



Reproductive biology of *Ophelia barquii* (Annelida, Opheliidae) along the Salento Peninsula (Mediterranean Sea, South Italy)

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Abstract

This study investigates two populations of a polychaete identified as *Ophelia barquii* Fauvel, 1927, collected in two beaches from the Adriatic (Alimini beach) and Ionian (Gallipoli beach) coasts from the Salento Peninsula. Each population was analyzed monthly from April 2017 to March 2018. In both beaches, populational density decreased from April to August with a complete absence of worms from August to the following January. We hypothesize that this trend results from horizontal migration of individuals for reproductive purposes. Sexual maturation was asynchronous between the two populations, with individuals becoming ripe earlier in Alimini than in Gallipoli. Significant differences in body size were recorded between the two populations, with specimens larger in Gallipoli than in Alimini, suggesting that they could belong to different species. However, life-cycle studies performed under laboratory conditions showed that both populations follow a similar developmental path and can cross-fertilize. The larval development featured a very short period of pelagic life, the shortest known so far for any species of *Ophelia*.

Keywords Distribution · Population dynamics · Reproduction · Larval development · Opheliid · Ionian · Adriatic Sea

Introduction

Species of the genus *Ophelia* Savigny, 1822 are presumed to play an important ecological role in intertidal, shallow sandy substrates exposed to high wave energy (Zaitsev 2012). To date, 32 species are known within the genus, most of them reported for the Atlantic area (Bellan and Dauvin 1991). *Ophelia bicornis* Savigny, 1822, the type species, was described from the Mediterranean Sea, but later presumed to have a worldwide distribution. It appears highly sensitive to grain size (Papageorgiou et al. 2006), probably due to the high specificity of larvae to sediment texture (Wilson 1948a).

According to Simboura and Zenetos (2002), it is also reported to live in clean waters and hence considered a sensitive taxon for the BENTIX Ecological Quality Index. *Ophelia bicornis* has a commercial interest, although only documented in the gray literature, due to its use as fish bait for leisure fishing in Turkey (Dağlı et al. 2015) and Italy.

Opheliids are gonochoric, and breed once per year, releasing a large number of gametes. They have a synchronized gametogenic cycle and spawning activity with simultaneous discharge by all the individuals from a given population (Giangrande 1997). Eggs of *O. bicornis* are located in the coelomic cavity; mature eggs usually appear disk-shaped. Fertilization is external, and development is indirect with the presence of a pelagic larval stage. According to Wilson (1991), species belonging to the genus *Ophelia* release their gametes at shallow depths over the sand, from where they are swept away by the tides or waves into the water column. The few species of *Ophelia* investigated as yet have a quite short larval phase (Wilson 1948a; Riser 1987), although Maltagliati et al. (2005) hypothesized that opheliid larvae are potentially prone to long-distance dispersal. The degree of isolation both within and between populations could be explained by current regimes constituting a barrier to gene flow (Vargiu et al. 2003).

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The taxonomic status of many Atlantic and Mediterranean species within the genus *Ophelia* is still a matter of debate. Among the European species of *Ophelia*, Fauvel (1927) distinguished *O. radiata* (Delle Chiaje, 1828) and *O. bicornis* and the variety *O. bicornis barquii* based on the number of gill pairs. However, this feature is highly variable and causes frequent misidentifications. For this reason, Bellan (1964), Cantone and Costa (1975), and Amoureux (1977) grouped *O. bicornis* and *O. bicornis barquii* into a single taxon identified as *Ophelia bicornis* sensu lato, characterized by remarkable morphological variability across Atlantic and Mediterranean localities. Pilato et al. (1978) separated *O. bicornis* from *O. barquii* according to the number of nephridiopores. The morphological taxonomy of the genus *Ophelia* was later supported by genetic markers, which confirmed the validity of *O. bicornis* and *O. radiata* (Britton-Davidian and Amoureux 1982) and the separation of *O. bicornis* and *O. barquii* (Vargiu et al. 2003; Maltagliati et al. 2004, 2005).

Interestingly, the two species can be found in sympatry along the Italian coast (Maltagliati et al. 2004). Their vertical zonation in the case of sympatry was studied by Iraci-Sareri (2006), who reported that *O. bicornis* is more abundant in the superficial layers of the substrate and the swash zone, whereas *O. barquii* is found in deeper sediment layers and the upper part of the shoreline. Such zonation seems to be absent when populations are not in sympatry (Castelli et al. 2006). The spatial and temporal distribution of *Ophelia* species is, therefore, an exciting topic in terms of adaptation to the sandy beach environment.

To fully depict spatial and temporal patterns, factors influencing the distribution of *Ophelia* species at the population level must be better investigated. It is well known that the larvae of *O. bicornis* are highly selective to substrate choice (Wilson 1948b, 1954, 1955), primarily concerning texture and microbial content. However, the spatial distribution of species of *Ophelia* was seldom studied within an ad hoc experimental design capable of catching both spatial and temporal variations (Harris 1991; Castelli et al. 2006). The spatial distribution of several populations of a species identified as *O. bicornis* in the Black Sea coasts of Turkey was studied by Dağlı et al. (2015), who hypothesized that individuals migrated within the sediment. Spatial and temporal patterns of biological and ecological features are known (Fonnesu et al. 2004; Orfanidis et al. 2007; Evagelopoulos et al. 2008), but they were not specifically investigated for *O. barquii*.

In the present paper, we investigated the spatial and temporal distribution of two populations of *O. barquii* in the beaches of Alimini and Gallipoli along the Salento Peninsula coast (Apulia, South Italy). The reproductive status of the individuals was analyzed and correlated with morphological and population dynamics features. The life cycles of both populations were investigated under laboratory conditions.

