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Research article

The spread of *Styela clava* Herdman, 1882 (Tunicata, Ascidiacea) in European waters

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Abstract

The immigrant ascidian *Styela clava* Herdman is native to the northwest Pacific. It was first found in British waters in 1953. The spread of this sessile invertebrate in western European waters during the fifty years since its introduction is described, and the current distribution is reviewed with reference to physical constraints and the dispersal methods available. Natural dispersion may account for the spread to neighbouring sites, but isolated populations can best be explained by man-aided dispersal. Suitable vectors are proposed and the potential for further spread is discussed.

Key words: ascidian, distribution, non-indigenous species, *Styela clava*, vectors

Introduction

The solitary ascidian *Styela clava* Herdman, 1882 (Figure 1) is native to the northwest Pacific (Millar 1960). Specimens were first collected in British waters in summer 1953 from Plymouth Sound and the estuary of the Lynher River, near Plymouth (Carlisle 1954); they ranged from 47 to 129 mm in length and some, if not all, derived from larvae that settled in the previous year. It was originally designated *Styela mammiculata* sp. nov. by Carlisle, but Millar (1960) demonstrated it to be synonymous with *Styela clava* Herdman, 1882 (Herdman 1882). It is probable that it was introduced into Plymouth Sound by military craft returning from the northwest Pacific during the Korean War (Minchin and Duggan 1988). It rapidly became established along the south coast of Britain, probably because the water temperature regime in the English Channel is similar to that of the northwest Pacific (Millar 1960).

S. clava is a large solitary ascidian, some individuals growing to > 200 mm total length. The firm body is elongated and club-shaped; a complete description is provided by Millar (1970). It is attached to the substratum by a short narrow stem-like stolon with an expanded membranous base plate; adults protrude from the surface and are rheophobic. It is hermaphroditic and oviparous; it spawns after one year, producing approximately 5,000 eggs (Davis and Davis, in preparation), which hatch after 12 to 15 h at temperatures of 16 to 20°C (Davis 1997). The pelagic lecithotrophic larvae are tadpole shaped and approximately 0.85 mm in length (Figure 2); they are negatively geotactic and exhibit high barokinesis (Davis 1997). They rarely travel more than a few centimetres in sustained swimming activity; their movement is mainly vertical. The larvae are active for approximately 12 h (Davis 1997).

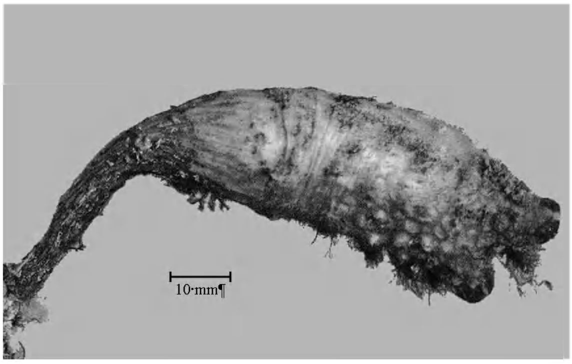

Figure 1. *Styela clava* Herdman, 1882 (Taken from Millar 1969)

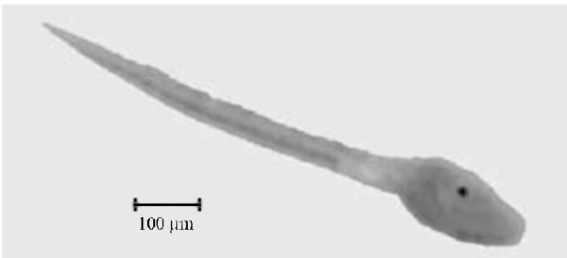

Figure 2. *Styela clava* larva (stained with Rose Bengal)

Since its discovery in Plymouth, *S. clava* spread around the coasts of Britain and Northern Europe, summarised by Lützen (1999). However, most observations have been opportunistic; and there is little recent information on the survival of the recorded populations. This study records the presence, and absence, of *S. clava* fifty years after its introduction employing a systematic survey technique. We also discuss the present distribution, constraints to dispersal and the likely further expansion of the species.

Methods

Populations in harbours often grow near the surface on floating substrata, e.g. pontoons, rafts, buoys and fenders, a consequence of the negative geotactic response of the larvae. We searched the near-surface areas of these floating objects either visually, where visibility was good, or by feeling the fouling community for the characteristic stolon; we also examined fouled ropes and other objects. Where there were no floating objects, we examined the harbour walls and some mooring chains by diving; a hand dredge was used to sample less accessible areas. Additional information was provided by fishermen, divers and aquarium staff. Although sub-littoral populations undoubtedly exist in some protected inlets, harbours, and particularly marinas, floating substrata could be more rapidly surveyed allowing us to survey large areas to verify a continued presence or to obtain new distribution records (Annex). A subjective ACFOR assessment of *S. clava* abundance (Abundant, Common, Frequent, Occasional, Rare) was made at each site.

