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## Nest-mediated parental care in a marine fish: Are large-scale nesting habitats selected and do these habitats respond to small-scale requirements?

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### Abstract

Fishes have evolved various reproductive strategies including mechanisms that involve parental care and demersal eggs laid into nests. *Symphodus ocellatus* has a seasonal reproduction period during which large, dominant males become territorial and build nests with fragments of algae, where they attract females to spawn and provide care to the developing eggs. Based on the hypothesis that the *S. ocellatus* males choose the reproductive habitat based on some characteristics of the substrate, here we assessed whether, on a coastal area scale, the distribution of this species changes during the reproductive period because of the selection of some suitable sites or substrates, and whether the nesting microhabitat used by this species responding to certain requirements in relation to different characteristics. From April to September 2010, at four locations and on three substrate types, the fish were counted in three periods related to different stages of reproduction. Furthermore, several physical and biological variables have been recorded around numerous nests to select those with more recurrence. We found that *S. ocellatus* prefers to live on rocky substrates populated by photophilic algae, regardless of the phases of the reproductive cycle. We identified depth (1.7–3.2 m), the presence of a hole, a 10–20 cm algal canopy, and high algal coverage of *Dyctiota* as nest requirements. *S. ocellatus* is mostly distributed in coastal sites sheltered from the action of waves. This allows the construction and maintenance of nests and the possibility to remain in a water temperature range similar to the reproductive physiological constraints.

**Keywords:** Nest-building; Labridae; habitat selection; habitat requirement; *Symphodus ocellatus*.

### Introduction

Many marine fish species inhabit shallow coastal areas, where anthropogenic disturbances tend to be most frequent and severe, representing the main threat to their survival. Because life history strategies of coastal fish are in association with high habitat specialization, these animals are particularly vulnerable both to habitat loss/degradation and to exploitation (Reynolds *et al.*, 2005). Fish species have evolved various strategies to increase their reproductive success, including simple processes (e.g., reproduction of pelagic species in the water column) and complex mechanisms that involve parental care as demersal eggs laid within nests (Andersson, 1994; Ballon, 1975; Taborsky *et al.*, 1987). *Symphodus ocellatus*

(Linnaeus, 1758) is a nesting wrasse that is widespread in shallow rocky and vegetated areas of the Mediterranean Sea, Black Sea, and Sea of Azov (Quignard & Pras, 1986). This species has a seasonal reproduction period (from May to early August) during which large, dominant males become strictly territorial and build nests with fragments of algae, to which they attract females to spawn and provide care to the developing eggs (Lejeune, 1985; Warner & Lejeune, 1985; Taborsky *et al.*, 1987; Sinopoli *et al.*, 2015). Dominant building males are often helped by satellite males (with a slightly different color pattern) to defend the nests from other opportunist males (sneakers) (Taborsky *et al.*, 1987; Taborsky, 1994; Alonzo *et al.*, 2000). Females visit multiple nests before selecting one for spawning (Taborsky *et al.*, 1987) and seem to

prefer those where other females have already spawned (i.e., mate-choice copying) (Alonzo, 2008) and with fewer sneakers present (Alonzo & Warner, 2000). Embryonic development lasts about 80 h and, after hatching, swimming larvae have a pelagic phase (PLD) of 8–11 days, after which they settle on shallow habitats among branched algae (Raventos & McPherson, 2001).

A study by García-Rubies & Macpherson (1995) reports that the larval recruitment of *S. ocellatus* occurs both on rocky bottoms with a high algal cover and, to a lesser extent, on *P. oceanica* from July to September. Some studies report that adults are more abundant in rocky substrates with algal coverage (Letourneur *et al.*, 2003; Lipej *et al.*, 2009). Other studies, however, show higher density on *P. oceanica* and other seagrass meadows (Guidetti, 2000; Guidetti & Bussotti, 2002). Mouillot *et al.* (1999) reported that larger fish are more abundant on rocky substrates, whereas smaller sized fish were more abundant on *P. oceanica* meadows.

