Bio-invasive ascidians in Ireland: A threat for the shellfish industry but also a source of high added value products

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ABSTRACT

In October 2016, a rapid assessment survey of ascidian species was conducted in shellfish farms at Killary Fjord, in the west of Ireland. Two non-indigenous solitary ascidians Ascidiella aspersa and Corella eumoyta were recorded for the first time in shellfish farms at this location. Both invasive ascidians have the potential to greatly reduce mussel production in Killary Fjord by competing with shellfish for food and habitat. Their high abundance also causes an increase in maintenance costs leading to economic losses for aquaculture farmers. Prompted by our finding of two invasive ascidians in Killary Fjord, we provide a brief review of the ecological role of ascidians and the potential of harnessing biomass from such invasive species for the production of high added value marine natural products.

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Introduction

A review of the literature indicates that more than 65 ascidian species are known from Irish waters, six of them being considered as invasive like Ascidiella aspersa, Didemnum vexillum, Styela clava, Botrilloides violaceus, Corella eumoyta and Perophora japonica. Several modes of introduction of NIA are possible into the region of Killary Fjord. The first is through ship ballast water, as the ascidian larvae are able to postpone settlement and survive for several days. Ascidians also represent one of the main biofouling species, particularly on ship hulls. These organisms can colonise all types of hard substrate both natural and artificial, especially in the environment characterised by low diversity fauna such as coastal lakes, lagoons, harbours and shellfish farms. The aim of this review is to report on the distribution of invasive ascidians in Killary Fjord, assess their environmental impact, the socio-economic implication and the potential in the discovery of marine natural products.

Impact of ascidians on shellfish farming

In 2013 mussel farmers in Killary Fjord identified some ascidian species as biofoulers of the shellfish lines and assumed they were not serious pests (Simon, Killary Fjords Shellfish Farm personal communication). From 2014 to 2016, the ascidian population increased massively.
expanding in Killary Fjord and other locations in Galway Bay. We subsequently recorded extensive biofouling of invasive solitary ascidians *A. aspersa*, and *C. eumoyata* along with other ascidians like *A. mentula*, *A. scabra* and *Ciona* sp (Fig. 1) at depths ranging from 3 to 15 m. The most common ascidians were the invasive species *A. aspersa* and *C. eumoyata*, both of which were recorded for the first time in Killary Fjords, and thus form new locality records. In previous studies, Minchin and co-workers reported the first appearance of non-indigenous ascidian species at various sites in Irish waters including *D. vexillum*, *S. clava*, *B. violaceous*, *C. eumoyata* and *P. japonica* while the biology of *A. aspersa* and *C. eumoyata* was reported. *A. aspersa* is reported to tolerate wide seasonal fluctuations of oceanic parameter changes and is more adaptable to climate change and shifting geographic ranges. The spawning of this species was noticed throughout the year, but mature development was observed only in autumn. Remar- kably, depth did not impact the reproductive cycle of *A. aspersa*. However, earlier gonad maturation and spawning were observed in individuals collected in deeper waters compared to shallow water depth.

Ojaveer and co-workers reported the major issues caused by marine invasive species in European waters. Invasive and native tunicates are of economic concern for shellfish farmers because they overgrow shellfish and pollute the long line gear, thereby adding weight and restricting water exchange and nutrients. Ascidians are benthic filter feeders, and can lead to a decrease in the shellfish production (both bivalves and gastropods) due to competition for food. The first evidence of PSP toxins saxitoxin and gonyautoxins were reported in *Microcosmus vulgaris* harvested from the farmed mussels (*Mytilus galloprovincialis*) at west coast of Istria Peninsula, Croatia. NIA species have caused serious impacts on aquaculture at Killary by smothering the stocks, making aquaculture production labour intensive, decreasing the overall production, reducing the size of mussels and causing loss of income for shellfish farmers.

Dense populations of the invasive ascidians *C. intestinalis*, *A. aspersa*, *S. clava*, *C. eumoyata*, *D. vexillum* and other species were frequently found on aquaculture structures and hard substrates more widely in the nearby marine environment of Killary Fjord suggesting that the invasive species significantly contribute to the fouling community and may also have become the dominating biofouling organisms in the region. Recently, Ardura et al. reported that the abundance of NIA is lower in coastal marine protected areas of Canada and French Polynesia. In Irish waters, the introduction of invasive ascidians is also reported to be lower in marine protected areas.

![Figure 1](image.png)

**Figure 1.** Invasive marine ascidians on blue mussels, Killary Fjord, Ireland. (a): Invasive ascidian communities attached to cultured mussels and biomass of cultured mussels is not even in longlines; (b) mussels with ascidians for machine cleaning and processing; (c) *Asciella aspersa*; (d) *Corella eumoyata*. 
areas compared to the coastal regions extensively affected by human activities e.g. Cork and Dublin harbour, Yacht clubs in Cork, Dublin, Howth fishing harbour, and aquaculture practice like in Killary Fjords, Galway. Previous reports of NIA distribution in Irish waters emphasized the importance of marine protected areas for marine biodiversity conservation and suggested that marine protected zones would confer efficient protection against NIA introduction. However, the lack of taxonomic expertise in the field of tunicate taxonomy, insufficient information on the distribution and origin of species and vectors of NIA species reported in the present study suggests that further research in this area is necessary.

