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Susceptibility of invasive tunicates *Clavelina oblonga* to reduced seawater salinities

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ABSTRACT

The intensive colonial tunicate *Clavelina oblonga* invasion was observed in the summer of 2020 at shellfish farms located on the western and eastern coasts of the Istrian peninsula (NE, Adriatic, Croatia). Aquaculture facilities create an ideal environment for ascidians, therefore, as a possible environmentally friendly eradication measure, laboratory experimental exposure of *C. oblonga* to different reduced seawater salinities of 37, 30, 20 and 11 was examined in November 2020. During the total observation time (14 days exposure + 14 days of recovery at 37) at all seawater salinities, there was no sexual reproduction of *Clavelina* specimens and larvae settlement in general. Salinities of 37 and 30 didn't provoke any significant effect in colonial corpus weight change and morphology. Salinities of 20 and 11 caused disorder in specimens feed uptake and feeding itself which led to change of colour, tissue necrosis and finally body fragmentation. Similar separate laboratory exposure of mussel *Mytilus galloprovincialis* specimens did not provoke significant differences in meat yield between control 37 salinity (14.0 %) and reduced salinities of 30 (14.2 %), 20 (14.6 %) and 11 (15.3 %) measured at the end of the recovery experiment. Based on our experimental results of *C. oblonga* salinity susceptibility and field observations (2020–2021), we can assume that field translocation of overgrown mussels and infrastructures to locations of seawater < 20 salinities could be a natural way for its eradication.

1. Introduction

In the Adriatic Sea, tunicates are a common occurrence. Some non-native species are invasive and can cause both ecological and economic losses (Mastrototaro and D'Onghia, 2008). An intensive ascidian invasion was observed in the summer of 2020 at shellfish farms on the western and eastern coasts of Istria, Northern Adriatic, Croatia. Based on collected specimens examined, it was confirmed that the present species is already known as invasive ascidian *Clavelina oblonga*, previously recorded in the Gulf of Trieste and Piran Bay in 2015 (Mioković, 2016). *Clavelina oblonga* (Ascidiacea, Aplousobranchia, Clavelinidae) is a distinctive and conspicuous epi/benthic colonial tunicate naturally distributed in the Gulf of Mexico's tropical area, on the west coast of the Atlantic, in the Azores and the west coast of Spain (Scarponi et al., 2018). It has been reported as invasive species in the Mediterranean Sea (Salfi, 1929; Monniot, 2001; Ordóñez et al., 2016; Shenkar et al., 2022).

According to the latest invasion in Northern Adriatic, the major impacts reported were associated with the bivalve industry. Aquaculture facilities create an ideal environment for ascidians, with abundant suspended food, unlimited locations and surfaces for attachment (Molnar et al., 2008; Mioković et al., 2018). *C. oblonga* physically interferes with the opening of mussels valves, competes for food resources, impedes food and oxygen procurement by reducing water flow and increases the weight on load-bearing infrastructure (holding nets, cages, ropes and buoys). The intense presence of overgrowth-biofouling communities in aquaculture increases production costs while decreasing product value (Fitridge et al., 2012; Watts et al., 2015), as well as the implementation of regulatory restrictions to reduce the risk of spread to unaffected areas (Adams et al., 2011; Sievers et al., 2013). Further, fouling community dominated by *C. oblonga* led to the loss of alternative community states and reduced species diversity (Christianson and Eggleston, 2021).

Clavelina oblonga is a tropical species and in its natural habitat temperatures do not fall below 10 °C, so regression was expected during the winter months and survival in the form of buds from which zooids sprout again when living conditions become favourable. Savudrija Bay is the northernmost point of *C. oblonga* distribution in the Croatian part of the Adriatic (Mioković et al., 2018). Unfortunately, according to our