Beyond this information, there are no studies indicating that, during the reproductive phase, *S. ocellatus* individuals choose to populate hard substrates more than soft substrates. Sinopoli *et al.* (2015) found that nesting males of *S. ocellatus* actively selected some algal species for nest building, particularly the coralline *Jania rubens* and the brown alga *Dictyota linearis*. The choice of these species was justified by their mechanical strength, their capacity to accommodate eggs, and their resistance to biochemical decomposition and wave action. Despite the evidence that *S. ocellatus* specifically chooses components to build the nest, it is still unclear if the choice of the nest site in *S. ocellatus* is influenced by specific characteristics of the microhabitat. Several studies regarding different nesting fishes highlighted difference in nests and nest sites, even if this difference led to variation in mating success (Wernerus *et al.*, 1989; Alonzo & Heckman, 2010). For species in which egg predation is common, many authors have suggested that the quality of the nest depends on some characteristic of the nesting site, such as camouflage (Sargent & Gebler, 1980; Kraak *et al.*, 2000; Candolin & Salesto, 2006).

However, since the structure of the habitats and the algal coverage determine the extent of the territory where courtship activities take place (Candolin & Voigt, 2001), sites that are overly hidden can be disadvantageous, making the nests poorly visible to potential partners (Mori, 1995). For the co-generic *Symphodus roissali*, substratum slope and the exposure to the wave action were identified as determinants of greater success (Raventos, 2006). However, few studies have identified any intrinsic physical characteristics of nests and nesting site that explain whether their building activities are in response to some particular characteristic or, instead, occur in a random pattern (Potts, 1985; Lipej *et al.*, 2009).

Based on the general hypothesis that the choice of the reproductive habitat can be influenced by the environmental characteristics of the substrate, we tried to answer two questions. (1) On a coastal area scale, is the

habitat occupancy of this species along several sites and substrates constant or does it change during the reproductive period because of a selection of suitable sites or substrates? If these hypotheses are true, we would expect to detect significant differences in the abundances of *S. ocellatus* when comparing the pre- and post-reproduction periods. (2) If nesting sites are selected based on certain physical, structural, or biological requirements/characteristics, we would expect to see a significant recurrence of specific variables near the nests.

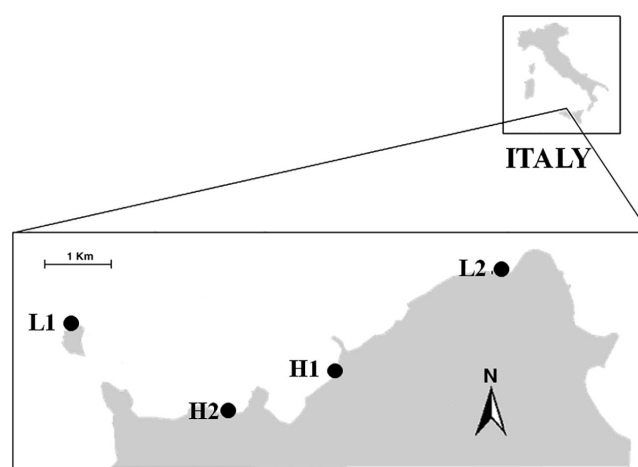
## Material and Methods

### Study area

The study was conducted in 2010 along the Tyrrhenian coasts of Sicily between Capo Gallo and Isola delle Femmine MPA (Fig.1). This coastal stretch of about 8 km is characterized by a high substrate heterogeneity with a mosaic of rocky substrates, *Posidonia oceanica* meadows, and wide patches of sandy bottoms. The algal community associated with the rocky substrate in the upper subtidal zone is dominated by photophilous Fucophyceae *Cystoseira brachycarpa* and *C. compressa* (Riggio & Raimondo, 1991). Furthermore substrate at the base of the erect algae and in the dark microhabitat, algal communities are dominated by sciaphyllous species, such as *Corallina elongata* and *Jania rubens* (Giaccone *et al.*, 1985; Mannino *et al.*, 2011).

