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Portuguese M. margaritifera populations being in the southern distribution limit are exposed to different ecological conditions at least on the water temperature that is far higher than in the pearl mussel's rivers from central and northern Europe. However there were more than ten known functional populations that existed in the beginning of the 20th century. Nowadays only two of these populations remain healthy and viable with an estimated one million in Rabaçal and fifty thousand mussels in the Tuela river. However, these populations are still threatened by anthropogenic influences responsible for the loss of the available habitat. A more comprehensive knowledge on the habitat requirements of M. margaritifera is essential to identify the best river management policies. The River Habitat Survey showed that Rabaçal and Tuela river sections have excellent quality. The preference curves for juveniles and adults of M. margaritifera, were shown to be similar to the water column velocity (0.10-0.20 $m.s^{-1}$) and bottom velocity (0-0.10 $m.s^{-1}$), dominant substrate (sand and gravel) and depth (30-40 cm), and different for the sub-dominant substrate (fine sediments for juveniles and cobbles and boulders for adults) and cover (boulders for juvenile and overhanging vegetation, roots and boulders for adults); Low levels of dissolved salts (conductivity <50 µS/cm), nutrients (N-Total <0.2 mg/L; P-Total <0.1 mg/L), Organic Matter (POM <4.5 mg/L and PIM < 0.01mg/L) and a high O2 concentration (> 10 mg/L) were detected, revealing a good water quality of these ecosystems.