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Biology of the invasive ascidian *Ascidiella aspersa* in its native habitat: Reproductive patterns and parasite load

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ABSTRACT

The European sea squirt *Ascidiella aspersa* is a solitary tunicate native to the northeastern Atlantic, commonly found in shallow and sheltered marine ecosystems where it is capable of forming large clumps and outcompeting other invertebrate fauna at settlement. To date, there have been relatively few studies looking at the reproductive biology and health status of this invasive species. Between 2006 and 2010 sampling of a native population took place to investigate gametogenesis and reproductive cycle and to determine the impact of settlement depth on reproduction. In addition, parasite diversity and impact was assessed. A staging system to assess reproductive development was determined. The study highlighted that from year to year the tunicate could change its reproductive strategy from single sex to hermaphrodite, with spawning possible throughout the year. Depth did not impact on sex determination, however, gonad maturation and spawning occurred earlier in individuals in deeper waters compared to shallow depth and it also occurred later in *A. aspersa* at sites further away from the open sea. Four significant parasite groups including eugregarines, ciliates, trematodes and turbellarians were detected and prevalence of parasite infections increased in *A. aspersa* at sites with a reduced water flow rate. This study demonstrates the high biotic potential of this ascidian bioinvader to have a negative impact on native fauna in an introduced ecosystem, due to its highly efficient reproductive and resource allocation strategies. Artificial structures such as mooring lines can harbour large aggregations of *A. aspersa*, however, these manmade habitats may facilitate the colonisation and establishment of this invasive species in the benthos. Additionally, the parasite communities that *A. aspersa* harbour may also exacerbate its negative impact, both ecologically and economically, in an introduced area by possibly leading to the emergence of new disease in native species i.e. pathogen spillover.

1. Introduction

Tunicates are a diverse group, which inhabit a range of marine environments worldwide (Quesenberry et al., 2003). Within the Class Ascidiacea, ascidians or sea squirts are conspicuous components of epibenthic marine communities globally (Pineda et al., 2013). This group is also among the most important marine invaders worldwide, with the capability of severely modifying coastal habitats (Whitlatch and Bullard, 2007; Pineda et al., 2013). Ascidians show major differences in their life history strategies and may be solitary, social living in clumps or compound living together in a gelatinous mass. Invasive solitary species can be found growing in dense monospecific aggregations and are frequently found outgrowing and outcompeting resident species, resulting in a significant reduction in species abundance and diversity in an introduced area (LeBlanc et al., 2007; Bullard and Carman, 2009). The rapid growth of ascidian species allows them to dominate available space (Bullard et al., 2007) and the ability of individuals to retain a large amount of water inside their body allows even solitary ascidians to cover a significant mass (Bullard and Carman, 2009). Globally, ascidians have a reputation as being the most prolific and devastating biofoulers to shellfish aquaculture operations (Adams et al., 2011; Fletcher et al., 2013). Ascidians can often physically compromise cultured shellfish by interfering with valve movement (Fletcher et al., 2013) thus reducing the availability of food to the shellfish and hindering growth (Lodeiros and Himmelman, 1996).

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Additionally, being filter feeders they directly compete for food with many commercial species such as oysters (Riisgård et al., 1995), mussels (Daigle and Herbinger, 2009; Fletcher et al., 2013) and scallops (Ross et al., 2004; Su et al., 2008).

A native tunicate species found around the coast of Ireland is the European seasquirt *Ascidiella aspera,* which as an invasive species in many other geographical locations is considered harmful and a moderate to serious threat (http://www.cabi.org/isc/datasheet/92557). To date, no studies have been published on this tunicate's biology and ecology in Irish waters. *A. aspersa* is native to the northeastern Atlantic, from the Mediterranean Sea to Norway (Cohen et al., 2000), including the British Isles and the Shetland Islands (Millar, 1966) and is euryhaline, tolerating salinities from 18 to 40 (NIMPIS, 2010). *A. aspersa* has a life span of 18 months and is a solitary tunicate but often forms unfused colonies in close association with each other on hard surfaces such as rocks (Curtis, 2005). This ascidian is often abundant in eutrophic estuarine habitats with high densities of plankton and organic matter (Mastrototaro et al., 2008) and is found in the lower intertidal to the sublittoral (80 m) (Curtis, 2005). Rajbanshi and Pederson (2007) described *A. aspersa* as an invasive species, which is more mobile, numerous and hardy than the highly invasive *Ciona intestinalis,* when studied in terms of settling ability. Additionally, *A. aspersa* has the potential to be a successful invader because of its rapid growth rate and tolerance to a wide range of environmental conditions (MacKenzie, 2011). In recent years, *A. aspersa* has expanded its range to a number of areas including the northwestern Atlantic coast of North America, New Zealand, Southern Australia, Tasmania and India (NIMPIS, 2010). Modes of introduction to new locations include unintentional movement with aquaculture materials, ship ballast water and ship or boat hull fouling (Fofonoff et al., 2003). Some biological invasions may affect the overall functioning of an ecosystem in terms of material flow between trophic groups, primary production, organic material decomposition and the extent of benthic-pelagic coupling (Occhipinti-Ambrogi, 2007). Outside of its native range *A. aspersa* has impacted on the environment by forming large populations and subsequent high amounts of biomass suspension feeding on a significant proportion of plankton in the water column (Pederson et al., 2003), which redirects energy pathways to decomposers in the benthos and not to higher trophic communities because it lacks many predators (Currie et al., 1998). This biofouling pest when introduced to new locations also directly competes with other native filter-feeding fauna of economic importance like scallops, mussels and oysters (Currie et al., 1998). The establishment of *A. aspersa* is increasing as global and ocean water temperature increases (Stachowicz et al., 2002).

