ABSTRACT: During the last 2 decades, the widespread temperate gorgonian Eunicella singularis has been among the species most affected by climate-induced mortalities. Recruitment and juvenile survival play crucial roles in the recovery process of this species, but turf algae may affect these early life history processes. We investigated the effects of turf algae on recruitment and juvenile survival of E. singularis using in situ turf-removal and turf-exposure experiments. The experiments were performed at a depth of 15 to 20 m off the island of Menorca (Balearic Islands, NW Mediterranean Sea) between April 2008 and July 2009. The turf-removal experiment indicated that exposure to turf algae caused up to a 5-fold reduction in the recruitment of the gorgonian species. The turf-exposure experiment revealed that transplanted juveniles exposed to turf algae overgrowth lost biomass and exhibited a threefold increase in juvenile mortality. These results demonstrate the negative effects that turf algae can exert on early stages of gorgonian species; in turn, this may affect their recovery capacity and population dynamics. Given that most Mediterranean invasive algae form a persistent turf, an increase in turf algae abundance may exacerbate these negative effects.

KEY WORDS: Turf algae · Gorgonian coral · Early life history stages · Recruitment · Survival · Mediterranean Sea

INTRODUCTION

Marine ecosystems are in serious decline because of overfishing, pollution, habitat destruction, invasive species and climate change (Jackson et al. 2001, Hughes et al. 2003, Halpern et al. 2008). Consequently, a major goal of ecologists has been predicting how these ecosystems will respond to local and global environmental changes. The Mediterranean Sea offers a unique opportunity to address the complexities of cumulative impacts because the rich biodiversity of the region has been affected by multiple stressors, and has changed more rapidly than many other world regions (Coll et al. 2010, Costello et al. 2010, Coma et al. 2011, Calvo et al. in press).

Recruitment is crucial for the persistence and resilience of marine benthic communities, and it is regulated by several early life history processes, including larval supply, settlement and post-settlement survival (Caley et al. 1996, Gosselin & Qian 1997, Hughes et al. 2000). Disturbances that disrupt these early stage processes can result in compromised or failed recruitment, which can profoundly affect future population dynamics (Gaines & Roughgarden 1985). Therefore, it is crucial to investigate the potential impacts of disturbances on the early life history stages of organisms.
Turf algae are dense, multispecies assemblages of filamentous benthic algae that are commonly found on many tropical and temperate intertidal and subtidal shores (Hay 1981, Steneck & Dethier 1994). In recent decades, evidence has emerged of a trend of turf algae increases in relation to enhanced levels of disturbance (e.g. sediment loads and nutrients) in temperate and tropical coastal ecosystems (Airoldi et al. 1995, Airoldi 1998, Vermeij 2006, Vermeij et al. 2010). Furthermore, in the Mediterranean Sea, some of the most invasive species consist of turf-forming macroalgae that are triggering substantial changes in the structure of benthic algal communities (Piazzi et al. 2001, Streftaris & Zenetos 2006). Turf algae have been shown to affect tropical coral recruitment negatively, either through preemption of settlement surfaces or by increasing the risks of post-settlement mortality (Edmunds & Carpenter 2001, Birrell et al. 2005, Vermeij 2006, Arnold et al. 2010, Vermeij et al. 2010). Therefore, we hypothesised that turf algae abundance would have negative effects on the recruitment of temperate sessile invertebrate species.

Gorgonians are emblematic species of sublittoral communities because they are one of the main contributors to the 3-dimensional structure of the community, comprise a major part of its diversity, and play an important role in plankton-benthos coupling (Gili & Coma 1998). However, gorgonians are among the most affected benthic species from escalating climate-related threats that affect the Mediterranean Sea (Coma et al. 2009, Garrabou et al. 2009, Linares & Doak 2010). Although certain gorgonian species exhibit low recruitment rates (Garrabou & Harmelin 2002, Coma et al. 2004, Linares et al. 2007), Eunicella singularis displays a size distribution that is dominated by early life history stages, suggesting that the species is not limited by recruitment (Linares et al. 2008a, Gori et al. 2011). However, mass mortality events have lead to the loss of approximately half of the gorgonian population (Coma et al. 2006). In the face of such large disturbances, larval recruitment is a critical step in the recovery process of gorgonian forests. Brooding and surface brooding are the dominant modes of development in Mediterranean gorgonians (Vighi 1970, Coma et al. 1995a,b, Ribes et al. 2007, Linares et al. 2008b) and contribute to the short-distance dispersal of the species that have been examined to date (Ledoux et al. 2010, Mokhtar-Jamai et al. 2011).

