



## Evaluating potential artifacts of tethering techniques to estimate predation on sea urchins



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### ABSTRACT

Measuring the strength of trophic interactions in marine systems has been central to our understanding of community structuring. Sea urchin tethering has been the method of choice to evaluate rates of predation in marine benthic ecosystems. As standardly practiced, this method involves piercing the urchin test, potentially introducing significant methodological artifacts that may influence survival or detection by predators. Here we assess possible artifacts of tethering comparing invasive (pierced) and non-invasive tethering techniques using the sea urchin *Paracentrotus lividus*. Specifically, we looked at how degree of confinement and high water temperature (first order artifacts) and predator guild and size of the prey (second order artifacts) affect the survival and/or detectability of pierced urchins. Our results show that first order artifacts only arise when pierced sea urchins are placed in sheltered bays with confined waters, especially when water temperature reaches extremely high levels. Prey detectability did not increase in pierced sea urchins for the most common predators. Also, test piercing did not alter the preferences of predators for given prey sizes. We conclude that the standard tethering technique is a robust method to test relative rates of sea urchin predation. However, local conditions could increase mortality of the tethered urchin in sheltered bays or in very high temperature regimes. Under these conditions, adequate pierced controls (within predator exclusions) need to be included in assays to evaluate artifactual sources of mortality.

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### 1. Introduction

Measuring the strength of trophic interactions has been central to our understanding of community structure (Estes and Palmisano, 1974; Paine, 1966). Estimating predation and its effects is critical to understand the ability of predators to control prey populations (Estes et al., 2011). This is especially important in marine systems, where such control often trigger cascading effects. While directly measuring rates of predation in real world ecosystems is generally unfeasible, researchers have developed assay techniques to obtain relative estimates that can integrate longer periods of time and avoid observer artifacts (Hairston, 1989). This has been done with the assumption that these techniques can serve, at the very least, as relative indices of actual predation rates that can still give valuable ways to compare ecosystems or track changes through time. In marine systems, measures of predation have relied heavily on tethering techniques, often using sea urchins as a model prey (McClanahan and Muthiga, 1989). In addition, sea

urchins are often themselves keystone herbivores in rocky reefs, coral reefs, seagrass meadows, and kelp forests. When sea urchin outbreaks take place, these communities can shift to a less productive and diverse state—termed “urchin barrens” (Pinnegar et al., 2000). In this context, estimating the ability of predators to control urchin numbers is critical to understand ecosystem functioning (Clemente et al., 2007; Farina et al., 2014; Heck and Valentine, 1995; Heck and Wilson, 1987; McClanahan, 1999; McClanahan and Muthiga, 1989; Pederson and Johnson, 2006; Shears and Babcock, 2002). Tethering experiments can provide insight on the degree to which differences in predation rates between different localities contribute to barren formation through cascading effects (Clemente et al., 2008). Nevertheless, these assays are artificial by design and invasive in their manipulation. It has, thus far, been difficult to assess how prone they are to methodological artifacts, precluding thus the evaluation of their reliability.

Tethering techniques have been extensively used in experimental ecology as a tagging and constraining technique to assess predation for different species in various ecosystems and conditions (Aronson, 1987; Herrnkind and Butler, 1986; Shulman, 1985; Watanabe, 1984; Wilson et al., 1990; Witman, 1985). This method consists of marking