Results and Discussion

The distribution of Styela clava

Annex records the presence of *Styela clava* in European harbours and ports during the period 1997 to 2006; these observations provide an indication of its current distribution, which can best be described as patchy. A sheltered and relatively high salinity site appears to be necessary for the establishment of a population in an area; however, it rarely spreads any distance along exposed coasts to neighbouring suitable habitats, with the notable exceptions of the Solent (southern England) and the Limfjord (Denmark).

Although *S. clava* is reported to be able to survive exposure to salinity of 10 psu (Kelly 1974), specimens have not been found in areas with salinity consistently lower than 22 psu; however, *S. clava* has been recorded in the eastern part of the Limfjord (Lützen 1999) where the salinity may drop below 20 psu for several days of the year. Of the total of 394 harbours and marinas examined in this study, populations were present at 156 sites (Figure 3; Annex), 75 of which contained water of salinity between 22 and 34.5 psu at the time of sampling. Salinity was not measured at the remaining 81 sites when the populations were discovered; however, 72 of these sites were in the Limfjord in areas regularly exposed to salinity greater than 20 psu. Where salinity was determined, the results are consistent with the laboratory observation that larvae cannot metamorphose at salinities below 20 psu (Kashenko 1996).

Of the 238 sites where *S. clava* was not found (Figure 3), 35 contained water of salinity less than 22 psu at the time of sampling, 129 contained water with salinity between 22 and 34.5 psu, and 45 contained water of salinity greater than 34.5 psu; salinity was not determined at the remaining sites. It is noteworthy that in the group of sites with salinity greater than 34.5 psu, 40 of the 45 sites were in the Mediterranean Sea (from Genoa, Italy to Benidorm, Spain); salinity at 16 of these sites was greater than 36 psu. In fact, 62 Mediterranean sites were inspected but no specimens of *S. clava* were found at any; however, we (MHD & MED) are currently investigating a reported observation in the Etang de Thau, near Sète (although adjacent to the Mediterranean Sea, the salinity in the lagoon is less than 34 psu). Most of the sites with salinity greater than 22 psu supported populations of other solitary ascidians (mainly *Ciona intestinalis* (Linnaeus) and *Ascidiella aspersa* (Müller) in the UK, together with the colonial *Botryllus schlosseri* (Pallas) in the Limfjord, and *Styela partita* (Stimpson) and *Molgula manhattensis* (De Kay) in southern European harbours). The sites with *S. clava* populations were often interspersed with apparently suitable sites where no specimens could be found, suggesting that the distribution is not extended by the colonisation of neighbouring sites resulting from the slow spread of benthic colonies or larval dispersion. However, it should be remembered that the inability to find specimens at a site does not necessarily mean that there are no populations present in the area.

