Stress resistance in two colonial ascidians from the Irish Sea: The recent invader Didemnum vexillum is more tolerant to low salinity than the cosmopolitan Diplosoma listerianum

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ABSTRACT

It is widely believed that non-native species possess a high tolerance toward abiotic stress that allows them to survive transport and to colonize new habitats. However, to date empirical support for this concept is scarce. We therefore tested whether Didemnum vexillum, which recently invaded the Irish Sea, is more tolerant to fluctuations in salinity than the closely related Diplosoma listerianum, a cosmopolitan species of wide ranging distribution. The two fouling organisms exhibit similar habitat requirements and co-occur in many hard-bottom sites along the coasts of Ireland and Wales. We collected adult specimens of both taxa in Holyhead Marina, Wales, UK, and exposed them to different levels of reduced salinity ranging from 10 to 27 in short-term laboratory experiments. In separate trials, we mimicked chronic (2 weeks) as well as episodic (2 h) low-salinity stress to account for the natural temporal variability in osmotic fluctuations. We compared the ascidians’ performances in the face of stress and found that the invasive D. vexillum showed higher growth and survival under low salinities than the cosmopolitan D. listerianum. This indicates that D. vexillum is generally better in adapting to fluctuating ion concentrations. This competitive advantage may facilitate the establishment and spread of D. vexillum, especially if osmotic conditions in coastal habitats will be altered by future climate-change induced shifts in local precipitation regimes.

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1. Introduction

The colonial ascidian Didemnum vexillum (Kott, 2002) is a rapid invader of inshore and offshore marine hard-bottom habitats (Bullard et al., 2007a; Coutts and Forrest, 2007; Gittenberger, 2007; Griffith et al., 2009; Minchin, 2007; Minchin and Sides, 2006). Its origin has not unequivocally been identified, but it presumably stems from Japanese waters (Lambert, 2007; Stefaniak et al., 2009). From there it dispersed with slow moving vessels and aquaculture as the most important transport vectors of D. vexillum along the west coast of North America. In the British Isles, D. vexillum was first found in two marinas on the east coast of Ireland in 2005 (Minchin and Sides, 2006) and, three years later, in Holyhead Marina in North Wales (UK) (Griffith et al., 2009). Intense traffic between North Wales and Ireland makes it likely that D. vexillum reached Holyhead Marina via fouled recreational vessels from infected areas.

D. vexillum may cause ecological and economic harm by altering marine habitats and damaging maricultures, especially shellfish farms (Coutts and Forrest, 2007; Gittenberger, 2007; Valentine et al., 2007a). It grows fast and forms massive colonies, which can colonize a wide range of submersed substrata including other sessile organisms, e.g. tunicates, sponges, mussels, and macroalgae (Coutts and Forrest, 2007; Gittenberger, 2007; Valentine et al., 2007a), as well as man-made structures such as docks, floats, ship hulls, pilings, ropes and moorings. In addition to its colonizing abilities, it has a wide thermal tolerance that ranges from ˂0 to ˃24 °C (Bullard et al., 2007a;
water and salinity levels were adjusted using a salinometer (cond315i experimental only). To achieve these different ion concentrations, (chronic stress experiment only) and d) 10 (episodic stress experiment only) Salinity levels covered the range of osmotic conditions that organisms "Valentine et al., 2007b). Furthermore, adult D. vexillum possess asexual dispersal mechanisms: Fragments containing brooded larvae can be released from colonies and drift until they re-attach to suitable surfaces (Lambert, 2009). These traits make D. vexillum an efficient invader that can dominate new habitats by rapid colonization and the competitive exclusion of native species (Bullard et al., 2007a; Lambert, 2007; Osman and Whilitch, 2007). In this study, we experimentally compared the ability to tolerate low salinities between the recent invader D. vexillum and the closely related Diplosoma listerianum (Milne-Edwards, 1841) from the Irish Sea. D. listerianum is a cosmopolitan species that was first described in England in 1841 (James T. Carlton, pers. com.), but its actual origin is still unknown (Carlton and Eldredge, 2009). Both species belong to the family Didemnidae, are filter feeding fouling organisms, grow to comparable sizes, and co-exist frequently. Information about the capacity of the two ascidians to cope with natural events of lowered salinity will, on the one hand, help to assess the potential of the invader to establish and spread in brackish environments. On the other hand, it may allow us to predict stress-induced shifts in the competitive symmetry between the two species in habitats that are prone to freshwater influx. In separate experiments, we mimicked chronic (2 weeks) as well as repeated episodic (2 h) low-salinity stress.