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and restraining target prey for a known period of time in natural conditions and documenting mortality. While it is commonly used in invertebrates, it has some disadvantages (Aronson and Heck, 1995; Peterson and Black, 1994). Individuals can be tagged by using different tethering techniques depending on the targeted prey, and some methods that clearly restrain the movement of the tethered individual can substantially increase the encounter rate by certain predators (Barbeau and Scheibling, 1994). The most effective and commonly used tethering methods involve piercing the target organism with a hypodermic needle. For instance, with sea urchins, this involves piercing the test from the oral to the aboral region and passing a monofilament line through the skeleton, which is then used as a tether (Ebert, 1965). Although sufficient care is taken not to affect the gonads inside the carcass, this procedure is still invasive and has a number of potential associated artifacts, which Peterson and Black (1994) have classified as first and second order artifacts. First order artifacts can arise if the wound caused by piercing increases the probability of infections under different environmental conditions; increased temperatures, pollution or nutrient levels, wave flushing, and other local factors could interact strongly to influence the disease susceptibility and survival of sea urchins (Girard et al., 2012; Lafferty et al., 2004), and likely also that of pierced organisms. In addition, second order artifacts could result from the leaking of coelomic fluids into the water column. These fluids could potentially act as chemical clues for certain benthic predators (Sloan and Northway, 1982; Valentinčič, 1973) increasing prey detectability, but not for others that base their predation on a more visual search. These biases can clearly affect the comparative estimates of predation when predator guild differs between sites. Despite these limitations, pierced tethering continues to be the most commonly used method to estimate comparative predation rates or predation risk in marine systems (Aronson and Heck, 1995). To reduce possible artifacts, some authors held tethered urchins in the laboratory for a period of time to allow urchins to heal as monitoring mortality revealed that field survival rates of tethered urchins were higher if they were maintained some days under laboratory conditions prior to using them in field experiments (Fagerli et al., 2014; Shears and Babcock, 2002), but often, this is unfeasible when using this field assay far from laboratories. Still, there have been a few attempts, although incomplete, to evaluate the possibility, magnitude, and sources of biases appearing as a result of first and second order artifacts due to

this experimental manipulation (McClanahan and Muthiga, 1989; Shears and Babcock, 2002).

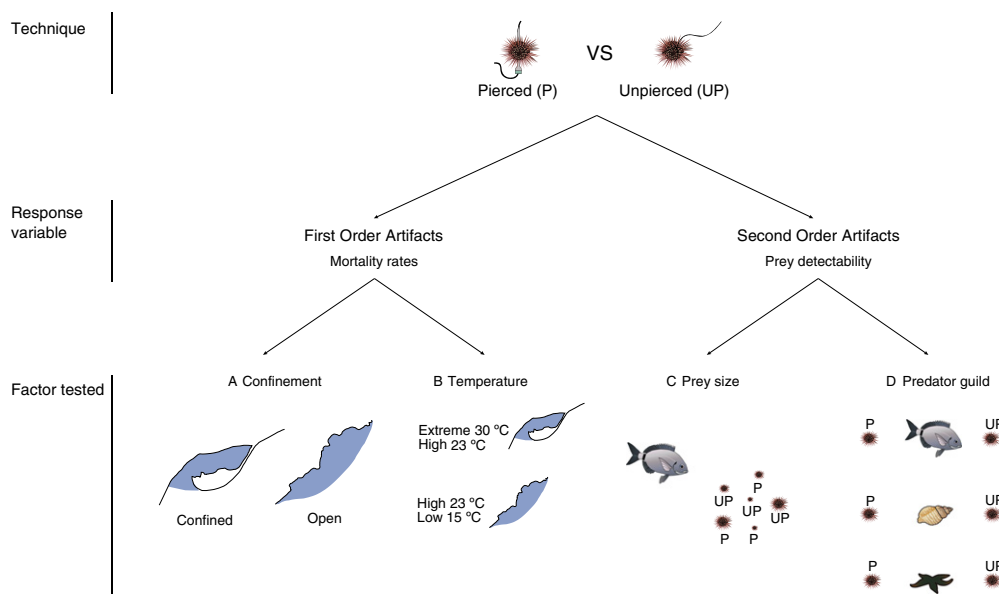
In this study, we investigate possible artifacts of tethering techniques, using the sea urchin *Paracentrotus lividus* (Lamarck), a key-stone herbivore in Mediterranean ecosystems. Pierced tethering has been employed extensively in this species and has been used to examine the importance of predation on *P. lividus* (Guidetti and Sala, 2007; Sala and Zabala, 1996), the importance of habitat-engineering species in providing refuge from predation (Farina et al., 2009), and the existence of indirect interactions between herbivores and predators in seagrass systems (Pagès et al., 2012), among others. In this work, we analyze, first, whether test piercing affects prey survival under different environmental conditions (first order artifacts), and second, whether this tagging technique enhances prey detectability under different sizes of the prey or for the most common predators (second order artifacts).