### Large scale site selection

To test the hypothesis that the habitat occupancy of individuals of *S. ocellatus* varies among the factors characteristic of substratum (CS), reproductive cycle phase (RP), and locations (LC), the following experimental design was developed: a) For CS factor three levels were chosen: sandy (SND), rocky (RCK) and *Posidonia oce-*



**Fig. 1:** Study area in northern Sicily (Tyrrhenian Sea); H1 = Barcarello, H2 = Cala Isola, L1 = Isolotto, L2 = Capo Gallo.

*anica* (POS); b) For the RP factor, we chose three levels: pre-reproductive (PRE; April), reproductive (REP; July), and post-reproductive (POST; September); and c) The choice of levels for the LC factor was based on preliminary observations made during the reproduction period. Indeed, at several locations along the coast, the density of nests varied greatly among locations with the same environmental characteristics. Two high (about 2 nests /10 m<sup>2</sup>, H1; Barcarello and H2; Cala Isola) and two low-density sites (about 0.2 nests /10 m<sup>2</sup>, L1; Isolotto and L2 Capo Gallo) were chosen. We hypothesized that *S. ocellatus* actively chooses high density sites. If true, in periods away from the nest building, *S. ocellatus* individuals should have comparable abundances in high and low-density of nest sites. This preliminary observation also confirmed that, at all locations, most *S. ocellatus* were found at depths of between 2 and 8 m, as has been reported in other studies (Mouillot *et al.* 1999; Ruitton *et al.*, 2000; Letourneur *et al.*, 2003). The fish were counted by underwater visual census (UVC). In this case, a modified version of the fixed point technique (D'Anna *et al.*, 1999) was adopted. Scuba divers, using metric references, defined a circle with a diameter of 5 m (19.6 m<sup>2</sup>). Inside the circle area, individuals of *S. ocellatus* were counted for 5 min. Each sample was replicated three times for each of the factors considered in the experimental design, for a total of 108 censuses. To avoid multiple fish counting, a minimum distance of 20 m was set between samples. The choice of this distance was justified by preliminary observations in which *S. ocellatus* showed high residence times and minimum displacement (Sinopoli *et al.*, 2015). Data were expressed as the mean  $\pm$  standard deviation (s.d.). To test the significance of the observed differences, an analysis of variance (ANOVA) was performed (Underwood 1997).

### Habitat requirements

With the aim of finding the highest number of nests possible, we designed a standardized protocol. In this protocol, the SCUBA diver explored a large extent of about 500 m<sup>2</sup>, making routes aimed at covering most of the area. During sampling, the following biotic and abiotic variables were recorded for each nest: the depth of each nest; nest height (i.e. the depth in centimeters of the concavity formed by the nest); average height (in centimeters) of the algal canopy surrounding the nest; type of cavities used for nest building; crevice type (fracture or fissure) in rocks with irregular margin, shape, and variable size; the presence of a hole (a hollow place or cavity with regular margin and circular shape and size constantly, around 10–15 cm in diameter); slope of the substrate in degree (°) being 0° the sea surface; slope Macro (the slope exclusively related to the nest); and the presence [as a percentage cover of the most abundant algal taxa (*Dictyota* spp., *Cystoseira* spp. and *Jania rubens*) or sandy

substrate in a circular area of 9 m<sup>2</sup>]. This surface corresponds to the range of activity during nesting estimated by Sinopoli *et al.* (2015).

To investigate the presence of high suitability zone for the presence of a nest, a count data matrix has been constructed, using the number of nests as the response variable, and three categorical covariates as independent variables. These were: 1) “Deep range”, with three levels (low, 0–1.6 m; medium, 1.7–3.2 m; and high, 3.3–5 m); 2) “type of cavities”, with two levels (hole and crevice); and “canopy height” (tall, 2–10 cm; medium, 10.1–20 cm; high, 20.1–30 cm). As our response variable was a count data, we implemented a generalized linear model (GLM) with Poisson family distribution. Moreover, a GAM model with Poisson family distribution has been used to investigate the relation between the number of the nest and the coverage percentage algal composition. For both models, we started with a full model using the complete interaction among covariates, and use the function step AIC to select the final model, which had the lowest AIC value and the largest explained variance. The complete check of residual's model and an over-dispersion value below the limit value of 2 have been used to validate our analysis (Zuur *et al.*, 2009).