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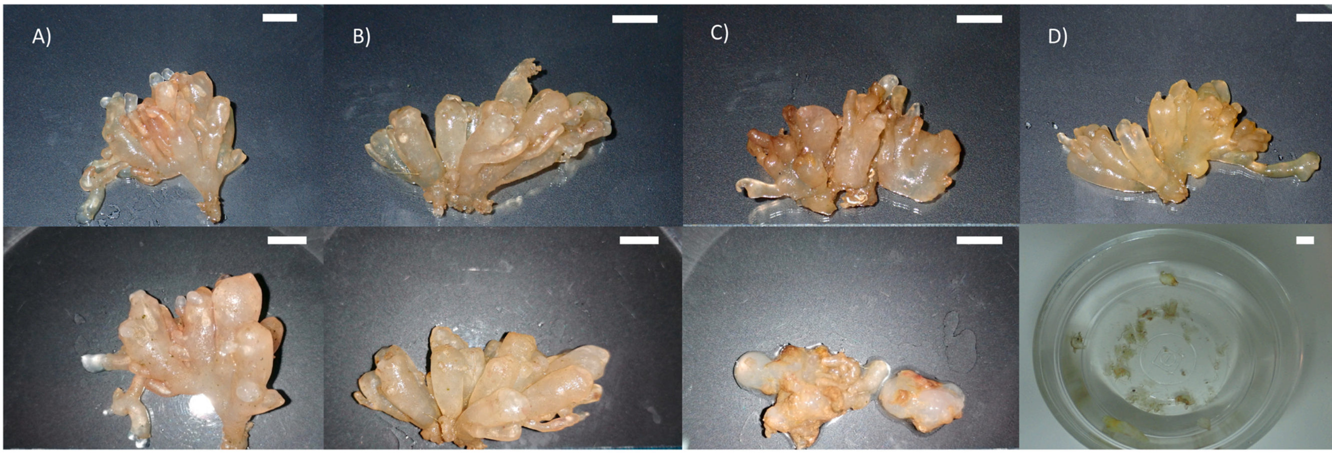

Fig. 1. Morphology of *C. oblonga* after 14 days of exposure to different salinities of A) 37, B) 30, C) 20 and D) 11 (upper row), and after 14 days recovery at 37 salinity (lower row). The bar represents 1 cm.

latest observations, this species has adapted to colder conditions and survived for six winters at this location. The growth of *C. oblonga* is correlated with the increase of seawater temperature and chlorophyll (Lins et al., 2018). Previously *C. oblonga* was shortly reported to be sensitive to lowered environmental salinity without any additional data (Goodbody, 2003). It is known praxis that shellfish farmers, e.g., in Lim Bay (western coasts of Istria) translocate their mussels to the beginning of the Bay, exposing them to the brackish water to eliminate overgrowth-biofouling organisms (Hamer et al., 2010; Pavičić-Hamer et al., 2016). On local shellfish farmers' community request for a natural removal treatment of overgrowth, *C. oblonga* susceptibility to reduced seawater salinities as a possible environmentally friendly eradication measure was examined under laboratory conditions.

2. Material and methods

2.1. Clavelina oblonga field observation and sampling

Following *C. oblonga* occurrence in the Lim Bay, Vabriga and Raša Bay aquaculture facilities, we conducted surveys in October 2020 to estimate the approximate invasion intensity and sampling. For instance, in Lim Bay *C. oblonga* colonies occupied on average ca. 25–100 % of space on the mussel nets and 10–50 % of other shellfish farm infrastructure, depending on locations. For experimental exposure of *C. oblonga* to reduced salinities, the whole colonial corpus with zooids (bulb) was sampled in Lim Bay at the end of October 2020. Until the beginning of experiments in the wet laboratory of the Center for Marine Research Rovinj, organisms were kept in 40 L basins under running seawater equipped with mechanical and bio-filter, including UV-C sterilisation before discharge in wastewater outlet.