## 2. Materials and methods

### 2.1. Sampling design

We designed a series of four separate experiments to test if the pierced tethering method applied to the sea urchin *Paracentrotus lividus* modify mortality rates and prey detectability. For first order artifacts, we conducted two experiments using predator exclusion cages to test the effect of a) degree of confinement (Fig. 1, A) and B) water temperature as factors increasing sea urchin mortality after piercing (Fig. 1, B). For the second order artifacts, we conducted two experiments: c) one to test the effect of pierced tethering on observed predation success for different prey sizes (Fig. 1, C) and the second to test d) the effect of pierced tethering in modifying prey detectability as a function of the predator guild (fish, gastropods, and sea stars, Fig. 1, D).

For all experiments, pierced urchins (P) where threaded according to the common methodology described for the target sea urchin species (Sala and Zabala, 1996). Unpierced urchins (UP) were used for the first order artifacts as controls. For the second order artifacts, unpierced urchins (UP) were restrained with a line directly wrapped around the sea urchin body twice and then tied to a weight or to experimental cages. This tagging method is useful to tether sea urchins for short



**Fig. 1.** Schematic description of the methodology used and the experiments developed to test tethering artifacts. Experiments are classified according to the explored variable A) confinement degree (under fixed temperature conditions; 23 °C), B) seawater temperature (for confined and opened conditions) for which we analyzed the prey mortality, and C) prey size, and D) predator guild type for which we analyzed prey detectability. In section A) and B), we present a drawing of the study sites; coastline (black line) and the water (shadow area) to show differences in the confinement degree of each site.

periods of time and keep them within the experimental area but is not useful for longer experiments as they manage to escape. All the experiments were conducted under field conditions rather than in the laboratory, since tethering methods are only relevant for *in situ* experiments and measures of predation rates.

## 2.2. First order artifacts/direct sea urchin mortality: confinement and water temperature

### 2.2.1. Confinement effect

We chose a site representative of an open Mediterranean coast (Fenals, 41° 41' 23" N, 02° 49' 42" E, total surface ca. 92 ha, aperture distance ca. 2500 m, maximum summer temperature ~23 °C) and an area with limited exchange with the open sea (Alfacs Bay, 40° 36' 38" N, 00° 39' 37" E, total surface ca. 3000 ha, aperture distance ca. 2500 m, maximum summer temperature ~30 °C) to assess the confinement effect (Fig. 1, A). Mortality was measured for 48 pierced urchins, 32 of them were placed in Alfacs Bay and the other 16 in Fenals under the same temperature conditions (23 °C). Thirty-two unpierced urchins were used as controls, 16 in each site. All pierced and unpierced sea urchins were placed in groups of 4 in 1.5 cm mesh exclusion cages (50 cm × 20 cm × 20 cm) at 1 m depth in Alfacs Bay and at 8 m depth in Fenals and tracked for 12 days. We test for the significance of differences in sea urchin mortality between treatments (pierced and unpierced) in the two sites using one-way ANOVA in the statistical software R (R Development Core Team, 2013).