## Results

### Large scale site selection

During the entire period of the study, 162 specimens of *S. ocellatus* were surveyed. The distribution of these fish along locations and habitats was not influenced by their reproductive phase (Fig. 2; Table 1). The density of *S. ocellatus* was significantly higher in the RCK habitat than in the SND and POS habitats. The distribution of *S. ocellatus* was higher at H1 and H2 localities than at L1 and L2, confirming the preliminary observation, but for all the phases of the life cycle (Fig. 2; Table 1).

### Habitat requirements

The characteristics of 84 nesting sites were recorded. The mean depth where nests were found was  $2.6 \pm 0.9$  m, the mean canopy was  $11.6 \pm 5.9$  cm, and the mean substrate slope was  $56 \pm 22^\circ$  (Table 2). *Dictyotales* resulted the most abundant algal group in the microhabitat with a mean presence of  $46.5 \pm 22.5\%$  (Table 2).

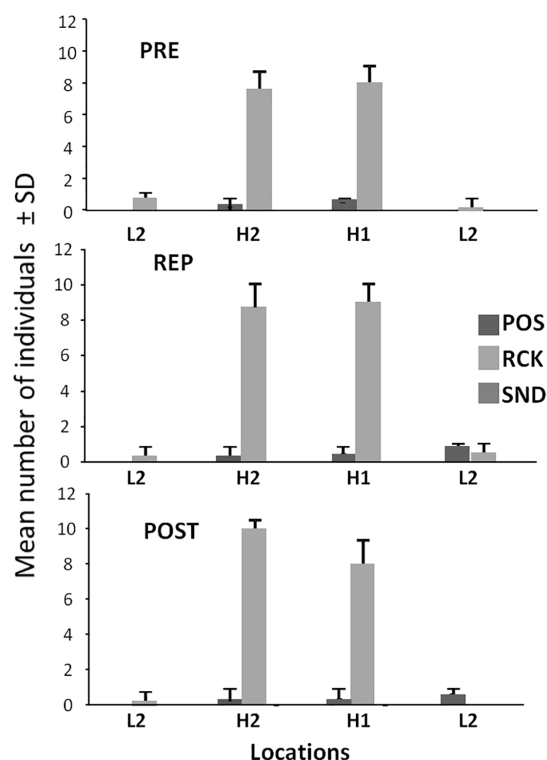
Our GLM model indicated that depth 2 (1.7–3.2 m), hole and canopy 2 (10–20 cm) were the abiotic nest features that represent nest requirements (Fig. 3, Table 3). Our GAM model highlighted a significant effect of the seaweed coverage (for all three seaweeds) on the number of nests encountered. The nests were composed of 40–50% of *Dictyotales*, 20–30% of *Cystoseira*, and 10–15% of *Jania* (Fig. 4, Table 4).

**Table 1.** Results of the ANOVA. RP = reproduction phases; LC =location; CS = characteristic of substratum; SND = sandy substrate; POS= *Posidonia oceanica* substrate; RCK = rocky substrate; H1 = Barcarello; H2 = Cala Isla; L1 = Isolotto; L2 = Capo Gallo; SNK = Student-Newman-Keuls test.