2.2. Experimental design – exposure to different salinities

a) Clavelina oblonga *treatment*

Eight colonial corpuses (length 50–73 mm, weight ca. 9–11 g) consisting of ten to twelve *C. oblonga* zooids (length 40–55 mm) were placed in 8 × 5 L glass with aeration and exposed to salinities of 37, 30, 20 and 11 for 14 days. Glass with seawater salinity of 37 was used as a control A) 100 %. For target salinities (30, 20 and 11) and seawater percentage adjustment of ca. B) 75 %, C) 50 % and D) 30 % we used distilled water. We placed three empty, clean, complete *Mytilus galloprovincialis* shells hanging on the rope in each glass, for possible *C. oblonga* larval settlement observation. After exposure, all used specimens were kept for additional 14 days in seawater salinity of 37 for recovery treatment. An experiment was performed in duplicate during November 2020 in the airconditioned room at + 16 °C, which approximately corresponds to sea temperatures. Everyday *C. oblonga* specimens were fed with algae *Tetraselmis chuii* (20 ml, 5 × 10^6 / ml) and observed for feeding behaviour (filtration, food uptake, faeces production). Tissue and corpus changes were noted, as well. 50 % of seawater was exchanged every second day, with daily salinity and temperature measurement using HI 98194 multiparameter probe (Hanna Instruments, USA). Exchanged seawater was filtered using 40 μm fine mesh and checked for the presence of larvae (sexual reproduction) under an optical Zeiss Primovert microscope (Karl Zeis GmbH, Jena, Germany). The whole *C. oblonga* colonial corpus with zooids was weighted and photo-documented before and after treatment with different salinities and at the end of the recovery period also.

b) Mytilus galloprovincialis *treatment*

Specimens of mussel *Mytilus galloprovincialis* of 4 cm size collected in Lim Bay were separately kept under the same treatment conditions as *C. oblonga* (5 L glass, aeration, feeding and salinities of 37, 30, 20 and 11) to avoid feeding competition between *C. oblonga* and *M. galloprovincialis* specimens. The mussels were daily observed for filtration (valve gaping), food uptake and faeces production. At the end of the recovery period, the mussel meat yield (wet tissue weight/ mussel weight * 100) was determined as general fitness indice (Pavičić-Hamer et al., 2016).

2.3. Statistical analysis

All experiments were performed in duplicates using *C. oblonga* colonial corpus with 10–12 zooids and 3 specimens of *M. galloprovincialis* per different salinity trial. Results of colonial corpus weight and mussel meat yield were expressed as average values with standard deviations. The significance of differences between the mean *C. oblonga* colonial corpus weight, as well as mussel *M. galloprovincialis* meat yield were tested using ANOVA, and post hoc Tukey test (Statistica 7.1, StatSoft, TIBCO Software Inc., Palo Alto, CA, USA).

3. Results and discussion

Laboratory experimental exposure of *C. oblonga* to different salinities (37, 30, 20 and 11) in November 2020 has shown that during the total observation time (14 days exposure + 14 days of recovery), there was no larval recruitment and sexual reproduction in general, which is in concordance with previous findings (Christianson and Eggleston, 2021). The effects of seawater salinity of B) 30 did not differ in comparison to A) control 37 salinity, while salinities of C) 20 and D) 11 provoked disorder in *C. oblonga* feed uptake and feeding itself, leading to change of

Table 1
Effect of different seawater salinities A) 37, B) 30, C) 20 and D) 11 treatment on *C. oblonga* colonial corpus wet weight. Results were expressed as wet tissue weight (g) and percentage (%) at the beginning of exposure, end of exposure (14 days) and at the end of the recovery period (14 days).

Seawater salinity	A) 37		B) 30		C) 20		D) 11	
C. oblonga weight	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)
Start of exposure	10.1	100.0	11.2	100.0	10.6	100.0	10.7	100.0
End of exposure	10.0	99.0	11.1	99.1	5.7#	53.8 * *	10.1	94.4 *
End of recovery	9.9	98.0	9.9	88.4 *	3.2#	30.2 * *	2.1##	19.6 * *

Corpus shrinking and change of colour, ## Corpus shrinking and fragmentation observed. Asterisk indicate the significance level (*) $p < 0.05$ and (**) $p < 0.01$.

