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Insights on the role of predation in Bay of Biscay anchovy eggs and larvae mortality; development and application of a real-time PCR based assay to potential invertebrate and vertebrate predators.

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Summary

A real-time PCR based assay, developed for the detection of predation on European anchovy (*Engraulis encrasicolus*) eggs, was applied to a set of potential predators, including sardine (*Sardina pilchardus*), sprat (*Sprattus sprattus*) and 52 macrozooplankton taxa during the 2010 Bay of Biscay anchovy spawning season. The two main findings are (1) that the previously neglected macrozooplankton impact in anchovy eggs/larvae mortality is in the same order of magnitude than that due to planktivorous fishes and that, (2) a distinct predation pressure corresponded to the two main spawning centers of Bay of Biscay anchovy.

Introduction

The Bay of Biscay anchovy, a species with large socio-economic impact in the Bay of Biscay area, experienced, since 2002, a succession of low recruitments, resulting in the collapse of the stock in 2005 that led to successive closures of the fishery until reopening in 2010. It has been suggested that anchovy recruitment in the Bay of Biscay could be partially controlled by early life stages (ELSs) predation (Irigoien *et al*., 2007). In this study, our objectives were to determinate the range of predators consuming anchovy ELS and to provide an estimation on the contribution of mortality by predation in Bay of Biscay anchovy eggs survival. To accomplish this, we designed, validated and applied (in the 2010 spawning season) a molecular method capable of detecting traces of *E. encrasicolus* DNA.

Material and Methods

European anchovy DNA detection assay.

Based on existing (GenBank) and newly produced sequences of the Cyt-b gene, we designed a real-time PCR based assay including an *E. encrasicolus* specific TaqMan probe for anchovy DNA detection in predators' stomach contents. The assay amplified a total of 87 bp including a 15 bp region matching the TaqMan probe. Inter- and intra-species specificity was tested against DNA extracted from wild specimens of the clupeid species potentially inhabiting the Bay of Biscay and, *in silico*, against publicly available clupeid sequences. This real-time PCR based assay was capable of detecting 0.005 ng of

anchovy DNA (roughly 1/100 of the DNA extracted from a single egg) and allowed detecting predation events up to 6h after ingestion by small zooplankton taxa. A total of 1069 macrozooplankton individuals, 237 sardines and 213 sprats were sorted for assay application.

Field sampling and assay application.

Both prey and predators were sampled within the 2010 BIOMAN survey (5-20 May). A total of 5 MIK (Methot Isaac Kidd) net samples, with a mesh size of 1 mm and a mouth area of 1 m^2, were collected at night in areas of relatively high anchovy eggs abundance in order to characterize the range of taxa feeding on anchovy eggs. Potential predators were sorted and kept in ethanol until DNA extraction. Sardines and sprats were obtained by pelagic trawling (a total of 14 hauls). The stomach (cardiac stomach and pyloric caeca) was dissected and preserved in ethanol until DNA extraction. The real-time PCR assay was applied to the extracted DNA and the percentage of positive signals per haul/station was computed. *In situ* data for both predator and prey abundances were available allowing the mortality estimation at haul location point. In the fish case we used acoustic biomass estimations from the coincident PELGAS 2010 survey (ICES, 2010) and thus mortality is provided by regional areas.

Results and Discussion

The large range of macrozooplankton taxa assayed (52 taxa) and the fact that, apart from anchovy, sardine and sprat comprised the bulk of planktivorous fish in the area, allow us to consider our results as a holistic view of anchovy eggs predation mortality. Both fish species and 32 macrozooplankton taxa showed remains of anchovy DNA within their stomach contents. Considered together, a distinct predation pressure corresponded to the two main spawning centers of Bay of Biscay anchovy. While relatively low mortality rates were recorded at the shelf-break spawning center, a higher predation pressure from both fish and macrozooplankton was exerted at the Gironde shelf centered one. While predation by sardine and sprat accounted for, respectively, 7 % and < 1 % of the daily anchovy egg mortality, macrozooplankton consumed around 1-4 % of the daily egg production in the shelf-break and between 14-89 % in the shelf stations where mysids and decapods larvae prevailed. Macrozooplankton and fish predation accounted for a large percentage of the anchovy egg mortality. It is noticeable that, at least for the Gironde shelf spawning center, the observed mortality due to macrozooplankton predation is in the same order of magnitude than the one computed for fish. Finally, reduced predation mortality at offshore environment has been proposed for both anchovy larva and juvenile stages (Irigoien *et al.*, 2007, Cotano *et al.*, 2008). Present results indicate that this applies also to eggs mortality. However other factors such as differential spawning intensity or advection patterns could be playing a role (e.g. Allain *et al.*, 2007). Further studies including a wider temporal window, both in number of years and in the extension of the sampled period, are needed to resolve the role of anchovy ELSs predation mortality on the species recruitment.

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