The aim of our work was to use field experiments to investigate the effects of turf algae on the recruitment and survival of juvenile colonies of the Mediterranean gorgonian Eunicella singularis.

MATERIALS AND METHODS

Study site

The present study was performed at 3 locations on the north coast of Menorca within the ‘Nord de Menorca’ Marine Protected Area (Balearic Islands, Spain, NW Mediterranean; Fig. 1). The study area remains largely unaffected by anthropogenic impacts because there are no sources of pollution (e.g. eutrophication, heavy metals, HAPs; Sales & Ballestros 2009, Sales et al. 2011). In addition, fishing has been prohibited since 2000. Eunicella singularis is an abundant species on the north-sloping underwater cliffs of the island, where it dwells in rocky bottom habitats in exposed areas either on walls or on large blocks from depths of 15 to 40 m (Coma et al. 2006).

In 1999, a regional mass mortality event severely affected this species at the study area, causing the
loss of ~46% of the population (Coma et al. 2006). In addition, the Balearic Islands are one of the regions most affected by invasive algae within the Mediterranean Basin (Zenetos et al. 2010). Caulerpa racemosa v. cylindracea, Asparagopsis taxiformis, Womersleyella setacea and Acrothamnion preissi are present in the littoral of Menorca Island, and populations of W. setacea and A. preissi in particular have been observed to develop dense and thick turfs in some habitats (Ballesteros 2004). These turf algae have been documented as threats to sublittoral habitats in various areas of Menorca and on other Balearic islands (Ballesteros 2004). Our assessment of algal biomass and species abundance at both study locations revealed that Haloptilon virgatum (Zanardini) Garbary & Johansen, Peyssonnelia squamaria (Gmelin) Decaisne & Sphacellaria citrosa (Roth) C. Agardh were the dominant native algal species of the turf community, which reached a biomass of ~300 g DW m⁻². The turf community was observed to consist of up to 30% invasive algae (W. setacea and A. preissi, ~60 g DW m⁻²). Within this community, we conducted a turf-removal experiment and a turf-exposure experiment to examine the effects of turf algae on the early life history stages of E. singularis.

Recruitment

We conducted a turf-removal experiment in situ to assess the effects of turf algae on the recruitment of Eunicella singularis. In the early spring of 2008 (before larval spawning; Ribes et al. 2007), we randomly marked 40 quadrats of 40 × 40 cm with plastic screws placed at a depth between 15 and 20 m in each of 2 localities (Cap Roig and Na Ponsa, Fig. 1). We chose a random distance from a spot within the community as a randomisation procedure to determine the place to locate each quadrant. Digital stopwatches were used to produce the random numbers (using numbers at the level of 1/100ths). Divers began at a spot within the community. The divers first chose a random direction (north, south, east or west, according to the numbers 1 to 4) and subsequently chose a random distance (numbers 0 to 9). A 50 cm ruler was used as the unit of distance (i.e. number 5 means 5 × 50 cm or 2.5 m from the initial spot). The 4 corners of each quadrat were marked with plastic screws, one of which was labelled for future identification. The total algal cover was scraped using a paint scraper until clean bare rock was exposed from 20 of the quadrats (the ‘Turf-removal’ experimental treatment). The algal cover was maintained at the other 20 quadrats (the ‘Non-removal’ control treatment). All of the colonies present in each quadrant were individually identified and mapped. At the end of July 2008, we counted in situ the number of new recruits (~3–5 mm height) in each quadrat with the aid of a hand lens.

Survival and fitness

Although desirable, long-term experimental plots to compare recruit survival in plots with and without turf algae were not used for several reasons. First, the rapid dynamics of turf algae (Airoldi 2000) make the maintenance of such experiments logistically difficult. Second, the periodic eradication of turf may affect the survival of gorgonian recruits. Third, periodic turf eradication favours the development and growth of invasive turf species (Klein & Verlaque 2011). Therefore, between July 2008 and June 2009, we estimated in situ the survival of post-recruitment Eunicella singularis colonies within 40 plots selected in both of the study locations (Cap Roig and Na Ponsa, Fig. 1), all of which were exposed to the natural overgrowth of the turf algae present at the study area.

