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# Benthic aliens in the plankton: investigating life cycle dynamics using metabarcoding on the larval stage

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## Abstract

The number of marine non-indigenous species (NIS) has never been so high and is still rising due to globalization and an ever-increasing international trade. Marine NIS are mainly reported in harbours and aquaculture sites, both being primary entry points for introduced species. As compared to these artificial environments, the proportion of NIS is low in natural habitats. This pattern might be due to ecological processes, such as limitations to dispersal or resistance of native communities, or to our inability to efficiently detect them. Most sessile marine NIS display a pelagic larval phase, the main dispersal agent. Studying this phase is, thus, of great importance to investigate NIS reproductive cycles, spread potential, connectivity between artificial vs. natural habitats, and its role in the pelagic ecosystem. Identifying larvae using morphology is notoriously difficult, sometimes even impossible, because of the lack of diagnostic criteria for many species. DNA metabarcoding, successfully used for species detection in various environments, could be a powerful tool to identify NIS larvae within pelagic communities. Here we assessed the efficiency of this approach to detect and monitor seasonal variations of benthic NIS larvae, by analysing plankton samples collected over 22 months in the Bay of Morlaix (Brittany, France). The DNA extracted from ‘bulk’ plankton samples was amplified for 18S and COI markers. Using metabarcoding, ten NIS were identified by at least one marker (5 with both markers). All were previously reported from the study area and displayed clear temporal variation patterns. For two species (the molluscs *Crepidula fornicata* and *Ruditapes philippinarum*), the temporal variations observed through metabarcoding agreed with those revealed by morphological identification or standard barcoding on individual larvae. This study indicates that DNA metabarcoding might be a suitable tool to assess the dynamics of NIS at the larval stage using semi-quantitative data.

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