2. Material and methods

2.1. Establishment of experimental colonies

Between May and September 2009, colony fragments (1 to 4 cm²) of both ascidian species were gently scraped from floating pontoons in Holyhead Marina, Isle of Anglesey, Wales (N 53°19'; W 04°38'). After transport to the nearby laboratory, the colonies were cut into similar sized fragments (~5 x 10 mm) using a scalpel and were then induced to re-attach onto 76–52 mm glass slides (carrier-slides in the following, method described in Epelbaum et al., 2009). Immediately before transplantation, colony surfaces were carefully examined for epibionts that were removed using a paintbrush. During culture and throughout all experiments, the ascidians were kept on a 15:9 h light:dark cycle in 0.9 L tanks (i.e. storage containers and experimental units) filled with seawater from the Menai Strait (salinity of 33–34) at 16 ± 1 °C and were fed twice a day with 2 ml of a Rhinomos rasatala suspension (1000 cells·μl⁻¹). Re-attachment of colony fragments was tested after 2 days by squiring the colonies with a pipette: Attached fragments remained in place, while the unattached fell off the slides (Bullard et al., 2007b). Only well-attached colonies were used in the experiments. Carrier-slides were suspended vertically from the top of the tanks using rods and clips (see Epelbaum et al., 2009), while the water within the containers was renewed every third day. On these occasions, tanks and glass slides were cleaned with a soft paintbrush. A total of 75 colonies per species were used. Prior to experimentation, colonies were kept in the laboratory for at least one week to allow acclimatization.

2.2. Experimental set-up

All experiments had a two-factorial design in which the factors “Species identity” (two levels: Didemnum vexillum and Diplosoma listerianum) and “Salinity” (maximum three levels) were orthogonal. Salinity levels covered the range of osmotic conditions that organisms can experience in Holyhead Marina: a) 34 (ambient in Holyhead Marina/Menai Strait), b) 27 (chronic stress experiment only), c) 20 (chronic stress experiment only) and d) 10 (episodic stress experiment only). To achieve these different ion concentrations, seawater from the Menai Strait was diluted with unchlorinated tap water and salinity levels were adjusted using a salinometer (cond315i from WTW). Ascidians were exposed to the diluted media abruptly by transplanting the carrier-slides from the storage containers to the experimental units, of which each represented one replicate of a given factor level. Each replicate contained one colony.

2.3. Growth and survival under chronic low-salinity stress

We measured growth rates of D. vexillum and D. listerianum under ambient conditions (salinity of 34) and permanent low-salinity conditions (salinity of 27 and 20) for two weeks. The first experiment started in mid-July (6–7 replicates per salinity level) and the second in early-August 2009 (11 replicates per level). In the latter experiment, we additionally recorded survival rates of both species under the different osmotic conditions. Mortality was recorded daily, while colony growth was documented weekly. To determine colony growth, each slide was photographed with a frame-mounted digital camera from a constant distance at an angle of 90°. The surface area of each colony was digitized using image analysis software (Image J). This approach only considers the lateral extension of the colonies, but does not account for an increase in thickness or colony folding. However, we observed no signs for neither of the two (Frederike Gröner, pers. obs.) and Millar (1952) stated that colonial ascidians mostly grow by increasing their surface area, but very rarely by gaining in thickness. Therefore, we believe that the potential bias associated with this sampling method was negligible.

The proportional change in colony surface size over two weeks served as a response variable and a two-way ANOVA tested for differences in growth rates. Tukey’s HSD was used for identifying significant differences between single treatment levels in case the global null-hypothesis was rejected. We used the Shapiro-Wilk’s-W test to test for the normality of data and the Levene’s test for confirming homogeneity of variances.

To determine the health status of the colonies, we checked whether a) the colony or single zooids detached from the glass slides, b) unicellular algae accumulated on the bottom of the tank, indicating that the zooids were not feeding, and c) colony color changed from pale yellow to brown-grayish in association with the appearance of small dark-brown spots on the surface of D. vexillum colonies. The spots were clumps of fecal pellets caught in the cloacal canals and cavities near the colony’s surface (Valentine et al., 2007a). Colonies were considered dead whenever at least one of these criteria was met; we never observed that a colony previously identified as moribund recovered. Differences in survivorship between salinity regimes were analysed using the Log-Rank test.

2.4. Survival under episodic low-salinity stress

We also tested for differences in survival rates of D. vexillum and D. listerianum under short-term low-salinity stress: Over a period of two weeks in August 2009, slide-attached colonies of both tunicates (n = 11 per species) were transferred from ambient salinity (34) to a salinity of 10 every second day for 2 h by transplanting the carrier-slides as described earlier. After this time, they were immediately returned to ambient conditions. By this, we mimicked abrupt drops in salinity, which can occur, for example, in tide pools during heavy rainfall. Death of colonies was recorded daily and survival curves were compared with the Log-Rank test.

3. Results

3.1. Growth under chronic low-salinity stress (July and August 2009)

Colony growth in both Diplosoma listerianum and Didemnum vexillum was significantly impaired when the salinity was hypo-osmotic to seawater, while the size of the effect differed between taxa (Fig. 1, Table 1). Under ambient salinity, colonies of D. vexillum
doubled their surface area (104% increase) within one week, while at salinities of 27 and 20 growth was reduced by two thirds (35% increase, 28% increase, respectively).

In contrast to the rapidly growing *D. vexillum*, *D. listerianum* colonies increased on average by 6% per week under ambient salinity, while there was no change in surface area at a salinity of 20. At a salinity of 27 all *D. listerianum* colonies died within seven days.