Materials and methods

Study area

Samples were collected in two beaches along the Salento Peninsula (Fig. 1). The first one, Alimini, located along the South Adriatic Sea coast (N 40° 11' 57"–40° 16' 11", E 18° 27' 35"–18° 27' 25"), is 6.1 km long and 36.2 m wide, on average. The beach tends to widen in its most southern part, reaching a width of 50 m. The second one, Gallipoli, located in the Ionian Sea coast (N 40° 02' 56"–40° 01' 00", E 18° 00' 08"–18° 01' 18"), has a length of 4.2 km and a median width of 19.4 m. The width is maximum in its central part, while both extremes are characterized by a reduction in width due to the transport of sediment to the central region (Fig. 1).

Both beaches are in an erosion state, with a high surf zone where waves dissipate their energy. The difference in sediment size among the sites is a combined effect of substrate characteristics and beach exposure due to varying hydrodynamic conditions. As a result, although both sites show a broad spectrum of sand grain sizes from fine to coarse, Alimini is characterized by medium-fine sediments (0.125–0.50 mm) abundant in quartz, plagioclase, and heavy minerals, while Gallipoli is characterized by a broader spectrum covering from 0.125 to 1 mm, and with a less represented fine component (0.125–0.25 mm), more than 60% being represented by medium sand (0.25–0.50 mm) made up of carbonate (Dilorenzo et al. 2001).

The seawater temperature and salinity were comparable among sites, 13–15 °C during winter and up to 24–30 °C during summer, with average salinity values ranging between 35 and 38‰.

Sampling methods

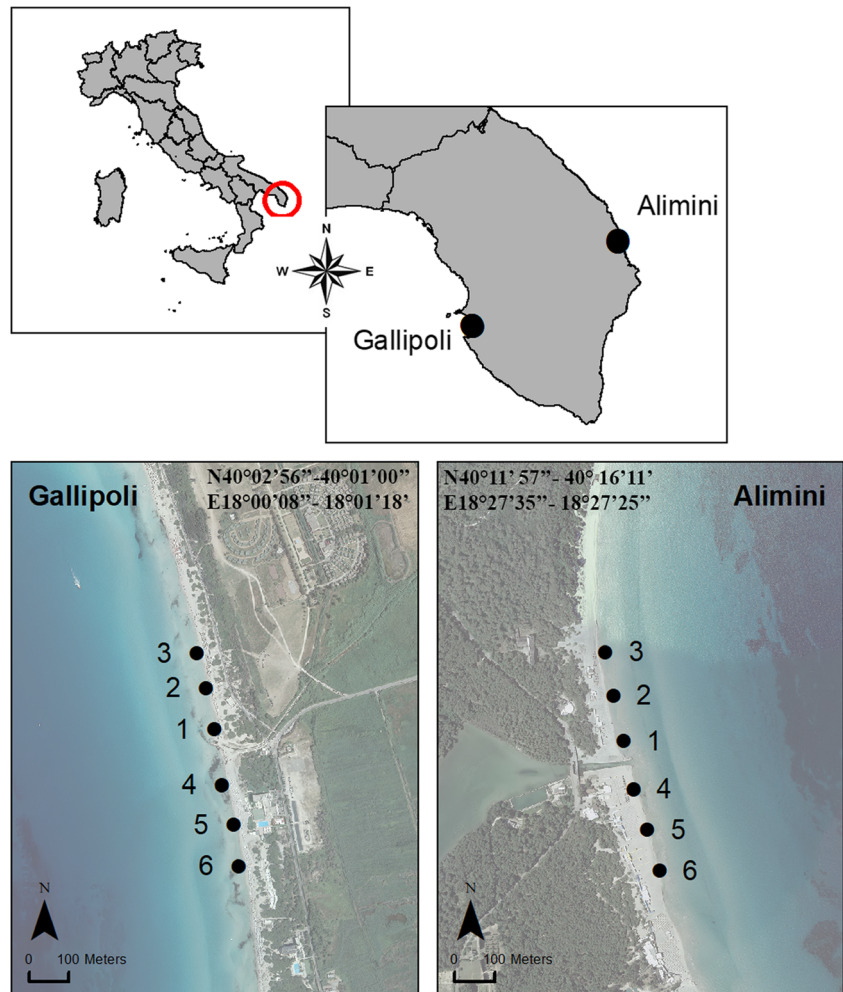
A box corer (17 cm × 17 cm × 15 cm) was utilized in the mid-littoral zone where waves regularly break (surf zone). Due to its precision, this sampling method is usually applied for soft-bottom research (Evagelopoulos et al. 2008; Pinna et al. 2017). Six plots were fixed on each beach, about 100 m distant from each other. Within each plot, three random replicates were collected monthly from April 2017 to March 2018.

The sand collected was sieved in situ with a net of 1 mm mesh size, and retained specimens were fixed in ethanol 70%, then brought to the laboratory to be identified, counted, and measured. Morphological identification was made utilizing the dichotomous keys provided by Rowe (2010) and Pilato et al. (1978). Total length (for biometric analyses), maturity, and presence/absence of germinal products inside the coelom were recorded for all individuals.

Life-cycle studies under lab conditions

Specimens for observations on larval development and cross-fertilization between the two populations were

Fig. 1 Study area showing the two beaches and sampling sites selected for the experiment. They are located in the Mediterranean Sea along the Salento Peninsula coast (Apulia, South Italy). Alimini beach is located in the Adriatic Sea coast while Gallipoli beach is located in the Ionian Sea coast



collected separately at the same sites, depth, and times of the material used for spatial and temporal analysis.

Live specimens were carried to the lab and kept in aquarium bowls. Saltwater was also collected from the same site and filtered with a mesh size of 45 μm . Males and females were isolated and placed under the stereoscope, where they were open, avoiding contamination of the dissecting bowl with blood and gut content. Artificial fertilization was performed in filtered seawater in a glass bowl, where spermatozoa were discharged over the isolated eggs. When fertilization occurred, the embryos were separated and placed in a glass. Once developed, larvae were moved into a new glass bowl and kept at 24 $^{\circ}\text{C}$, i.e., the temperature recorded in the natural environment during the reproductive period. The water was daily cleaned up from organic debris to maintain as much as possible optimal conditions and avoid contamination by bacteria and protozoans. Fertilization was tested in three experiments: two of them utilizing male and female from the same population, and the third utilizing males and females from the two populations to obtain cross-fertilization.