**Commercial biocides against ascidian larval settlement**

The prevention method used to deter marine biofouling organisms may vary depending on the species and the age of the shellfish being cultured. Removing the ascidians manually requires additional labour by shellfish farmers, leading to increased maintenance costs. The following techniques have been effectively utilised for the removal/control of marine biofoulers on shellfish; the application of lime, dilute bleach, vinegar, plastic wrapping and exposure to air and using biocides. All such practices can account for up to 25-30% of the operational cost of shellfish farmers.

The commercially important anti-fouling biocides Irgarol-1051, Sea-Nine 211 and Chlorothalonil significantly inhibit embryonic development in *C. intestinalis*. The biocide, Chlorothalonil showed maximum toxicity against embryonic stages of *C. intestinalis* (at concentration $EC_{50}$ 33 $\mu$g/L), and following that, Sea-Nine 211 ($EC_{50}$ 105 $\mu$g/L), Irgarol-1051 ($EC_{50}$ 2115 $\mu$g/L) and zinc pyrithione (Zpt) ($EC_{50}$ 108 nM) also inhibited larval settlement. Over all, Chlorothalonil was revealed 70 times more toxic than Irgarol-1051 and was three times more toxic than Sea-Nine 211.

Furthermore, Sea-Nine 211 and Chlorothalonil showed the reduction of haemocyte functionality of colonial ascidian *Botryllus schlosseri* at concentration 1 and 10 $\mu$M, which is comparable to that of Tributyltin (TBT). Similarly, the other biocides Diuron and TCMS pyridine showed sublethal activity on haemocytes of *B. schlosseri* by changing the cytoskeleton and cell morphology and inducing the impairment of DNA. Additionally, the synthetic catamine and medetomidine induced metamorphosis and reduced the larval settlement of ascidian *S. clava* at concentration 20 mg/L. Further, medetomidine increased the rate of larval immobility with $EC_{50}$ values of 3.8 mg/L in 2 h. The use of commercial anti-fouling painting maintains the colonization of the ascidian *Styela clava* on boat hulls to a minimum, even after 12 weeks in seawater. The results of the previous studies suggest that these biocides can control biofouling communities and non-target benthic organisms. However, such biocides have to be used with caution as ecotoxicological studies on other micro and macro organisms of the ecosystem are still needed. Further investigation and long-term monitoring data are required to evaluate the bioaccumulation of synthetic biocides by benthic organisms before their use against NIA species in finfish/shellfish aquaculture systems.

**Anti-fouling marine natural products**

Settlement and metamorphosis are subsequent critical events in the life cycle of sessile marine organisms to obtain a suitable habitat for food and reproduction. Although diverse physical factors have been reported that influence larval settlement and metamorphosis, metabolites derived from the adults, from prey, or from bacterial films are believed to be more important. In addition to purely synthetic compounds, a number of natural products have been reported from marine organisms such as sponges, ascidians, corals etc that could affect these physiological processes. The anti-fouling Tirol compounds (callytriols A-E) derived from the marine sponge *Callyspongia truncata* showed potent metamorphosis inducing activity against larval settlement of the ascidian *Halozynthia roretzi* at ED50 values of 0.24–4.5 $\mu$g/mL. Further, Haliclonacyclamine and halaminol A were isolated from other haplosclerid sponges and inhibited the larval settlement of ascidians *C. intestinalis* and *Herdmania momus* at concentrations as low as 5 $\mu$g/mL. Both compounds significantly induced metamorphosis and changed larval morphologies after reaching the tail-resorbed stage, and inhibited larval settlement.