### 2.2.2. Water temperature effect

For the temperature experiment (Fig. 1, B), we compared pierced sea urchins with unpierced ones (controls) during high temperature conditions in summer (23 °C) and during low temperature conditions in spring (15 °C) in Fenals. We also checked the effect of extreme high conditions of temperature that occur only in very confined areas in the Mediterranean. To do this, we compared mortality of pierced and unpierced urchins in extreme high temperatures in summer (30 °C) and high temperatures in spring (23 °C) in Alfacs Bay. A set of 16 sea urchins was pierced using a 0.8 mm needle and 16 unpierced sea urchins were used as controls in each site and temperature condition. Urchins were placed in 1.5 cm mesh exclusion cages in groups of 4 (50 cm × 20 cm × 20 cm) at 1 m depths in Alfacs Bay and at 8 m depths in Fenals and tracked for 12 days. We used one-way ANOVA to test for differences in mortality between treatments (pierced and unpierced) in the two sites and temperature conditions.

## 2.3. Second order artifacts/prey detectability: prey size and predatory guild

### 2.3.1. Prey size effect

To test if the treatment (pierced versus unpierced) influenced predation success by fish (Sala, 1997) depending on prey size (small; 1–3 cm diameter without spines, TD, medium; 3–5 cm TD, and large individuals >5 cm TD) (Fig. 1), we performed an experiment in Medes Islands MPA (42° 02' 47" N, 03° 13' 11" E) where predation impacts on sea urchin populations is known to be very high (Hereu et al., 2005). The experiment was conducted during daylight hours because nocturnally active urchin-feeding fishes are uncommon (Sala, 1997; Savy, 1987). A total of 90 sea urchins were used for this experiment: 30 small, 30 medium, and 30 large. Test diameters were measured with a caliper to determine size classes. Pierced and unpierced urchins were tethered to a 1 kg weight and randomly distributed on a macroalgal habitat (5 m depth) within a total rocky area of around 330 m<sup>2</sup> to avoid transmission of the chemical clues due to currents or waves action. The principal predator of this urchin is *Diplodus sargus* (Sala, 1997); large individuals of this species can consume the entire range of *P. lividus* sizes, while smaller fishes are potential consumers of only juvenile sea urchins (Sala, 1997). The experiment was repeated on 3 different days; on each day a total of 5 individuals of each size class

were pierced (P) and other 5 unpierced (UP) were used as controls. Three experienced divers were responsible for visual observations to track the experiment from a certain distance to avoid biasing the information. Each experiment was terminated when 50% of the total initial urchins (P + UP) had been eaten by fish (average time around 45 minutes), and the percentage of both P and UP eaten in each size class were recorded. Generalized Linear Models (GLMs) with binomial distributions were used to evaluate predation impact. The state of the urchin (dead/alive) was analyzed as the response variable. Explanatory variables selected were 'size' (S, M, L) and 'treatment' (P/UP). These analyses were developed using the R software (R Development Core Team, 2013).

### 2.3.2. Predatory guild effect

Prey detectability of pierced (P) and unpierced (UP) sea urchins was assessed for the principal urchin predators (fish, gastropods, and starfish, Fig. 1, D) (Boudouresque and Verlaque, 2001). We used guild-specific methods to assess prey detectability by each one of these organisms according to response times and behaviors (see below). We used Wilcoxon matched paired tests using R software (R Development Team, 2013) in order to determine prey detectability for fish and benthic predators (see below).

Prey detectability by fish: To test if pierced tethering enhances prey detectability by fish, predation was monitored on 24 sea urchins (3–5 cm of test diameter, TD), of which 12 were pierced and 12 were unpierced, using underwater video cameras. We used medium size urchins as this is the main targeted size by fish predators (Sala, 1997). The experiment was done in Medes Islands MPA, where the density of predatory fish is very high. GoPro Hero 2 (10MP, FullHD) cameras were placed in front of pierced and unpierced urchins randomly distributed in a rocky macroalgal habitat. The experiment was done on three different days (8 cameras were placed each sampling day). For each video, predator species were identified, and the time of first attack was measured as a proxy of detectability, with shorter attack times indicating faster detection.