Treatment of data

The population density of *O. barquii* was measured in each replicate and expressed as the number of individuals per square meter (ind/m^2). Monthly comparisons between Alimini and Gallipoli were performed using the Student's *t* test. The patchiness within each beach was tested by a two-way ANOVA analysis, based on the factors "plot" and "month." The *post hoc* Tukey HSD test was applied to assess significant differences in all coupled combinations between the two factors. In Gallipoli beach, *O. barquii* species was consistently present in only 3 of the 6 plots throughout the year. Therefore, only three plots were considered for statistical analysis.

Results

Specimen identification

The specimens collected from both sites of Adriatic and Ionian Seas fit well the original description of *O. barquii*:

the body shape was cylindrical, enlarged in its anterior part, with 32 segments and a variable-length reaching a maximum of about 38 mm. The body was divided into two regions, the anterior part with a small and conical prostomium, characterized by the absence of the ventral groove that becomes well defined starting from the 10th chaetiger, and ended 6–7 chaetigers before the pygidium, where two anal papillae were visible. Twelve cirriform-like branchiae were present starting from the second chaetiger. Lastly, 5 nephridiopores could be detected. Females appeared greenish-dark when full of eggs, while males appear pale-cream. In contrast, juvenile and empty specimens were pink colored (Fig. 2).

Spatial and temporal variability

A total of 2247 individuals of *O. barquii* were collected and examined: 978 individuals in Gallipoli and 1269 in Alimini. While in Alimini worms were found in all the six sampled plots, except for plot 2 in February 2018, the species was always absent in the three southern plots in Gallipoli (Fig. 3). However, density for single plot was significantly higher in Gallipoli than in Alimini (Student's *t* test, $p < 0.05$) (Fig. 3). In both sites, a significant temporal variability among sampling times was observed, i.e., monthly (ANOVA, $p < 0.05$), as well as a density decline progressively from April 2017 to July 2017. In August 2017, worms completely disappeared until February 2018, with the highest density recorded in April 2017 in Gallipoli and in March 2018 in Alimini (Fig. 4).

Whenever the species was detected, it was possible to observe a quite homogeneous distribution in the plots (Fig. 3). Significant differences in density at the Alimini site were always due to plot 6 in April 2017, plot 3 in June 2017 and July 2017, and plot 6 in March 2018 (Table 1). At the Gallipoli site, as for the plots 1, 2, and 3, the only significant differences were detected in April 2017 and February 2018, in both cases relatively to plot 3 (Table 2).

Population dynamics

The mean worm length observed at Gallipoli beach was $31.87 \text{ mm} \pm 3.47$, while in Alimini, the mean length was $22.63 \text{ mm} \pm 5.65$. The population structure during the

monthly samplings from April 2017 to March 2018 was similar in the two investigated beaches: unimodal but without any definite growing trend. However, while in Alimini, the length seemed to remain similar each month (Fig. 5a); in Gallipoli (Fig. 5b), a change in size distribution was observed between April 2017 and May 2017, and February 2018 and March 2018, with the largest sizes recorded in April 2017.

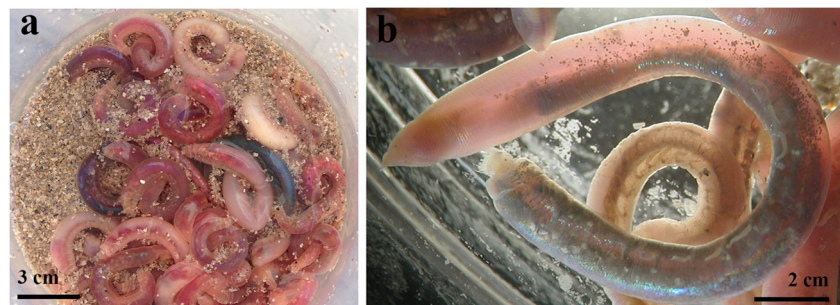
In both beaches, almost half of the population was found in a reproductive stage from April 2017 to May 2017 and in March 2018. The smallest size at reproduction was observed to be 13–15 mm in Alimini and 19–21 mm in Gallipoli. However, according to the size, empty specimens could belong to different categories: immature specimens and specimens that have already spawned (spent phase). In both populations, larger individuals found in April 2017 (from 40 to 51 mm) were all ripe. In July 2017, most of the population was in a spent phase in both sites; yet, it was not possible to assess if they were immature or already spawned specimens.

Similar size distribution was present in 2018. When the specimens reappeared in February 2018, none of them was ripe. A trend similar to April 2017 was observed in March 2018 in Gallipoli, with the absence of large individuals.

Reproduction

Egg size was similar in both populations, averaging $150 \mu\text{m}$. Mature eggs were found free in the coelom; they were greenish-brown and oval-shaped with a nucleus in the central region (Fig. 6a). Sperms were typically oval-shaped joined together in a membrane and splitting up when released. Two artificial fertilization trials were performed in the laboratory during the year 2017. The first trial was conducted in April 2017 using the Alimini specimens because in this site, most of the individuals appeared ripe. The second trial was conducted later in June 2017, using specimens from Gallipoli because in Alimini, most of the specimens appeared in a spent phase. A third artificial fertilization trial was conducted across populations in March 2018, when in both sites, most of the specimens were ripe. Similar results were obtained for the three trials: artificial fertilization gave 100% of fertilized eggs; fertilization occurred after a few minutes and was asynchronous. One day later, most of the eggs appeared at the bottom of the