We conducted a turf-exposure manipulative experiment to examine the effects of overgrowth of turf algae on juvenile colonies of Eunicella singularis. Small apical tips (<5 cm), each of which was obtained from a different colony, were placed in 2 different environments: ‘non-overgrowth’ (i.e. not exposed to contact with turf algae; experimental treatment) and ‘turf-overgrowth’ (i.e. exposed to overgrowth by turf algae; control treatment). The apical colony tips of 80 healthy E. singularis were haphazardly sampled at Cova de Ses Bruixes between 15 and 20 m of depth (Fig. 1). Each colony’s apical tip (hereafter known as colony) was attached to the experimental plates (Fig. 2). These plates were built from 3 PVC disks (each 10 cm in diameter). Two rubber layers were used to separate the PVC plates. Each PVC disk had 8 holes (1 cm in diameter) for the colonies. The entire set was held together with screws. The colonies were attached to the experimental plates without putty but were held mechanically simply by perpendicular cuts that had been previously made in the rubber layers (one cut per layer). Each experimental plate had labels to distinguish the experimental treatments and colonies within the plate. Forty colonies were placed in 5 experimental plates (8 per plate), and 40 colonies were placed in 5 control plates. The sampled colonies were randomly attached to the plates under-
water by divers less than an hour after collection. The 5 control plates were kept on a support slightly above the rocky substrate (‘non-overgrowth’) at the minimum distance needed to prevent overgrowth by turf algae and limit the exposure to variation in environmental conditions to those occurring on the substrate (previous preliminary experiments had been conducted to determine this distance). The other 5 plates were installed such that they would be completely attached to the rocky substrate leading to the turf algae overgrowth (‘turf-overgrowth’, Fig. 2).

Special care was taken to distribute all of the plates randomly within the same area (separated <1 m) and at the same depth to expose all of the colonies to similar environmental conditions. The experiment lasted from April 2008 to July 2009. At the end of the experiment, we calculated the survival rate and the mean diameter of the colonies that survived on each of the 5 experimental and 5 control plates. To analyse the data, we did not take into account the detachment of a transplanted colony from the experimental plate (~10% on both treatments and occurring shortly after the beginning of the experiment) because we considered these events to indicate methodology failures in contrast to the mortality caused by turf algae that resulted in the loss of the living tissue of the colony.

The mean diameter of the colonies was used as an indicator of gorgonian fitness based on the relationship between biomass and diameter. Biomass has been shown to be a good indicator of gorgonian fitness because biomass falling below a critical threshold indicates the first appearance of partial mortality (Coma et al. 2009). At the end of the experiment, 10 apical tips from the experimental plates (which were not exposed to algal turf) and 10 from the control plates (which were exposed to algal turf) were collected. Their diameters were measured in the laboratory using a micrometer under a stereomicroscope. It should be noted that although we installed 40 apical tips at each treatment, the low number of colonies that was used was due to the low survivorship obtained from the control plates.

**Statistical analyses**

The effects of treatment and location on gorgonian recruitment were assessed using a 2-way ANOVA. Alternatively, a t-test was used to examine the differences in survival and diameter between the experimental and control colonies at the end of the turf-exposure manipulative experiment. The values of survival from the turf-overgrowth treatment found in the manipulative experiment were also compared with the in situ survival of small colonies obtained by monitoring the installed plots using a t-test. For all of the variables, the homogeneity of variances was verified by Cochran’s C-test, and the normality was assessed by the Kolmogorov-Smirnov test prior to the analysis. If the data did not follow a normal distribution, the Wilcoxon rank sum test was used. All of the statistical analyses were performed in Statistica 8.0.
RESULTS

Recruitment

At both study locations, recruitment was significantly higher on those quadrats exposed to the ‘Turf-removal’ treatment (experimental) than on those exposed to the ‘Non-removal’ treatment (control; 2-way ANOVA, p < 0.01). Turf algae removal increased recruitment approximately 5-fold at the Cap Roig location. The recruitment rates at Cap Roig were also 4-fold higher than those obtained at Na Ponsa (site; 2-way ANOVA, p < 0.05). Within the ‘Turf-removal’ quadrats, the mean number of recruits recorded in Cap Roig and Na Ponsa were 14.7 ± 15.5 (±SD) recruits m$^{-2}$ and 2.7 ± 2.2 (±SD) recruits m$^{-2}$, respectively (Fig. 3). Notably, no recruitment was recorded in the Na Ponsa location control quadrats (Fig. 3).