Prey detectability by gastropods and starfish: To test if pierced tethering affects prey detectability by benthic invertebrate predators, experiments were conducted at locations where gastropods (*Hexaplex trunculus*) and starfish (*Marthasterias glacialis*) were abundant (Alfacs Bay and Fenals, respectively). Predation rates of these predators are very low, so rather than depending on random, low-probability encounter rates, we placed one predator and two sea urchins (one pierced and one unpierced) into 1.5 cm mesh cages and evaluated the detectability of each predator for each type of urchin. We did prior assessments to analyze mobility of the benthic predators to determine the size of the cages and the variable to be measured. We observed that *M. glacialis* followed a less directional path compared with *H. trunculus* that presented a more ballistic movement to the prey. According to the predator behavior, we used different cage sizes and different variables to test preferences for pierced and unpierced sea urchins. For *H. trunculus*, we deployed 20 cages measuring 50 × 20 cm in Alfacs Bay, while for *M. glacialis*, we used 6 cages of 100 × 30 cm, deployed in Fenals. One pierced and one unpierced sea urchins (3–5 cm TD) were placed at each side of the cage while the benthic predator was placed in the center. The side for the pierced and the unpierced urchins was randomly selected for each trial to avoid biases to a particular direction due to currents or waves. For the *H. trunculus* experiments, prey preference was estimated as the number of times the predator was found at each of the sides of the cage that had either a pierced or an unpierced urchin after 30 minutes of visual observation. We expressed the variable as a percentage of the total number of observations. If the gastropod remained at the center of the cage (10 cm wide), it was recorded as no preference and not included in the analyses. For the *M. glacialis* experiments, we estimated prey preference by video recording the time the predator spent in the cage area near the pierced or the unpierced urchin, expressed as percentage of total time in the cage. The time that sea

stars spent at the center of the cage (20 cm wide) was recorded as no preference.

### 3. Results

#### 3.1. First order artifacts: increased mortality due to manipulation

Mortality of pierced urchins was significantly affected by the degree of confinement (Table 1,  $p = 0.024$ ). Mortalities (around 20%) were found exclusively for pierced urchins in confined waters from the sheltered Alfacs Bay compared with no mortality (0%) in the exposed location Fenals (Table 1). Unpierced sea urchins (UP, control) did not show any mortality (0%), even inside the bay, indicating that the mortality observed was a direct result of the manipulation (piercing). Extreme high temperature also significantly increased mortality in pierced urchins in the confined site (Table 2,  $p = 0.001$ ). Around 60% of pierced sea urchins died in extreme conditions of high temperatures (30 °C). Once again, this mortality was attributable to the combination of piercing and temperature, since controls (unpierced sea urchins) did not show any mortality. Meanwhile, both pierced and unpierced sea urchin controls under the high (23 °C) and low (15 °C) temperatures in open coast (Fenals) did not show any mortality.

#### 3.2. Second order artifacts: increased mortality due to a higher detection rate by predators

Prey size significantly influenced predation rate by fishes (Table 3;  $p = 0.001$ ). Smaller sea urchin sizes were more vulnerable to predation (Fig. 2). The highest predation rates were found for small (~80%), closely followed by medium (~75%), while large urchins attracted the lowest overall predation (~30%) (Fig. 2). Nonetheless, the influence of treatments (pierced versus unpierced) on predation rates was not significant regardless of the size class considered (Table 3). Of the three experiments designed to detect guild-specific differences in detection rate (or mortality) of predators between pierced and unpierced sea urchins, we recorded only a slight preference for unpierced urchins by gastropods, while no trend was observed for fish and starfish (Table 3). Fish chose equally pierced and unpierced urchins (Fig. 3). Only half the *Hexaplex trunculus* showed a preference either for pierced or unpierced urchins. Of the individuals that did display preference, unpierced urchins were slightly preferred (Fig. 3;  $p = 0.04$ ). Of the total experimental time in which individuals occupied the areas with the urchins, *Marthasterias glacialis* spent the same amount of time in pierced than in the unpierced sea urchins areas (Fig. 3;  $p = 0.81$ ).