Fig. 2 **a** Alive specimens of *Ophelia barquii*; **b** particular of a ripe female



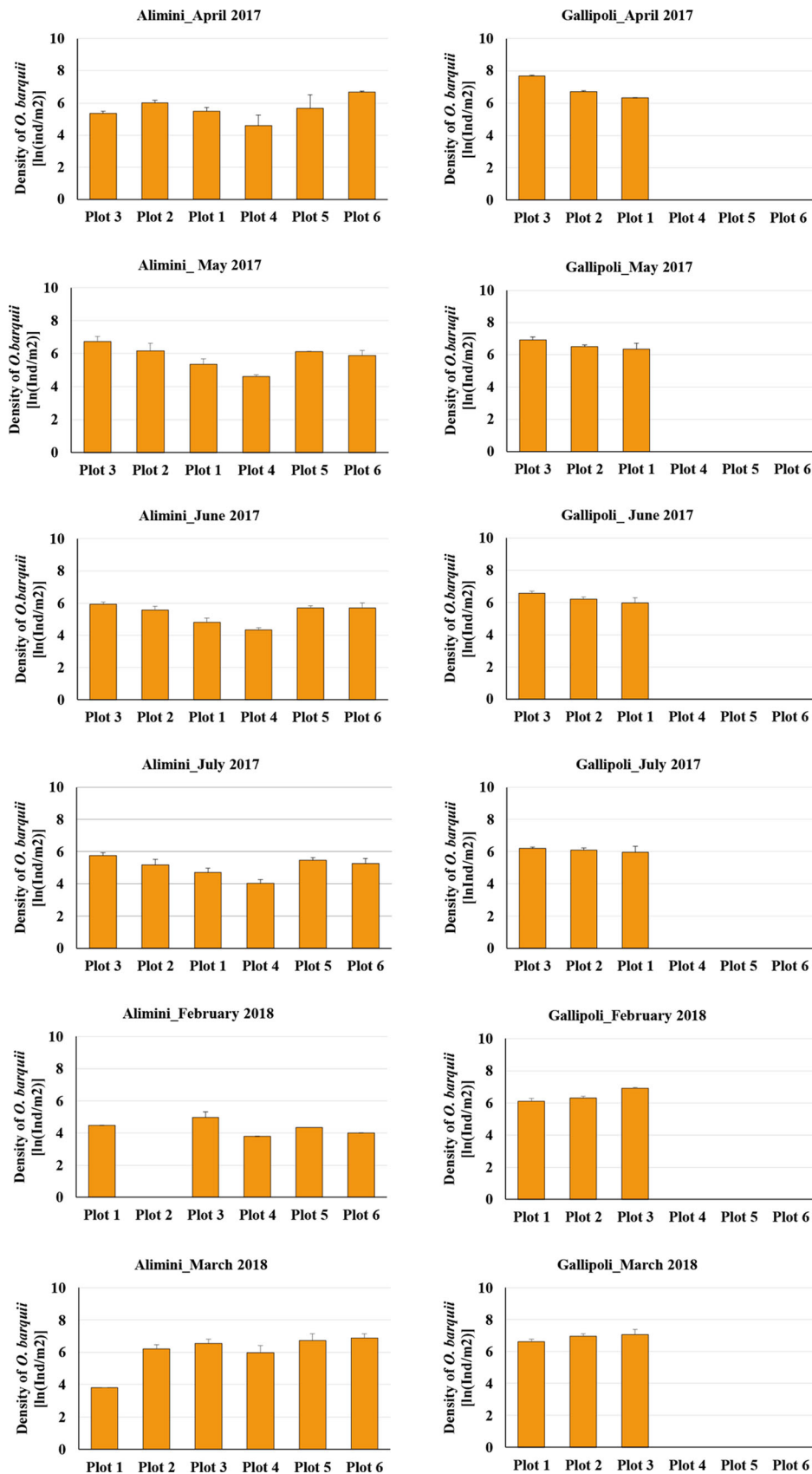


Fig. 3 Spatio-temporal variability of *Ophelia barquii* density in each plot of Alimini and Gallipoli beaches. Density is reported as [ln(ind*m⁻²)]

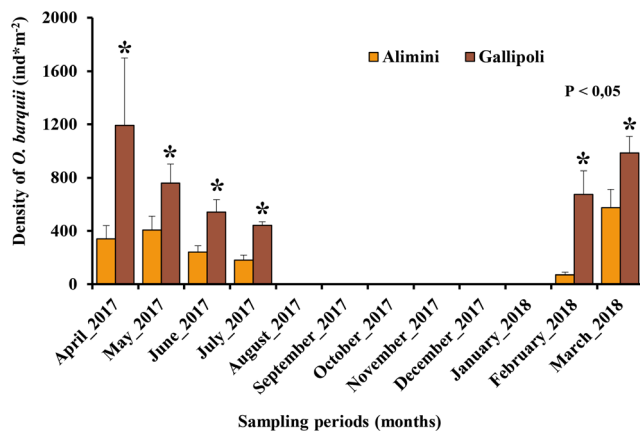


Fig. 4 Population density of *Ophelia barquii* from April 2017 to March 2018. In the y-axis, the density of the specimens is reported as the number of individuals per m⁻² (ind*m⁻²) while the x-axis corresponds to sampling times (months)

glass bowl, with only a few trochophores swimming in the water column. Trochophores' development stopped at 4 days of age.