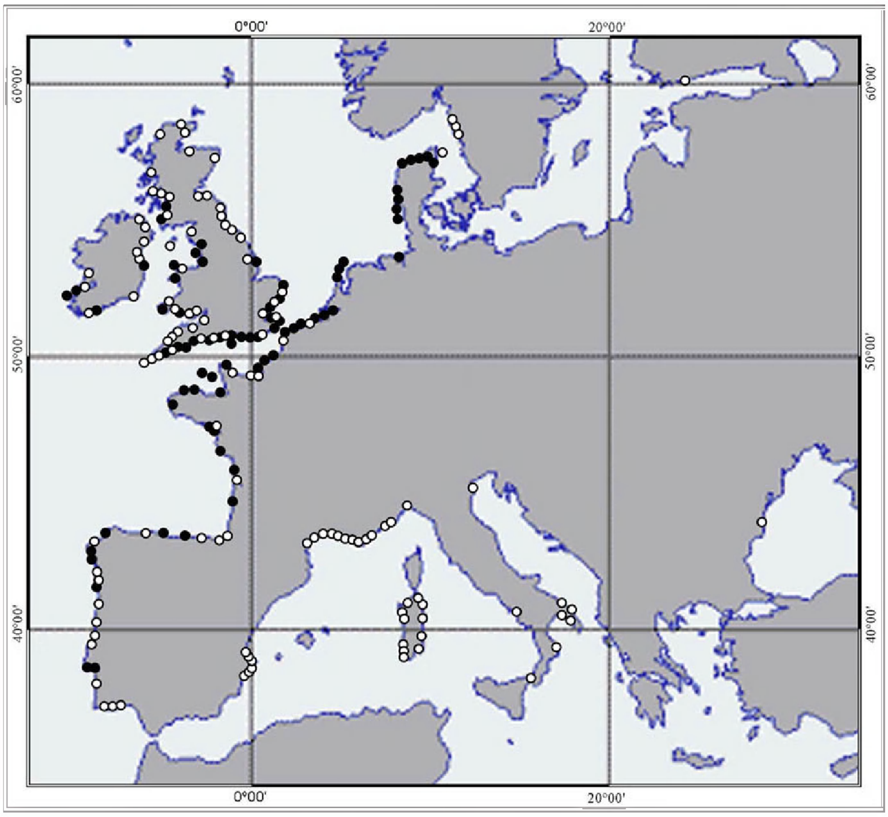

Figure 3. Current distribution of *Styela clava* in European waters (filled cycles indicate records of *S. clava*, open cycles – absence of the species. Adjacent sites are included in a single symbol. See also Annex)

Physical restrictions to the spread of S. clava

Styela clava adheres to a solid substratum. This usually takes the form of natural rocks, mussel shells or concrete in harbours, and metal or plastic in marinas. It has been found attached to seaweed, e.g. *Laminaria saccharina* and *Sargassum muticum*, but in general a hard substratum is required. Although it is commonly found near the surface in marinas, it can survive in the sub-littoral zone; individuals have been dredged from 10 m depth in the Solent (Barnes et al. 1973), 15 m depth in the Eastern Scheldt (Buizer 1980) and up to 40 m depth off the French coast (Dauvin et al. 1991). However, it is rarely found on exposed rocky shores, suggesting that it is intolerant of wave exposure.

The species is intolerant of continuous low salinity. It may survive for three days out of water if kept moist (Lützen and Sørensen 1993); specimens are often found attached to pebbles on beach exposed at low water spring tide, and attached to jetty supports up to a metre above the low water level.

Gonad maturation occurs when water temperatures exceed 8°C and spawning occurs when temperatures exceed 15°C (Holmes 1968). Larval settlement in Southampton Water was observed when water temperatures exceeded 16°C (Holmes 1968, Davis 1997). Thus, ambient water temperatures must exceed 16°C for several days for successful reproduction and settlement, and longer for successful recruitment. This will restrict the northward spread of *S. clava*. In fact, no UK populations were found north of Ardrossan (Lat. 55° 38'N) and Grimsby (Lat. 53° 34'N) on the west and east coasts of Britain respectively, which may represent the northern limits of UK distribution. Water temperature data from the Firth of Forth (eastern Scotland), Dunstaffnage (western Scotland) and Larne Lough (Northern Ireland), sites that are close to the assumed northern distribution limit, indicate that water temperatures have not exceeded 16°C over the last few years. Nevertheless, it is possible that insolation could increase the summer water temperatures of shallow marinas and coves to above 16°C, so undiscovered isolated populations of *S. clava* may exist further north. The northernmost known locality is the Limfjord, Denmark (Lat. 57° 04′N), where the average surface water temperature exceeds 16°C for two months in summer. As yet we have no information on the maximum water temperature at which *S. clava* can survive, but high water temperature may contribute to its absence from the Mediterranean Sea.

In general, a sheltered, mild environment appears necessary to establish a population. This may explain why the majority of the sites where it has been recorded are ports or harbours.

Methods of dispersal

Four methods of dispersal have been proposed for the spread of *Styela clava*, two natural and two man-aided (Lützen 1999):

i) as planktonic eggs and larvae, carried by tidal and other horizontal currents;

ii) as sessile adults attached to drifting flotsam, e.g. *Sargassum muticum*;

iii) as settled juveniles attached to oysters that have been transported and re-laid;

iv) as established adult animals attached to the hulls of ships.

Recent studies have suggested two further man-aided methods:

v) as planktonic eggs and larvae transported short distances in ballast water;

vi) as established adult animals attached to the interior of ships' sea-chests.

Natural dispersion

The total time spent as planktonic egg and larva is 24-27 h; at the end of this period the larva must find a settlement site. A larva rarely travels more than a few centimetres in sustained swimming activity. Thus larval dispersion is mainly dependent on water movement, which, in an estuary or harbour, has a maximum range of little more than the tidal excursion in the time available. This method of dispersion, probably with sub-littoral "stepping stone" populations, may account for the colonisation of adjacent inlets and harbours, for example within the Solent where the majority of suitable harbours support *Styela clava* populations; it may also be responsible for the slow eastward colonisation of the Limfjord from the original introduction site in the West. However, it cannot explain the establishment of distant populations; numerous deep water 'stepping stones' would be required between populations and, although specimens attached to stones and shells have been dredged from depths of 40 m off the French coast (Dauvin et al. 1991), a population spreading slowly along the sea-bottom is unlikely not to colonise interjacent protected harbours. Nevertheless, the spread of a sparse, continuous population of *S. clava* attached to suitable hard objects on the sea floor could explain the colonisation of some neighbouring sites.

