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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.

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 keystone 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

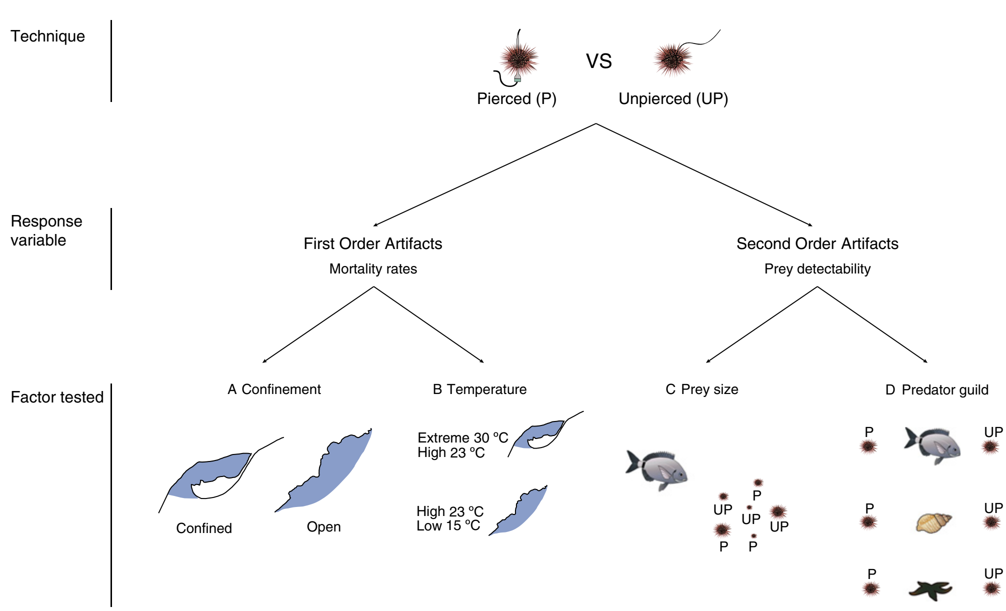

Fig. 1. Schematic description of the methodology used and the experiments developed to test tethering artifacts. Experiments are classified according to the explored variable A) confinement degree (under fixed temperature conditions; 23 °C), B) seawater temperature (for confined and opened conditions) 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.