Ascidiella aspersa are hermaphroditic and contain both an ovary and a testis, but previous studies indicated that they are protandic meaning that the male reproductive organs mature before the female ones. Upon reaching a size of 30 mm, male gonads are mature while at 40 mm both sex organs are fully mature (Millar, 1952). Ascidians exhibit variations in reproductive cycle patterns, fluctuating between a strict seasonal spawning per year (Sahade et al., 2004) to a continuous gamete release throughout the year (Lambert, 1968). The reproductive seasonality of ascidians can vary widely between species (Pérez-Portela et al., 2007; Fletcher et al., 2013) and all factors controlling their gametogenesis are still not fully understood (Bourque et al., 2007), however, temperature is considered a major variable (Sahade et al., 2004). It is believed temperature determines whether reproduction takes place year round or exclusively during a warm season (Millar, 1970; Svane, 1984; Durante and Sebens, 1994). Generally temperate ascidians predominately spawn during the summer months followed by a decline, occasionally resulting in a halt in reproduction in colder months (Coma et al., 2000; Lambert, 2005). Svane (1984) found species such as *Ascidia mentula* at sites of 15 m in depth followed a more seasonal pattern of gonad development while below 15 m breeding was more continuous. Bolton and Havenhand (1996) noted that there was synchronicity with light and spawning under laboratory conditions. The fertilisation and embryogenesis of solitary ascidians is an external event and the ascidian larvae usually spend no more than 24 h in the plankton before settling (Svane, 1984; Svane and Young, 1989). Ascidians possess limited dispersal capabilities due to the short larval period resulting in more localised natural recruitment (Davis and Butler, 1989; Davidson et al., 2005).

Invasive species and their associated parasites when introduced to a new area can pose a serious threat to highly susceptible local populations of cohabiting species. Few studies have been conducted on the micro-parasite communities of *A. aspersa*. Copepod species, including *Doropygus* spp., *Doropygella psyellus*, *Gonophysema gullmarensis, Lichomolgus canui* and *Notodelphys* spp., have been reported as ectoparasites living on the outer wall of the atrial cavity of this species (López-González et al., 1998; MacKenzie, 2011). Due to the large quantities of water that *A. aspersa* filters, it can potentially act as a carrier or reservoir of parasites for other host species and facilitate the introduction of disease such as the protistan parasite *Bonamia ostreae,* which is the causative agent of bonamiosis a fatal disease of the native European oyster *Ostrea edulis* (Lynch et al., 2007). It is recognised that fouling communities may facilitate the introduction of fish parasites including nepten liver disease, amoebic gill disease, the nematode *Hysterothylacium anduncum* and the sea louse *Lepeophtheirus salmonis* (Braithwaite et al., 2007).

The aims of this study were to determine if reproductive strategy is a key element in the success of an invasive species by investigating the reproductive patterns and health status of *A. aspersa* from different depths and current flow regimes where it is indigenous. Findings of this study might provide a better understanding of *A. aspersa* as an invasive species and the associated risks with such an introduction. An increased knowledge of *A. aspersa* biology and the factors influencing recruitment in its native habitat will better inform management in introduced areas.

2. Materials and methods

2.1. Study site

The present study was carried out at Lough Hyne Marine Reserve, Co. Cork, Ireland (51°70′N 9°40′W) (Fig. 1). Lough Hyne is an enclosed marine sea lough 1 km long and 0.5 km wide with a catchment area of 2.89 km^2. The lough is a designated statutory marine reserve located on the south west coast of Ireland with a high biological diversity and exceptionally varied habitats within a localised area (Bell and Barnes, 2003). Lough Hyne is connected to the Atlantic Ocean at the southeast corner (South Basin) by a narrow (25 m) and shallow stretch of rapids (5 m at high water) and due to constriction at this point there is an unusual tidal regime, which results in water flooding for 4 h but ebbing for 8.5 h (Ballard and Myers, 1997; Bell et al., 2003; Bell and Okamura, 2005). There is a strong inflow and outflow at the rapids (Kitching et al., 1952) and as the current flows around the lough it dissipates to low water flow in the Western Trough and negligible water flow in the North Basin (Greenwood et al., 2000). The North and South Basins are approximately 20 m deep and are connected by a deeper (50 m) trough in the western part of the reserve (Jessopp and McAllen, 2007; Sullivan et al., 2013). The average salinity in Lough Hyne is 34.06 ± 0.543, making it more marine rather than estuarine in nature (Jessopp and McAllen, 2007). Permanent loggers in the lough show a minimum/maximum seawater temperature of