Some marine natural products isolated from ascidian species themselves reveal promising in-vitro activity such as anti-bacterial, anti-fungal, anti-viral, anti-malarial and anti-cancer activity. Therefore, an obvious question is whether any compounds from biofoulers such as ascidians could be found and developed for use as antifouling
agents. Crude extracts from the Mediterranean ascidian *Polysyncraton lacerai* exhibited good protection against the epibiosis of *Loxocalyx* sp on the colony surface and inhibited anti-mitotic activity in sea urchin eggs at concentration 8 ppm. Other marine alkaloid compounds, Eudistomis G and H were isolated from the colonial ascidian *Eudistoma olivaceum* collected in the Indian River lagoon, United States. Both compounds effectively showed anti-fouling activity against the larvae of the bryozoan *Bugula nertitina* at concentration 0.5%. The ascidian *E. olivaceum* showed strong anti-fouling activity against larval settlement of *B. nertitina* compared to *E. capsulatum*. In addition, Murugan and Ramasamy reported modest anti-fouling activity of crude compounds from the Indian ascidian *Distaplia nathensis* against byssal thread production and attachment in the mussel *Perna indica* with EC$_{50}$ and LC$_{50}$ values of (50, 150 $\mu$g/mL). In further research, Trepos et al. reported novel anti-fouling compounds Synoxazolidinones A and C (1), Pulmonarins A and B from the sub-Artic ascidian *Synoicum pulmonaria* and screened them against sixteen bacterial and micro algae strains. Synoxazolidinone A showed potential anti-bacterial inhibition against the marine bacterial strain *Halomonas aquamarina* at a concentration of 20 $\mu$M. Synoxazolidinone A also inhibited the settlement of the microalga *Cylindrotheca closterium* and reduced the settlement of barnacle larvae at a concentration of 15 $\mu$M, which is lower than the anti-fouling activity of marine sponge *Agelas sp.* and higher than the sponge derived compound bastadin-9 and iantelline at 1 $\mu$M. Remarkably, a bicyclic derivative Synoxazolidinone C showed promising growth inhibition of *Balanus improvisus* at a concentration of 2 $\mu$M, which is a higher inhibition value comparing to the commercial anti-fouling product Sea Nine-211. Furthermore, Pulmonarins A exhibited strong anti-bacterial activity against *Vibrio natriegens*, and *Roseobacter littoralis* at an outstanding concentration of 30 nM, but no significant activity against micro algal species was evidenced. From this literature survey, it is clear that ascidians contain a vast pool of anti-fouling resources. Further exploration of novel biomolecules might provide promising candidates to control the benthic marine biofouling community.

**Chemical ecology of ascidians**

Marine ascidians contain a broad range of natural products that can serve as important source of new therapeutics but also chemical defenses. Metabolomics provides a snapshot of the metabolites present in a living organism. It can be used in: functional genomics to analyze fluxes in metabolic pathways and to decipher the biological relevance of each metabolite, to differentiate inter and intra specific variation of marine organisms, in chemotaxonomic classification, and also in understanding the interactions of organisms with their environment. Modern developments in analytical methods have resulted in many different platforms of metabolome investigation of marine flora and fauna using mass spectrometry (MS) and Nuclear Magnetic Resonance (NMR) combined with multivariate statistical analysis. In recent years, LC-MS and NMR-based metabolomics are increasingly utilized for their systematic manner of profiling chemical fingerprints of individual samples, either plant or animals. A comparative metabolomics profiling requires a large number of samples to generate results that are statistically robust. Besides, highly sensitive and accurate instrumentation, powerful bioinformatic tools (e.g. XCMS-METLIN) are essential to address the vast amount of data generated by these experiments.

Tianero and co-workers reported that microbiomes and metabolomes of ascidians contain species-specific and location-specific components. To assess the chemical diversity of ascidians, the authors applied an untargeted LC-MS based metabolomics approach. High amounts of lipids (phospho-glycerolipids, glycerolipids) were detected in ascidians and specimens were discriminated via geographical location rather than by species. These observations might be due to the variation of surface water temperature, available food sources and biogeographical affinity of species. Species-specific bacteria were represented by abundant OTU of the microbiomes sampled and these major components of the microbiome may be responsible for the production of the secondary metabolites. The results of this study confirmed that bacterial associations with didemnid ascidians may be related to the production of defensive chemicals in ascidians, implying a strong selection for specific bacteria. Metabolomic profiling of two Mediterranean ascidians *Styela plicata* and *Ascidia mentula* using mass spectra and multivariate analysis suggested that secondary metabolites could apparently account for chemical defence in *S. plicata*. They also recommended that the LC–MS based metabolomics method could be used as a reliable tool for taxonomic classification of marine ascidian species. Therefore, we have started some investigations into the chemical ecology of the invasive species...
found in Ireland, using the metabolomics approaches with the aim to find compounds of interest for future development.

In conclusion, we first report the occurrence of non-indigenous ascidians in shellfish farms in Killary Fjord, West coast of Ireland. The potential impact of NIA on the wider Irish shellfish industry needs further investigation. It is important to introduce long-term monitoring of the population of invasive ascidians *A. aspersa* and *C. eumoyata* in a longer term to determine if they persist or increase in numbers during this period. Investigation of NIA effects on blue mussel growth rate, chemical defense and other interaction in the marine environment is also recommended. In further research, symbiotic relationship with microbial communities, and MS/NMR based metabolomic profiling of these ascidians could enable straightforward detection of defense metabolites. With the high potential of secondary metabolites for the development of products that prevent marine benthic biofouling community, we believe that biodiscovery research on invasive ascidian species will have bright future.

**Disclosure of potential conflict of interest**

No potential conflicts of interest were disclosed.

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