Fertilized eggs lost the central nucleus, became spherical, and started the development of the “activation cone” (Fig. 6b). Some of the fertilized eggs began the first step of segmentation (Fig. 6c), passing through different stages in less than 1 hour. The last stage of the embryonic development, swimming in the water column, rounder, and with the surface entirely covered by cilia, was considered a protrochophora stage (Fig. 6d). The 1-day old larva was a small trochophore swimming in the water column, provided by an apical tuft and the metatroch equatorial, cilia for locomotion (Fig. 7a). At this stage, some larvae started to elongate in the posterior part (Fig. 7b). The 2-day old larva showed still a spherical shape of the “head” provided by a tuft, while in the equatorial region was still visible, and the segmentation appears (Fig. 7c). In the 3-day old larva, two eyespots appeared together with the chaetae in the segmented body and two papillae at the posterior extremity of the body (Fig. 7d). At this stage, the larvae started to produce mucus. After the fourth day, the larvae became benthic (Fig. 7e–f); they became much more elongated while the prototroch started to shorten, and segmental grooves appeared. The larvae had 3 segments complete with chaetae. The anus opened behind the dorsal gap of the telotroch, and larvae appeared fixed to the bottom with the anal papillae.

Table 1 Two-way ANOVA analysis testing for differences in *O. barquii* density between different months (April, May, June, July, February, March) and across the studied plots (plot 1, plot 2, plot 3, plot 4, plot 5, plot 6) within the Alimini site

	df	SS	MS	F	p
Intercept	1	8838.231	8838.231	276.9962	***
Month_Year	5	2583.713	516.743	16.1951	***
Plot_Stat	5	1590.269	318.054	9.9680	***
Month_Year*Plot_Stat	25	1943.454	77.738	2.4364	**
Error	72	2297.333	31.907		

***= $p < 0.001$; **= $p < 0.01$

Discussion

Spatial and temporal distribution

Quite homogeneous distribution of the species was observed in both investigated areas, taking into account the small-scale variability tested. However, medium-scale patchiness was consistently recorded in Gallipoli beach, where *O. barquii* was always absent in three of the six sampled plots. This is a well-known trend in organisms inhabiting soft-bottom environments exposed to high wave energy, which are likely to show small-scale patchiness as well as cross-shore variations depending on beach topography, hydrodynamic forces, sediment moisture, and food availability (Defeo and McLachlan 2005, 2011). The difference in abundance between the two sites may be due to varying beach morphodynamics. Indeed, the sand composition and the granulometry, which are expected to affect sandy beach macrofauna and, more specifically, soft-bodied burrowing organisms like polychaetes (Barboza and Defeo 2015), clearly differ between the two beaches due to their varying hydrodynamic conditions.

Gallipoli beach, which stretches along a semi-closed bay located along the Apulian Ionian coast in the western part of the Mediterranean Sea basin, is characterized by a significant accumulation of organic fragments and a high percentage of coarse sand. Conversely, the sediment of Alimini beach (located on the eastern side of the Mediterranean Sea basin—Apulian Adriatic coast) is 50% medium sand and nourished mainly by the Ofanto River runoff. The different origins of the sediments, which ultimately define the mineralogical composition of the sand (Dilorenzo et al. 2001), may have also influenced the observed biotic patterns. Morphodynamic features could further explain the high polychaete abundance in Gallipoli. In fact, even if the larger particle size can indicate that Gallipoli is a more exposed site, it is an enclosed beach in its southern part and features an artificial channel in the central part of the coastal stretch, which can act as physical barrier causing an accumulation of organic matter in the northern part, where the species was found very abundant. The presence of this channel could also be responsible for the absence of the species in the southern part.

Temporal variability was higher in both beaches, with the absence of specimens from August to January. This trend

Table 2 Two-way ANOVA analysis testing for differences in *O. barquii* density between different months (April, May, June, July, February, March) and across the studied plots (plot 1, plot 2, plot 3, plot 4, plot 5, plot 6) within the Gallipoli site

	df	SS	MS	F	<i>p</i>
Intercept	1	28,428.17	28,428.17	614.0484	***
Month_Year	5	3205.50	641.10	13.8478	***
Plot_Stat	2	2956.33	1478.17	31.9284	***
Month_Year*Plot_Stat	10	2396.3	239.63	5.1761	***
Error	36	1666.67	46.30		

***= $p < 0.001$

could be explained by the presence of a monotelic 1-year cycle. However, the strategy of committing the population continuity to a single synchronized reproductive episode is quite uncommon and risky, even for a species that does not suffer from competition with other similar polychaetes on the same beach. The few available data on opheliid life span indicate up to 6 years for *O. verrilli*, about 2 years for *O. bicornis*, and only 1 year for *O. limacina* (Giangrande 1997). In both the examined sites, no sign of size increase could be detected. Even though a targeted sampling for recruitment collection was not part of our design, it was unlikely that the large specimens that reappeared after January were derived from the growth of the single recruitment occurred the previous year (especially considering that after their reappearance, the size of the worms remained quite constant for several months). It is more likely that what appeared as a unimodal distribution of the population was the product of at least two overlapped generations. This is supported by the presence of “non ripe” specimens, which, according to size, can be actually immature and/or already spawned individuals. Therefore, we suggest that the species can live more than 1 year and that the disappearance of specimens from the surf area may be due to mortality after spawning but also to a sort of “reproductive migration” of the remaining specimens. Defeo and McLachlan (2005) indicated that large macrofauna inhabiting beaches exposed to high wave energy can regulate their position by moving fast and by other behavioral adaptations. The marked variation in the temporal distribution could thus reflect active seasonal migration. Our observations on *O. barquii* agree with those already reported for other species within the same genus. The occurrence of horizontal movements leading to “seasonal disappearance” had previously been hypothesized by Dağlı et al. (2015) to explain the temporal patchy distribution from *O. bicornis*. Wilson (1948b) has also mentioned that *O. bicornis* generally moves to deeper zones when hydrodynamic conditions are adverse in the mid-littoral zone. By contrast, Öztürk and Ergen (1994) noticed that *O. bicornis* shows homogenous spatial distribution during high-energy hydrodynamic conditions, moving deeper into the shores with lower hydrodynamics to find an optimal moist environment. Finally, Riser (1987) in reporting the spawning of *O. verrilli* during spring and early summer hypothesized that individuals migrate to the upper layers of the substrate to

allow for egg spawning and sperm release. At the end of this period, individuals return to the lower layers, probably to avoid the risk related to the high wave energy swashing the littoral.