### 4. Discussion

Although pierced tethering has been criticized as a tagging method because of the possibility of introducing artifacts by altering either prey mortality or predator behavior, our study shows that first order artifacts, linked directly to prey mortality due to experimental manipulation, only arise under very specific conditions of extreme high temperature or very low water turnover rate, both of which occur in confined waters, such as closed bays. In contrast, along exposed coastlines and under normal high (summer) temperature conditions, piercing and tethering the sea urchins do not induce any mortality. Interestingly, second order artifacts,

**Table 2**

Temperature effect (first order artifacts). Mortality found under each temperature condition in confined and open waters, expressed in percentage for the whole experimental period. SE: Standard error.  $p$  values of one-way ANOVA tests for differences between pierced and unpierced urchins in each experimental condition.

	Extreme (30 °C)					High (23 °C)				
	Pierced		Control		ANOVA	Pierced		Control		ANOVA
	Mean	SE	Mean	SE		Mean	SE	Mean	SE	
Confined	59%	9%	0%	0%	0.001	22%	6%	0%	0%	0.024
	High (23 °C)					Low (15 °C)				
	Pierced		Control		ANOVA	Pierced		Control		ANOVA
	Mean	SE	Mean	SE		Mean	SE	Mean	SE	
Open	0%	0%	0%	0%	–	0%	0%	0%	0%	–

arising because of increased prey detectability by predators, also do not represent an important confounding effect when applying this technique. Taken together, we demonstrate that tethering sea urchins with piercing is a reliable and robust method for assessing comparative predation rates in *Paracentrotus lividus* for most common conditions in the Mediterranean. The present study is, to the best of our knowledge, the first to explicitly examine the possible artifacts that could bias results when using such a method and provides support for earlier and future studies using these techniques for field-based estimates of predation.

Our results indicate that only the effects of water confinement and unusual extreme high water temperature appear to significantly increase prey mortality after piercing, while in most common conditions, high temperature seems not to affect mortality rates. In large areas of the North Western Mediterranean, summer temperatures do not surpass 23 °C ( $\pm 1$  °C) (data since 1969 from Josep Pascual; [www.meteoestartit.cat](http://www.meteoestartit.cat)) except in shallow bays with high degree of confinement where seawater temperature can register values up to 30 °C. However, when working in other areas, where temperatures can cross this limit even under exposed conditions, these second order artifacts are likely to be important. For instance, exposed waters in the Eastern Mediterranean, or areas in the South Western Mediterranean, may standardly experience higher summer temperatures, and experiments done here should consider the possible by-side effects of piercing. We believe that under extreme temperatures and in high confined areas, keeping sea urchins under control after piercing and using the ones that resist these effects to test predation will improve the method. Mortality of sea urchins due to piercing was accompanied by an evident deterioration in the body's external tissues and a loosening of the spines. Recent studies have demonstrated the negative effects of an increase in sea water temperature on the ability of urchins to resist pathogens, and a positive effect of waves and water renewal that diminish their susceptibility to disease (Clemente et al., 2014; Girard et al., 2012). It is also possible that the mortality found in confined waters would also be associated with pathogens, even at normal temperatures with increased abundance of pathogens. There is a high diversity of pathogens that can cause urchin diseases, making it difficult to identify the exact cause of elevated mortalities in such conditions.