Survival and fitness

Three months after the beginning of the experiment, the control plates were overgrown by turf algae in a manner similar to that observed in the nearby benthic community, whereas the experimental plates were not overgrown. Periodic inspections indicated that the turf algae did not overgrow the experimental plates throughout the manipulative experiment. At the end of the manipulative experiment, 90 ± 6% (mean ± SD) of the transplanted colonies survived under the non-overgrowth conditions. In contrast, the survival of transplants exposed to the overgrowth of turf algae was 30 ± 7% (mean ± SD). Thus, the manipulative experiment demonstrated that the survival rate of juvenile colonies exposed to overgrowth by turf algae was 3-fold lower than that of unexposed colonies (t-test, p < 0.01; Fig. 4a). The manipulative experiment also indicated that Eunicella singularis colonies exposed to turf algae overgrowth displayed a diameter that was 23% smaller (2.1 ± 0.4 mm, mean ± SD) than that of unexposed colonies (2.8 ± 0.4 mm, mean ± SD) (t-test, p < 0.01; Fig. 4b).

The survival rate of small colonies on the 40 non-manipulated plots (i.e. those exposed to turf algae) between July 2008 and June 2009 did not vary between locations (39 ± 38% in Cap Roig vs. 12 ± 25% in Na Ponsa; t-test, p = 0.25) and exhibited a mean value of 26 ± 18% (mean ± SD). The survival rate of colonies in these non-manipulated plots did not differ from that observed of the colonies exposed to overgrowth by turf algae in the turf-exposure manipulative experiment (t-test, p = 0.88).

DISCUSSION

This study offers the first quantitative data on the effects of turf algae on the process of recruitment of a Mediterranean marine invertebrate, specifically, the widespread gorgonian Eunicella singularis. Our
results demonstrate that turf algae have the capacity
to affect gorgonian population dynamics by reducing
recruitment and juvenile survival, as well as by
inhibiting gorgonian growth.

Differences in recruitment between the study loca-
tions are probably related to the spatial variability in
the recruitment of gorgonian species (Gotelli 1988).
However, the turf-removal experiment produced a
marked increase in recruitment, indicating that the
presence of turf algae sharply reduced the recruit-
ment of *Eunicella singularis*. The effects of turf algae
on the gorgonian recruitment appear to depend on a
decrease in larval access to suitable substrata for
settlement and on the effect of physical stress of algal
overgrowth on the colonies, as has been observed in
coral reefs (Arnold et al. 2010).

The turf-exposure experiment demonstrated that
the survival of juvenile gorgonians exposed to the
turf algae was 3-fold lower than that of the unex-
posed juveniles (30% vs. 90%, respectively). The survival rate of juvenile colonies exposed to turf
algae in the turf-exposure experiment did not differ
from that exhibited by the small colonies in the non-
manipulated plots (~25%). The reduction in biomass
of the juvenile colonies exposed to turf algae from
the turf-exposure experiment suggests that turf algae
might reduce juvenile colony survival through the
physical stress that algal overgrowth exerts on the
gorgonian, which affects its feeding capacity and
leads to a loss of biomass. These effects jeopardise
the viability of the gorgonian because tissue mortal-
ity has been observed to begin after a threshold of
biomass loss has been reached (Coma et al. 2009).

The negative effects of turf algae on the examined
gorgonian species are similar to those documented
for other coral species (Birrell et al. 2005, Vermeij &
Sandin 2008, Arnold et al. 2010). However, as high-
lighted in other studies, other mechanisms could be
involved in the observed negative effects caused by
the turf algae beyond the physical effects of over-
growth and space occupation on the recruitment and
survival of gorgonians, such as allelopathic inter-
actions of macroalgae and cyanobacteria with corals
(Kuffner et al. 2006), or the effects of algae-asso-
ciated pathogens (Nugues et al. 2004).