However, in most of the papers, there is a lot of confusion concerning vertical and horizontal opheliid migration, and it is not clear if also vertical migration into the sediment occurs, although it was clearly reported for *O. barquii* when in sympatry with *O. bicornis* to avoid competition (Castelli et al. 2006).

We hypothesize that the animals “disappear” due to horizontal migration, since none were found in the deeper layers of the sampled sediment, although we checked them deeper than 15 cm. However, our results are only indicative of adult migration, as the use of a 1 mm mesh size excluded smaller specimens.

As a whole, the high temporal variability of *O. barquii*, with the presence/absence of individuals in a particular period of the year, is probably related to the reproductive period and conditioned by changes in hydrodynamic conditions. Under low-energy conditions, individuals can live in the mid-littoral area, while under high-energy conditions, the species may move to low mid-littoral, and maybe infra-littoral zones to cope with the putatively adverse effects of waves and turbulence. The spawning period should, therefore, occur under particular hydrodynamic conditions, when the organisms go towards the upper level to allow for egg spawning and sperm released for fertilization, and probably, migration has also the scope to concentrate individuals. At the end of this process, individuals return at low level probably to avoid stressful conditions due to seasonal storms.

Reproduction

The reproductive period of *O. barquii* in Mediterranean spans from March–April to July; reproduction is reported from spring to autumn for *O. bicornis* (Dağlı et al. 2015), and in spring and early summer for *O. verrilli* (Riser 1987). However, the length of the reproductive period slightly differed between the two populations, since the Gallipoli specimens seem to become ripe earlier with a prolonged spawning period compared with Alimini. Indeed, large individuals collected from Gallipoli in early April 2017 appeared swollen, probably because they had already spawned, compared with the Adriatic specimens that were still full of gametes in the

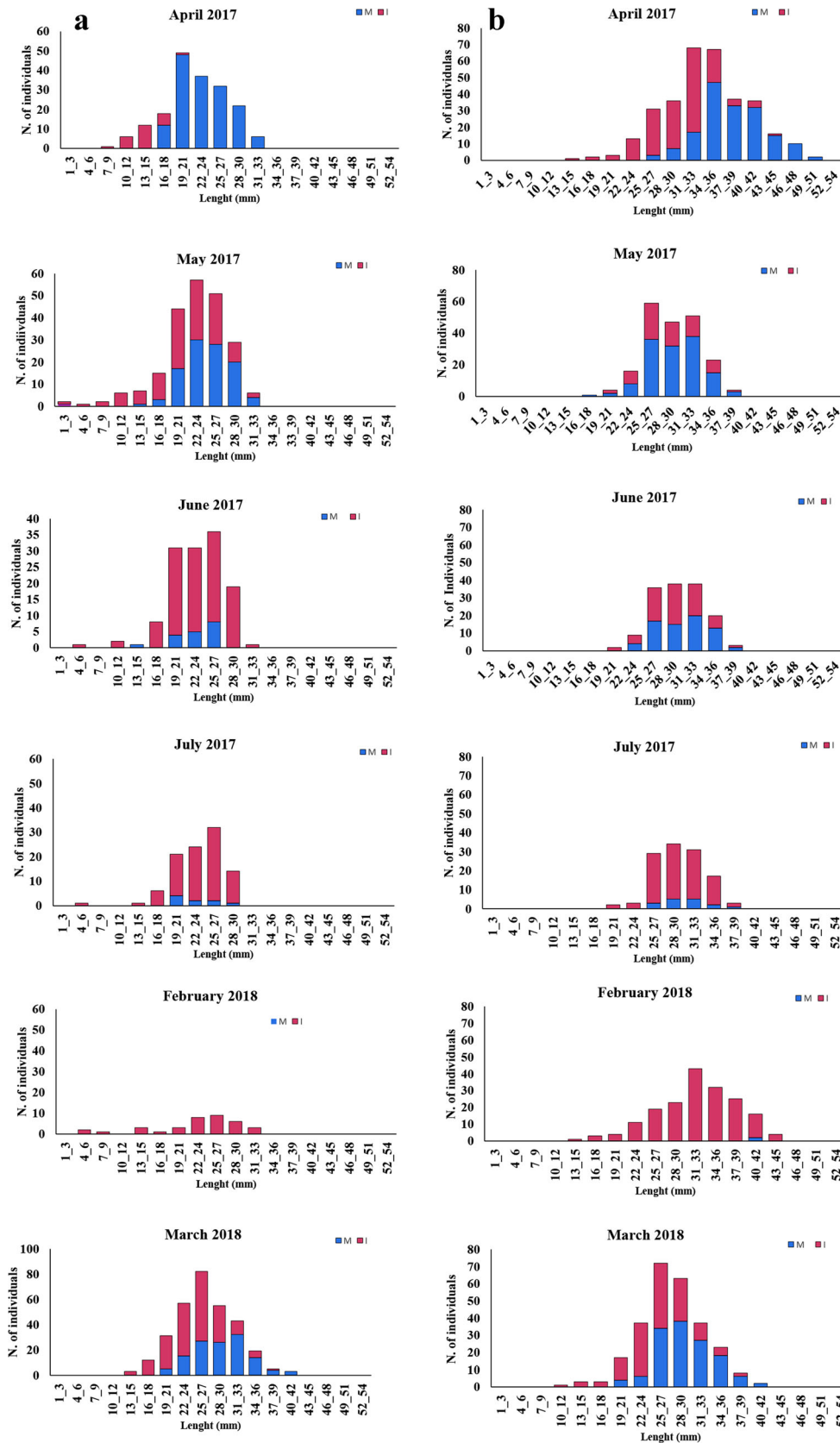
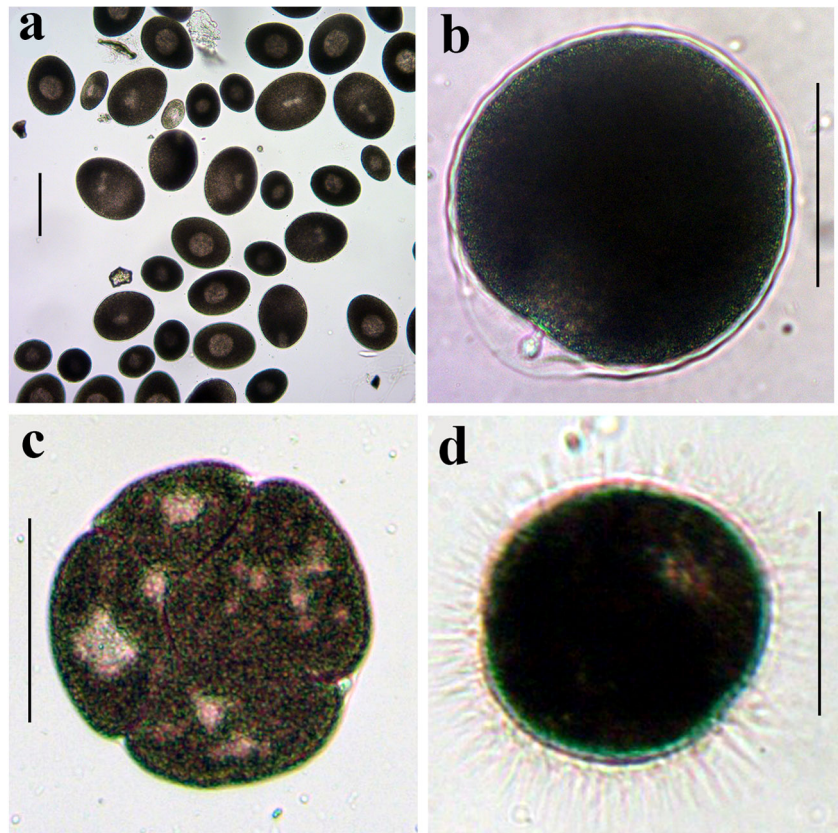