Interestingly, in our work, none of the studied predator species preferred pierced urchins, which, *a priori*, were more likely to emit chemical cues. Only the *H. trunculus* showed preferences for unpierced urchins (10% more than pierced urchins), but low significance was found in statistical analysis. This does not, however, imply that these predators were unable to detect and respond to chemical cues but that differences between the emitted signals by a pierced and unpierced sea urchin were undistinguishable for the predator under natural field conditions. In fact, it is known that benthic predators, such as gastropods and starfish, are cryptic chemotactic organisms, relying heavily on chemical cues for their predation success in highly structured habitats such as seagrass meadows (Farina et al., 2014). However, the lack of preference for

**Table 1**

Confinement effect (first order artifacts). Mortality found at 23 °C, expressed in percentage for the whole experimental period in the confined site (Alfacs Bay) and in the open site (Fenals). SE: Standard error.  $p$  values of one-way ANOVA test for differences between pierced and unpierced urchins in each confinement condition.

Confined					Open				
Pierced		Control		ANOVA	Pierced		Control		ANOVA
Mean	SE	Mean	SE		Mean	SE	Mean	SE	
22%	6%	0%	0%	0.024	0%	0%	0%	0%	–



**Table 3**

Second order artifacts analyses. Generalized Linear Models (GLM) analysis for predation according to treatment (pierced and unpierced) and size (juveniles, young adults and adults). We present the initial complete model and the selected one after a stepwise process (AIC criterion). Wilcoxon matched paired test for preferences of each predator guild (fish, gastropods, and sea stars). Significant p values are presented in bold for each of the analyses.

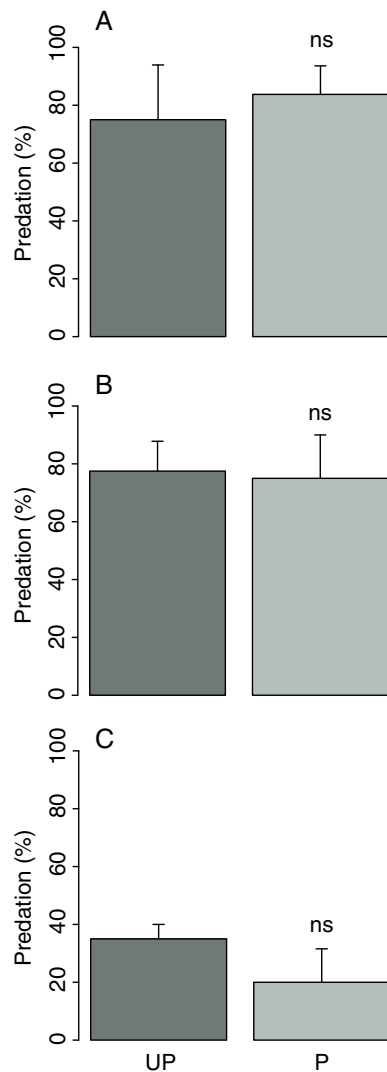
Analyses	Type of artifact	Model	Selected	Effects	Chisq	p
GLM	Predation	Status ~ Treatment * Size (binomial)	Status ~ Size (binomial)	Size Treatment	25.814 0.370	<b>0.001</b> 0.543
Wilcoxon	Preferences	Fish	–	–	–	0.962
		<i>Hexaplex trunculus</i>	–	–	–	<b>0.037</b>
		<i>Marthasterias glacialis</i>	–	–	–	0.809

pierced urchins indicates that second order artifacts are minimal for fish and benthic predators. This was also true for the main fish predator observed in video trials; the prey detection time of the sparid *Diplodus sargus* was not influenced by piercing. Once the urchin was detected and preyed on, several other fish species were attracted to the kill, and scavenged of it (i.e. *S. aurata*, *D. vulgaris*, and *L. merula*). This has been previously described in other studies in the Mediterranean (Guidetti, 2004; Sala and Zabala, 1996).