Source	DF	MS	F
RP	2	0.5	0.2 <sup>ns</sup>
LC	3	69.7	34.6**
CS	2	219.0	109**
RPxLC	6	0.4	0.2 <sup>ns</sup>
RPxCS	4	0.6	0.3 <sup>ns</sup>
LCxCS	6	71.0	35.3**
RPxLCxCS	12	0.4	0.2 <sup>ns</sup>
RES	72	2.0	
TOT	107		

SNK			
SND	POS	RCK	
L1 =L2=H1=H2	L1 =L2=H1=H2	H1= H2 > L1 =L2	
L1	L2	H1	H2
SND=POS=RCK	SND=POS=RCK	RCK > POS = SND	RCK > POS = SND



**Fig. 2:** Mean ( $\pm$  standard deviation) number of *Symphodus ocellatus* individuals in the three reproductive phase (PRE = before reproduction, REP = during reproduction, POST = after reproduction) on the three substrates (SND = sandy substrate, POS = *P. oceanica* substrate, RCK = rocky substrate) and in the four locations (H1 = Barcarello; H2 = Cala Isla; L1 = Isolotto; L2 = Capo Gallo)

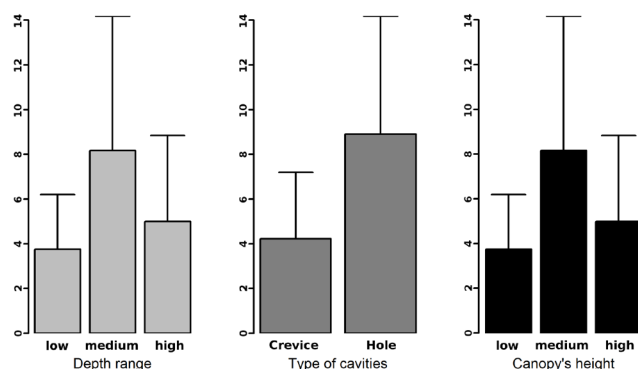
**Table 2.** Mean value ( $\pm$  standard deviation) of all the variable in the 84 nest sampled for habitat requirements ).

Variables	Mean	S.D.
Depth (m)	2.7	0.9
Hole/Crevice	1.3	0.5
Nest height (cm)	7.2	2.9
Canopy mean	11.7	5.8
Slope (°)	56.4	22.7
Slope macro (°)	2.0	0.8
% Dictyotales	46.5	22.5
% Cystoseira	24.6	20.1
% Jania	18.4	19.5
% Sand	1.5	6.3

## Discussion

We found that the overall density of *S. ocellatus* is comparable with that observed in other studies carried out in other areas of the Mediterranean sea using the visual census technique (Francour, 1997; Mazzoldi & De Girolamo, 1998; Guidetti, 2000; De Girolamo & Mazzoldi, 2001; Guidetti & Bussotti, 2002; Letourneur *et al.*, 2003). The higher density of the ocellated wrasse found associated with rocky substrate than with other substrates was also reported in a one-year study in the Lavezzi Islands (north-western Mediterranean Sea; Mouillot *et al.*, 1999) but only for larger size specimens. A higher number of *S.*





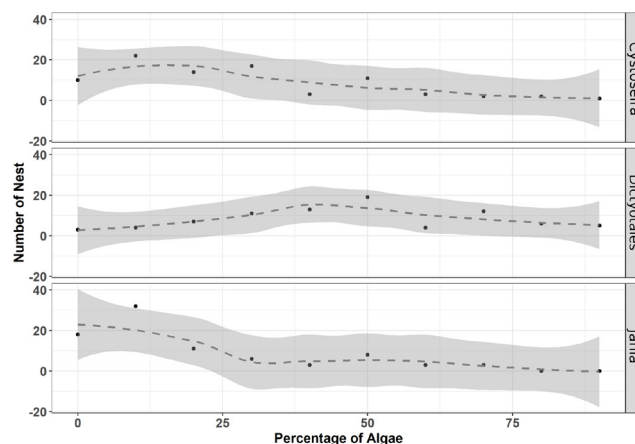
**Fig. 3:** Mean ( $\pm$  standard deviation) depth and size of the hole/crevice and canopy of the 84 studied nests.

**Table 3.** GLM (General Linear Model) results performed on three categorical independent variables. Significance value is given as \*\*\*p value < 0.001, \*\*p value < 0.01, \*p value < 0.05.