Fig. 5 a Population dynamics in Alimini specimens; b population dynamics in Gallipoli specimens. The figure included only the months in which specimens were found. M = mature specimens; I = Immature specimens

Fig. 6 Photos of the embryonic development of *Ophelia barquii* undergoing through different stages after 1 h from fertilization: **a** unfertilized eggs; **b** fertilized eggs with the formation of the “activation cone”; **c** morula at the second segmentation stage; **e** last stage of segmentation. scale bars: (a) 100 micrometers; (b, c) 50 micrometers; (d) 80 micrometers



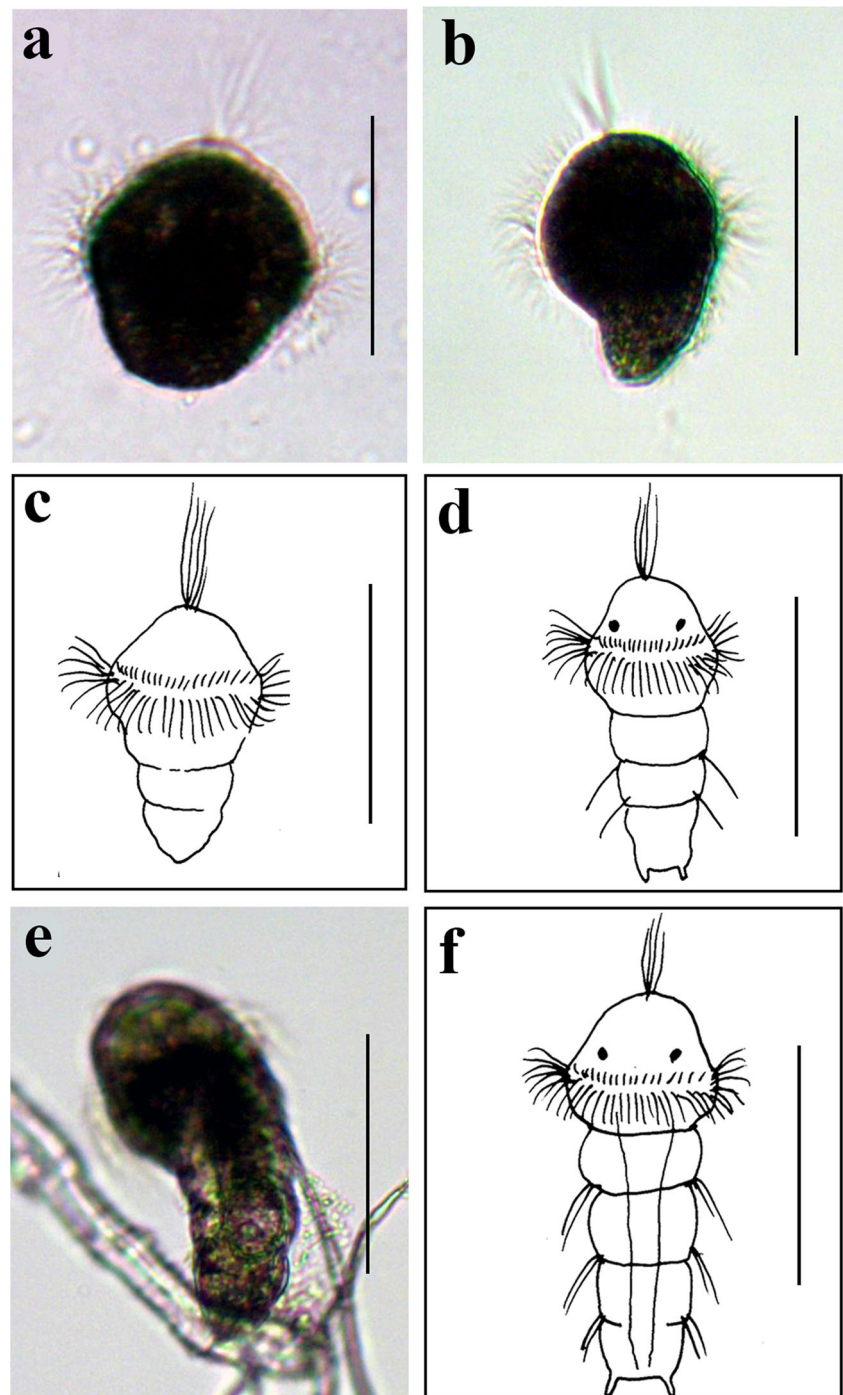
same period. In the following month of May 2017, however, both populations were still showing reproductive activity. Finally, by the end of June 2017, almost all the individuals from Alimini seemed to have already spawned and started to appear swollen and pinkish. A similar pattern occurred in Gallipoli a month later. The reappearance of individuals in February 2018, which were more abundant in Gallipoli, also supports this hypothesis. Therefore, Gallipoli specimens seem to be ripe and to start to reproduce 1 month in advance, especially during April 2017, while Alimini individuals were mostly ripe in May 2017. Changes in size distribution between April and May 2017 were observed only in Gallipoli, due to the likely death of specimens that had already reproduced.

