warming of 1.4 °C at 20 m (Lejeusne et al., 2009). Also in the Aegean Sea, satellite sea surface temperature data from the last 20 years showed a significant 1 °C warming trend (Lejeusne et al., 2009). Therefore, since *F. affinis* and *C. peregrina* are probably warm-water species, the presence of them in any season of the year could be an indirect indicator of the warming of the Mediterranean Sea.

In conclusion, through the present study new data on the seasonality and lifestyles of *F. affinis* and *C. peregrina* along the central-eastern coast of Sicily has been reported. These results were interesting since reported information on these two species different from the cited literature:1) both species along the Ionian coasts of Sicily may be present in any season of the year with a high number of specimens; 2) *F. affinis* along the Ionian coasts of Sicily is more abundant, and probably more competitive, than *C. peregrina*; 3) the two species showed a less photophilous lifestyle than that usually reported in literature, since in this study both were found in a deeper bathymetric range; 4) if our hypotheses are correct, *F. affinis* and *C. peregrina* are warm-water species and their strong presence in cold seasons might be used as an indicator of the increase in the seawater temperature.

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