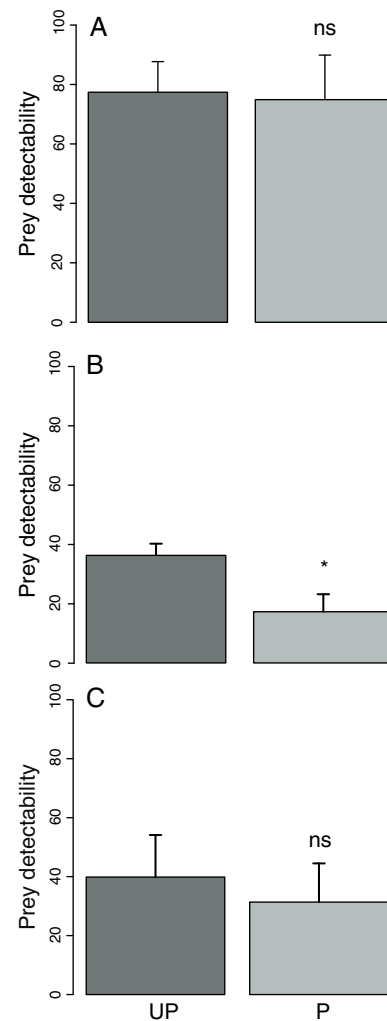
Fish did not display a preference for pierced urchins in any of the size classes. This suggests that fish predation success under field conditions is primarily driven by visual cues and they are not sensitive to any

potential enhanced chemical cues derived from puncturing the urchin. Our video analysis shows that there was no difference in prey detection time between pierced and unpierced urchins, confirming this pattern. It has been previously shown that predator–prey interactions between sea urchins and its fish predators are size dependent (Sala and Zabala, 1996). Smaller sizes have a larger predator guild (Guidetti, 2004), and they typically rely on finding adequate refuge to escape predation (Sala and Zabala, 1996). While larger urchins have fewer available refuges, their size itself serves as a refuge, making them invulnerable to all but the largest extant fish predators.

To summarize, our results show that, at least for the tethering method most commonly used to estimate rates of urchin predation, artifacts are negligible in most standard environmental conditions in the



**Fig. 2.** Predation rates (%) for pierced (P) and unpierced (UP) sea urchins (mean values  $\pm$  SE) according to prey size classes A) small (1–3 cm test diameter; TD), B) medium (3–5 cm TD), and C) large (>5 cm TD). Differences between pierced and unpierced sea urchins were non-significant in all cases (Table 2).



**Fig. 3.** Prey detectability (mean values  $\pm$  SE) of unpierced (UP) and pierced (P) urchins by each predator guild; A) predation impact by *Diplodus sargus*, B) percentage of detection times for *Hexaplex trunculus*, and C) percentage of time preference for *Marthasterias glacialis*.

Mediterranean (open coast with low to moderate water residence times and normal high temperatures). Thus, except for uncommon conditions (extreme temperatures, high water confinement), we can confirm that pierced tethering is a very useful tool to mark individuals of this sea urchin species in the Mediterranean when assessing predation rate for comparative purposes (e.g. between regions and treatments, between different predators or to evaluate the effectiveness of MPAs). This is encouraging given the critical importance of assessing predation in marine benthic ecosystems, many of which are strongly controlled by top-down processes, often showing non-linear responses to changes in predation rates. Whether this method is a useful test of absolute (natural) rates of predation will depend on the ability of urchins to properly find refuges as they would if they were not tethered, on non-altering their attachment strength and on maintaining the relative sea urchin abundances at the study sites. In fact, this technique can potentially alter encounter rates between certain predators and the tagged prey if its movement is strongly limited (Barbeau and Scheibling, 1994). Nevertheless, in this study, we observed that even for comparative studies, it is important to account for the environmental conditions at sites in which the experiment will be deployed to effectively apply this technique, since high temperatures and water residence times could potentially bias the results. In conclusion, we consider that the tethering method used to evaluate comparative predation impacts on the sea urchin *P. lividus* in the Mediterranean is a robust technique useful to provide accurate results and that ecological biases in measuring predator–prey interactions are negligible at least in the most common conditions. Our work confirms the robustness of pierced tethering as a valuable technique in the marine field ecologist's toolbox to measure essential functional rates that shape communities and ecosystems.

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