Table 2
Different seawater salinities treatments of *M. galloprovincialis* resulted without significant effect ($p < 0.05$) on mussel fitness measured as meat yield (%) at the end of the recovery period (14 days).

Seawater salinity	A) 37		B) 30		C) 20		D) 11	
Meat yield (%)	AVG	SD	AVG	SD	AVG	SD	AVG	SD
End of recovery	14.0	2.7	14.2	1.9	14.6	3.7	15.3	3.2

corpus colour, tissue necrosis and finally body fragmentation (Fig. 1, Table 1).

To prove that mussels *M. galloprovincialis* can survive such overgrowth treatment, they were separately kept under the same treatment conditions as *C. oblonga*. Results showed that during all 14 + 14 days no mortality was noted, and finally, no significant fitness decrease measured as meat yield was determined between the treatment group A) 37, B) 30, C) 20 and D) 11 at the end of the recovery experiment (Table 2). Only the first day of exposure to seawater of 11 salinity and recovery period (glass D) no mussel filtration activity-faeces production was observed. The slight increase of wet tissue content expressed as meat yield can be explained by osmosis, increased tissue water uptake of mussels after reduced salinity treatment and adequate, satisfactory feeding during the whole experiment (Hamer et al., 2008).

Based on our results, we can assume that hypothetical translocation of mussels and whole cages with holding nets to locations of seawater < 20 salinities for 7–10 days could be a natural and effective way for the eradication of this invasive species. The effectiveness cannot be directly compared with the results of mussel mechanical cleaning and pressurized seawater antifouling treatment, as well as reactive pest treatment with commercial products (Arens et al., 2011; Cahill et al., 2021), but can help and prevent further spreading of *C. oblonga* invasion.