The second experiment in August 2009 yielded similar results: Growth rates differed strongly between species as well as between salinity levels (Fig. 2, Table 2). However, overall growth rates were much lower in August than in July. Colonies of the invasive *D. vexillum* grew only by 37% per week under ambient salinity, while moderately lowered salinity (27) reduced growth to 0.4% per week. Furthermore, colonies at a salinity of 20 shrank due to the death of zooids (5% loss in surface area per week; Fig. 2). The cosmopolitan *D. listerianum* grew only by 7% per week under ambient salinity, while all stressed colonies shrank (37% and 17% loss in surface area per week at salinities of 27 and 20, respectively; Fig. 2).

3.2. Survival under chronic low-salinity stress (August 2009)

Under ambient salinity conditions (34), both species, *D. listerianum* and *D. vexillum*, showed negligible mortality during 14 days: only one colony per species died (Fig. 3a). At a salinity of 27, however, mortality in *D. listerianum* was generally higher than in *D. vexillum*. After seven days, 64% of the *D. listerianum* colonies were dead, but only 5% of *D. vexillum* colonies. *D. listerianum* showed 100% mortality after ten days, whereas 72% of the invasive colonies survived until we terminated the experiment after 14 days (Fig. 3a). Kaplan-Meier survival curves were significantly different between species (Log-Rank test: \( \chi^2 = 19.7; p \leq 0.001 \)). When salinity was at 20, the general picture was the same, while the effect of low-salinity stress was more pronounced: 92% of *D. listerianum* colonies died within six days, but only 18% of the colonies of *D. vexillum*. All *D. listerianum* were dead at day 10, while 55% of the invasive *D. vexillum* survived until day 14 (Fig. 3b). Survival curves were again significantly different (Log-Rank test: \( \chi^2 = 14.6; p \leq 0.001 \)).

### Table 1

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## 3.3. Survival under episodic low-salinity stress (August 2009)

When exposed to severe osmotic stress (salinity of 10) for 2 h every second day, mortality in *D. listerianum* was significantly higher than in *D. vexillum* (Log-Rank test; \( \chi^2 = 24.6; p \leq 0.001 \)). The latter showed no mortality at all, while 55% of the *D. listerianum* colonies died within one day and 100% within eight days (Fig. 4).

4. Discussion

We compared the performances of the recent invader *Didemnum vexillum*, a colonial ascidian that is known from the Irish Sea since 2005, and the closely related *Diplosoma listerianum* in the face of chronic and episodic low-salinity stress. In both cases, *D. vexillum* showed higher growth and lower mortality rates than *D. listerianum*, indicating that it is more tolerant to fluctuations in ambient salinity.

Temperature and salinity have repeatedly been proposed as important environmental determinants controlling the distribution of ascidians (Groszholz, 2001; Millar, 1971; Toop and Wheatly, 1993). Moderate temperature rise increases growth and recruitment rates (Brunetti et al., 1980; Stachowicz et al., 2002), while previous findings suggest that sudden declines in salinity cause mass mortalities among ascidians (Goodbody, 1961; Lambert, 2005; Lambert and Lambert, 1998). It has been suggested that tunicates perform best at salinities above 25 and that they rarely tolerate brackish conditions (Lambert, 2005). They are stenohaline osmotic conformers and mostly lack tubular excretory organs that could assist in ionic and osmotic regulation (Goodbody, 1974; Prosser, 1973). Therefore, brackish conditions, for example in enclosed and semi-enclosed sea areas such as the Baltic Sea, are viewed as a major barrier for the distribution of these organisms (Groszholz, 2001; Millar, 1971; Toop and Wheatly, 1993). In contrast to this general notion, the two species we investigated have been reported from brackish systems. *D. vexillum* is known to inhabit the Kiso River estuary, Japan (Lambert, 2009) and the Mystic River estuary, USA, (Osman and Whitlatch, 2007), while *D. listerianum* can be found in the Lagoon of Venice (Brunetti et al., 1988). However, the majority of reports of the two species come from habitats with oceanic salinity (Lambert, 2009;
During the entire experiment (data not shown), n=11 in both groups. Days at which episodic stress was applied are underlined. Survival of colonies under ambient salinity conditions (34) was 100% during the entire experiment (data not shown), n=11 in both groups.

Fig. 3. Survival (%) of Diplosoma listerianum (white circles) and Didemnum vexillum (black quadrats) colonies at salinities of (A) 34 (circle in quadrat due to perfect overlap) and 27, (B) 20 during two weeks. n=11 in all groups.

Fig. 4. Survival (%) of Diplosoma listerianum (white) and Didemnum vexillum (black) colonies that were exposed to a salinity of 10 for 2 h every second day. After seven days, we terminated the experiment and let the colonies recover under ambient conditions (salinity of 34) for another seven days. Days at which episodic stress was applied are underlined. Survival of colonies under ambient salinity conditions (34) was 100% during the entire experiment (data not shown), n=11 in both groups.
in physical stressors and species introductions to clarify whether the one begets the other.

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