Variable	Df	R <sup>2</sup>	Chi <sup>2</sup>	p <sub>value</sub>
Depth Range	2	8.82	0.012	*
Type Of Cavities	1	10.28	0.001	**
Canopy's Height	2	25.78	2.5*10 <sup>-06</sup>	***

*ocellatus* on rocky substrate was also found in the summer in the Frioul Archipelago (north-western Mediterranean Sea; Letourneur *et al.*, 2003), while Lipej *et al.* (2009), in the North Adriatic Sea, found that the density of *S. ocellatus* individuals was higher in rocky vegetated substrates during the reproductive period (June to August). By contrast, Guidetti (2000) found that *S. ocellatus* was more abundant in *Posidonia oceanica* substrate than on rocky substrate, whereas the species was not observed on sandy substrate. The high density of small sized specimens of the ocellated wrasse on seagrass meadows was also found by Mouillot *et al.*, (1999). Guidetti & Bussotti (2002) found high densities of this fish associated with two other seagrasses (*Cymodocea nodosa* and *Zostera noltii*) in the coasts of Sardinia (Northern Tyrrhenian Sea). However, they did not include rocky bottoms in their experimental design.

Our results show that the reproductive phase did not influence the distribution nor the density of *S. ocellatus* along different substrates. No studies have compared the density of this species along different substrate strictly tied to reproductive phases. The higher presence of ocellated wrasse associated with rocky substrate was observed over all seasons in the north-western Mediterranean (Mouillot *et al.*, 1999). The habit of fish to not change the used substrate during the season was already



**Fig. 4:** The abundance of nests and the percentage cover of the three principal algal species around the nests.

**Table 4.** GLM (General Linear Model) results performed on three principal independent algal variables. Significance value is given as \*\*\*p value < 0.001, \*\*p value < 0.01, \*p value < 0.05.

Variable	eDf	Chi <sup>2</sup>	p <sub>value</sub>
<i>Cystoseira</i>	2.97	53.2	***
<i>Dictyotales</i>	2.67	20.86	***
<i>Jania</i>	5.01	93.78	***

recorded by Guidetti (2000), even if in this case fishes remain associated with *P. oceanica*. This contrasts with the needs of the reproductive male of building a nest in the hole of a hard substrate using algal fragment (Sinopoli *et al.*, 2015). The evidence that a part of the population does not live closely associated with rocky substrates could be justified by the fact that not all individuals are involved in reproduction during the nest building period. In fact, based on several factors (e.g., sexual maturity stage or energy reserves accumulated by sexually mature specimens), a portion of the population sometimes skips the reproduction season (Taborsky *et al.*, 1987). This part of the population shows a different pattern of schooling behavior and a different use of substrate (Budaev, 1997). This hypothesis is partially confirmed by Mouillot *et al.* (1999), who found larger individuals (probably sexually mature) associated with the rocky substrate and smaller individuals associated with seagrass.

Despite the comparability of substrate features in terms of presence of patches of rocky, sandy and *P. oceanica* dominance, depths and slope, the high-density zone remains the most populated by the ocellated wrasses over all of the season, including the non-reproductive periods. Other factors can influence the choice of these areas. Francour (1997), sampling on seagrass meadows of different sites, recorded a higher density of *S. ocellatus*

in small gulfs and sites protected from exposure to open water than in sites exposed to wave action. Mazzoldi & De Girolamo (1998) reported a greater abundance of this species in the rocky habitats less exposed to the currents and the wave action compared to open sea sites. Even our high-density sites are located in more sheltered areas. Therefore, it seems that *S. ocellatus* prefers sheltered than wave-exposed sites, independent of the reproductive period. This choice is in agreement with the findings of Raventos (2004), who reported a higher risk of nest destruction in areas with high exposure to waves.