The lack of data on the recruitment and survival
rates of *Eunicella singularis* in areas without turf
algae prevented us from contrasting the results
obtained in the present study with those obtained
from other locations and areas. However, in contrast
to other Mediterranean gorgonians, which typically
exhibit low recruitment rates (Garrabou & Harmelin
2002, Coma et al. 2004, Linares et al. 2007), the size-
frequency distribution of various *E. singularis*
populations obtained in previous studies indicates a high
recruitment rate and a high post-settlement survival
of the species (Linares et al. 2008a, Gori et al. 2011).
Consequently, the present study shows that an
increased abundance of turf algae may negatively
affect gorgonian population dynamics by reducing
recruitment and juvenile survival. These effects
would slow down the recovery capacity of the popu-
lations and reduce the long-term resilience of gor-
gonian forests, especially those exposed to recurrent
disturbances.

The negative effects on the early life stages of
*Eunicella singularis* were found in the presence of a
biomass of turf algae that reached a value of approx-
imately 300 g DW m⁻², from which the invasive algae
*Womersleyella setacea* and *Acrothamion preissii*
accounted for ~30% of the biomass. This biomass of
turf algae was high compared to those observed in
other Mediterranean locations (i.e. 90–150 g DW m⁻²
at Calafuria, Airoldi 2000), but similar values are
commonly observed in areas that have been densely
occupied by invasive turf species (i.e. 500 g DW m⁻²,
Cabrera National Park, Balearic Islands, Cebrian &
Ballesteros 2009, 2010).

Native Mediterranean turf algae exhibit a seasonal
reduction of turf cover and biomass during the winter
months (Airoldi 2000). In contrast, this pattern does
not occur in invasive species, such as *Womersleyella
setacea* (Rodriguez-Prieto et al. 2010), which persist
throughout the year. In some Mediterranean locali-
ties, various turf-forming invasive species can consti-
tute up to 80% of the algal biomass (e.g. Scandola
Nature Reserve, Corsica; authors unpubl. data). The
replacement of perennial canopy-forming algae by
opportunistic taxa, such as turf-forming algae, affects
not only temperate areas, such as the Mediterranean,
but is also a source of concern in tropical ecosystems
(Krause-Jensen et al. 2008, Vermeij et al. 2010). En-
hanced loads of sediment in coastal habitats and
water eutrophication have been identified as the
main drivers of the shift from the dominance of
canopy-forming species to algal turfs (Airoldi et al.
1995, Benedetti-Cecchi et al. 2001, Gorgula & Con-
nell 2004). Although the effects of algal turfs on
macrophyte assemblages have been explored in tem-
perate systems (Kennelly 1987, Airoldi 1998, Gorman
& Connell 2009), the outcome of the interactions
between turf algae and marine invertebrates remains
largely unknown. An increase in turf algae abun-
2010), in combination with the fact that some of the
most invasive Mediterranean algae form a thick and
persistent turf over the substratum, might indicate an increase of the potential negative effects of turf algae on the population dynamics and recovery capacity of *Eunicella singularis* and, probably, of other benthic invertebrates.

**Acknowledgments.** The authors thank Dr. E. Ballesteros for his help in algal identification. We also thank Dr. J. Garrabou and C. Marchal for providing the PVC disks for gorgonian transplants. We are grateful to the entire staff of the ‘Reserva Marina del Nord de Menorca’ and ‘Direccio General de Pesca del Govern de les Illes Balears’ for providing logistic support. Financial support was provided by the ‘Conseil Insular de Menorca’ and the ‘Ministerio de Ciencia e Innovació (MCI)’ projects CGL2010-18466 and CTM2009-08045. C.L. and E.C. were funded by a Juan de la Cierva Postdoctoral Fellowship from the MCI and are part of the Marine Conservation research group (2009SGR174); R.C. is a member of the Marine Biogeochemistry and Global Change research group (2009SGR142) from the Generalitat Catalunya.

**LITERATURE CITED**


Jackson JBC, Kirby MX, Berger WH, Bjorndal KA and others (2001) Historical overfishing and the recent collapse of coastal ecosystems. Science 293:629−637


Editorial responsibility: Matthias Seaman, Oldendorf/Luhe, Germany

Submitted: June 10, 2011; Accepted: January 5, 2012

Proofs received from author(s): April 5, 2012

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