Generally, ripe eggs have an oval shape of about 150 μm and are usually dark green or greenish-brown. As observed by Benham (1896), eggs appear enclosed in a well-defined jelly membrane wholly transparent and invisible, several micra thick. The shape of the fertilized eggs changes and they become spherical and smaller with a diameter of around 95 μm . This change is coupled with swelling up of the jelly layer.

No morphological differences were detected during the larval developmental stage between individuals from Alimini and Gallipoli. Larval development, examined separately in the two populations, appeared similar, showing both a lecithotrophic pelagic stage of only 4 days, very short if

compared with that found by Wilson (1948a) for *O. bicornis*, who reported a planktotrophic, free-swimming larval stage of 6–8 days. This author stated that at this stage, larvae develop 3 chaetigers, change behavior, and swim downward adhering to the sand grains by using their anal papillae. The apical cilia, prototroch, telotroch, and neurotroch disappear, and the larva loses its swimming ability. The body elongates, and the worm crawls among the sand grains gradually developing into the juvenile phase. Wilson (1948a) was the first author to give a detailed description of the larval stage of a species of *Ophelia*, also highlighting the difficulty of obtaining the settlement of the larvae. Earlier, Brown (1939) had described the development of *O. cluthensis*, whereas McGuire (1935) and Benham (1896) recognized for the first time that *Ophelia*'s eggs were enclosed in a jelly structure. Later, Riser (1987) reported the life cycle of *O. verrilli* as lecithotrophic, with a pelagic phase lasting for 12 days. At this time, when two chaetigers are already fully formed, the larva attaches itself to the sand grains and is ready to metamorphosis. They recognized three juvenile stages: in the first stage (18 chaetigers), the larva feeds itself actively on diatoms and foraminiferans. The second stage is characterized by a slight increase in the number of chaetigers and chaetae per parapodium. During the third stage of development, the blood vascular system is complete; the worms become pinkish with the typical adult shape, losing their adhesive capability.

Fig. 7 Photos and drawing of larval development of *Ophelia barquii*: **a, b** 1-day old larva; **c** 2-day old larva; **d** 3-day old larva; **e, f** 4th day larva. scale bars: 100 micrometers



Apical cilia and branchial develop and become functional. Similar results were also obtained within the third trial, carried out to test cross-fertilization between the two populations.

All our trials stopped at the stage just before the metamorphosis, when worms had three chaetigers and started to secrete mucus to attach them to any small structure found in the dish. Therefore, the developing larvae did not complete their entire life cycle, and further comparison of later developmental stages was not possible.

During the short phase of pelagic development obtained in the present study, larval morphology of *O. barquii* matched that of the congeneric species investigated by Wilson (1948a). The only significant difference found was related to the short duration of larval development. However, we do not discard the possibility of development being affected by the temperature of larval rearing, which was higher in ours when compared with Wilson's experiment.

Conclusions

The reproductive period of both populations of *O. barquii* goes from March–April to July. The distribution of “empty” specimens, especially from April 2017 to May 2017, led us to hypothesize the occurrence of a monotelic reproductive cycle over at least 2 years.

Temporal patchiness is probably due to the migration of large sized adults, which display faster movements and fully developed behavioral adaptations. The disappearance of specimens from the surf area is due to the co-occurrence of both mortality after spawning and a sort of horizontal “reproductive migration” of the remaining adults.

The populations from the two sites differed in size, and time and length of the reproductive period. Individuals from Gallipoli appeared ripe earlier than those from Alimini, which were ripe mostly in May. All these differences coupled with the differences in substrate granulometry between the two beaches, at first, led us to hypothesize that the two studied populations did not belong to the same taxon. However, observations of their life cycles also after cross-fertilization indicated that the two populations are divergent forms of the same taxon adapted to different environmental conditions, and that this species do not show the high selectivity behavior during settlement, as found for the congeneric *O. bicornis*. Additional analyzes are, however, needed, not only on the larval development and settlement but also on the organic matter content and microbial composition bot with and between beaches. Lastly, molecular analyses would better clarify if genetic exchange occurs between the two populations, considering their very short pelagic stage and the restrictions imposed by water circulation between the Ionian and Adriatic seas (Boero et al. 2016).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

Sampling and field studies No permits were necessary for sampling and observational field studies.

Data availability All data generated or analyzed during this study are included in this published article.

Author contribution LF and MP conceived and designed research. AG, IG, MT and ML conducted experiments. MdP and MP analyzed data. AG, ML and MP wrote the manuscript. All authors read and approved the manuscript.

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