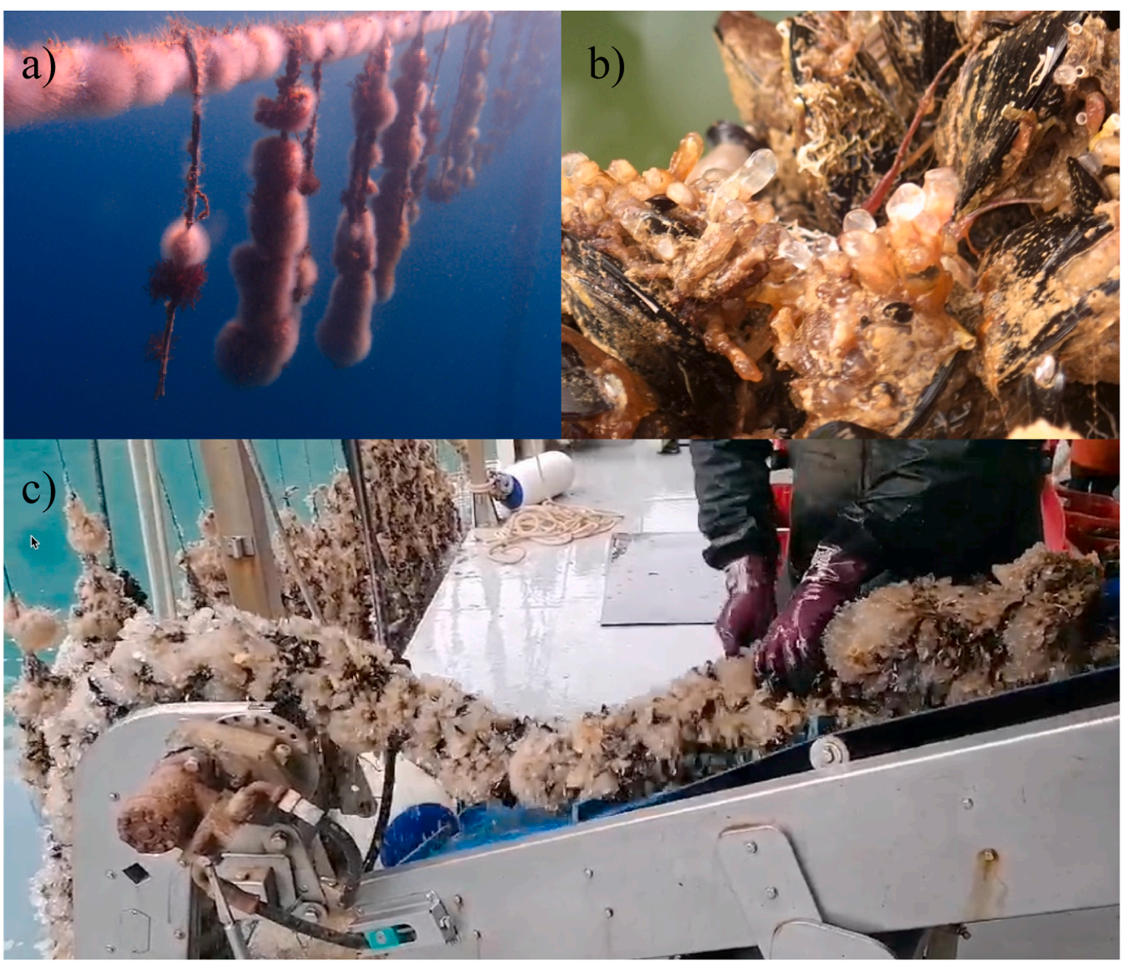

Fig. 2. Field observation of *C. oblonga* occurrence - invasion status at endangered aquaculture location in Lim Bay: a) overgrown mussels in the nets (September 2020); b) overwintering remaining parts of colonial corpus buds with young zooids as a result of vegetative reproduction (February 2021); d) mussels overgrown with ascidians after sexual reproduction and invasion (September 2021).

Further, in February 2021, our assumption was confirmed during a field inspection of affected aquaculture locations after an intensive rain period during winter 2020–2021. *Clavelina oblonga* colonial corpus reduction-fragmentation on mussels and infrastructure up to 4 m depth was observed. Unfortunately, young active live specimens (zooids) were observed in deeper waters and sporadically in the upper water layer, on the remaining reduced old *C. oblonga* colonial buds (Fig. 2. b), probably due to vegetative reproduction. Field surveys taken in August and September 2021 also revealed the huge presence of this species at mariculture locations in Lim Bay (Fig. 2. c). This was indication that the ascidians population have successfully over-wintered and continued its invasion by sexual reproduction(Alié et al., 2021).

While observed *C. oblonga* biofouling decreases mussel growth and production efficacy, at the same time it increases production costs by demanding additional mechanical cleaning efforts and time. It appears that *C. oblonga* is currently in the dangerous stage of introduction in western and eastern Istrian aquaculture areas, and its further spread is expected (Rocha et al., 2009, 2012). Such colonization may potentially cause severe impacts depending on local environmental conditions (Mckindsey et al., 2007; Sievers et al., 2013, 2017). Until now its occurrence in natural habitats outside aquaculture areas was not observed in the region. In coordination with shellfish farm owners, further monitoring of *C. oblonga* occurrence, distribution and abundancy is organized, as well as salinity and temperature continuous measurement using data loggers. The global redistribution of this species is a prominent impact of climate change and human-mediated biological invasions with negative impacts on shellfish production.

4. Conclusion

Based on our experimental results on the susceptibility of *Clavelina oblonga* to lower salinities and field observations, we can assume that field translocation of overgrown mussels and infrastructures to locations of seawater < 20 salinities could be a natural way for the eradication and preventing spreading of this invasive species.

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CRediT authorship contribution statement

Nina Majnarić: Investigation, Formal analysis, Data curation, Methodology, Writing – Original Draft. **Dijana Pavičić-Hamer**: Formal analysis, Data curation, Methodology, Validation, Writing – Original Draft. **Andrej Jaklin**: Investigation, Formal analysis, Data curation, Writing – Original Draft, Writing – Review & Editing. **Bojan Hamer**: Conceptualization, Supervision, Investigation, Methodology, Resources, Writing – Original Draft, Writing – Review & Editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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