Nest site characteristics were specifically investigated for *Simphodus roissali* in the northwest Mediterranean Sea (Raventos, 2006) and for *S. roissali*, *S. ocellatus*, and *S. cinereus* in North Adriatic Sea (Lipej *et al.*, 2009). In these studies, the authors used an approach similar to the one used in our research. Indeed, to determine the main microhabitat requirements of the nests, they have focused on those features that often recur near nests.

Nesting males of *S. roissali* select nest sites mainly on flat substrata and close to the margin in the rocky littoral strip (Raventos, 2006). A preference for flat substrates was also found by Lipej *et al.* (2009) together with a significant presence of *Cystoseira barbata*. Both authors justified this by suggesting that nesting males actively select sites that are more visible to females. Some similarity was found between the microhabitat characteristics of *S. ocellatus* nesting reported by Lipej *et al.* (2009) and our results. Indeed, in this study, we showed that the choice of site for nest building is related to the presence of brown algae of the order *Dyctiota*les brown algae and algal canopy in the microhabitat. It is difficult to give a functional meaning to this feature of the nesting sites. The reproductive success in nesting fish species might depend on the trade-off between nest concealment (that ensures protection by predators and opportunistic males) and nest visibility (that is crucial for attracting the highest possible number of females) (Lejeune, 1985; Wernerus *et al.*, 1989; Alonzo & Warner, 2000; Uglem & Rosenqvist, 2002). Algal canopy height increases nest camouflage. For *S. roissali*, this effect was compensated because nests were near the margins of the rocky littoral strip. This made nests more visible to *S. roissali* (Raventos, 2006).

The presence of a circular hole in the substrate was identified as an important feature of the nest microhabitat and as preferable to crevices. Circular holes are present in the hard substrates and are the result of the corrosive actions of water movements (Alexander, 1932; Wentworth, 1944). From previous studies (Sinopoli *et al.*, 2015) it was noted that these holes are the ideal initial nucleus for nest construction. This is because the holes help to keep algae together by countering the forces of water motion. Although crevices can be used to build the nest, they do not have the same qualities in retaining the algae.

Our study suggests that a depth between 2.7 and 3.2 m is most suitable for *S. ocellatus* nesting. Lipej *et al.* (2009) reported 5.5 m as the mean depth of the nest presence; however they did not give any functional justification

on the depth of the nests. In our opinion this shallow depth could be related to reproductive physiological constraints. In fact, *S. ocellatus* sexual maturation was optimal when the sea water temperatures were 22–23°C in the Gulf of Naples (Tyrrhenian sea; Bentivegna & Benedetto, 1989). During May and June, in our study areas water temperature begins to increase from the most superficial part directly exposed to solar irradiation. For this reason, the depths between 0 and 4 m reach 20°C first.

Some studies attempted to define the reproductive habitat requirements of nesting fish species, and salient features of the nest sites were highlighted (Raventos, 2006; Lipej *et al.*, 2009). Although a functional role has been attributed to each of these features, no studies have correlated these features with reproductive performance and success. This is because fish are thought to be able to compensate for suboptimal habitats by gains in mating performance (Milazzo *et al.*, 2016).

In conclusion in this study we can reject the first hypothesis since the ocellated wrasse in their pre-reproductive and reproductive periods remain associated with the rocky habitat in which it build nests. We can accept the second hypothesis because of the significant recurrence of four variables (middle sized canopy, around 3 m in depth, circular holes and the availability of *Dyctiota*les) near nests. However, the presence of *Dyctiota*les deserves further reflection. The presence of this algae could be due to co-occurrence, rather than being a strict microhabitat requirement. Indeed, this order of algae is included in the ecological group that is only present at sites that are less exposed to wave action and open water flows (Boudouresque, 1984). This observation confirms that *S. ocellatus* prefers the low-hydrodynamic sites, as observed in other studies (Francour, 1997; Mazzoldi & De Girolamo, 1998). This choice, in addition to the advantage of nest protection from wave motion (Raventos, 2004), decreases the remixing of the water, allowing the temperature to quickly reach optimal levels for the maturation of the gonads (Bentivegna & Benedetto, 1989).

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