Similarly, although dispersion as settled animals attached to drifting flotsam should cover a wider area, since wind could enhance tidal displacement, it would still probably be of local importance only (Lützen 1999). Furthermore, much flotsam washes up on the strand line (high tide mark) on beaches, where it may remain drying for several days before re-immersion. So the spread of organisms by this method would be opportunistic.

Man-aided dispersal: oyster movements

As natural dispersal appears to have a limited range, the spread of *Styela clava* has generally been attributed to the inadvertent introduction by man. It has been suggested that long distance dispersal could occur if juvenile animals were transported attached to oyster shells when the oysters were re-laid. This dispersion method would account for the appearance of *S. clava* in oyster culturing areas such as West Mersea in the UK, and the small harbours of Brittany, France; it is also probably that this was the vector by which *S. clava* was introduced into the Limfjord (Lützen 1999). However, this vector would only account for a few of the isolated populations.

Man-aided dispersal: ship hull fouling

An alternative hypothesis is that settled animals may be moved from one harbour to another attached to the hulls of ships. Transportation on slow moving wooden-hulled ships has been proposed as the method by which many of our apparently indigenous (cryptogenic) species were introduced over the last few thousand years (Carlton and Hodder 1995, Carlton 1999). But the hulls of modern operational ships are coated with anti-fouling agent to inhibit the attachment of organisms. Nevertheless, recent studies suggest that attachment to ships' hulls, particularly in areas of reduced flow, continues to be an important dispersal method for some immigrant species (Gollasch 2002). However, mature specimens of *S. clava* are rheophobic; their firm bodies protrude from the surface to which they are attached through the boundary layer into turbulent water. Hydrodynamic analysis indicates that they are unlikely to survive sustained high-velocity movement through water when the ship is in service; a force of 20 Newtons, equivalent to the hydrodynamic drag experienced at 8 knots, is typically sufficient to pull a 65 mm individual from an untreated pontoon within five minutes (Davis and Davis, in prep.). It would therefore appear that this method of dispersal is most likely to occur when a ship is in a colonised port for sufficient time to allow breakdown of the antifouling coating and subsequent larval settlement, and is then moved (with attached juveniles) at low speed to a new port and remains there long enough for the animals to reach maturity and spawn. Such a series of events is unusual, but may account for the appearance of *S. clava* in Loch Ryan, in southwest Scotland.

A survey of Loch Ryan in May 2003 revealed that there was a small population of *S. clava* in Stranraer Harbour and a few individuals around the ferry terminal (Cairnryan), but it was very abundant on and around a derelict jetty further down (seaward) the loch. There is a native oyster fishery in Loch Ryan and it is possible that stock movements may have introduced *S. clava*; however, the high population density on the jetty piles suggests a major inoculum was introduced in the vicinity of the jetty. This jetty had been the site of the breakers yard at Cairnryan; warships were moored to the jetty during dismantling. The paid off warships usually spent several years deteriorating in their final ports, most of which were infested with *S. clava*, and a further few years being de-equipped; they were towed slowly to Cairnryan, and then spent several years moored to the jetty whilst being dismantled - ideal conditions for the transfer of *S. clava*. A few examples, taken from Holme (1997), will suffice to illustrate how transfer could have occurred. The aircraft carrier HMS EAGLE (43,000 tons) was de-equipped over eighteen months in Portsmouth, laid up in the Hamoaze (Plymouth) for six years, then towed slowly to Cairnryan (approximately 650 km) over four days; demolition took over two years. The aircraft carrier HMS ARK ROYAL (43,000 tons) was de-equipped over two years at Devonport, and then towed slowly to Cairnryan over six days; demolition of the Ark Royal took over three years. HMS BULWARK, a 23,300 ton commando carrier, was de-equipped over three years at Portsmouth, and then towed slowly to Cairnryan over seven days; demolition took over two years.

Could the animals survive such a voyage? The average towing speed of HMS ARK ROYAL and HMS BULWARK was approximately 1.5 m s^{-1}, less than the typical flow in power station cooling water intake culverts (2 m s^{-1}) in some of which *S. clava* has become established. Furthermore, a recent hull examination of HMS SCYLLA, a 2,500 ton Frigate, revealed that *S. clava* could survive being towed over 200 miles from Portsmouth to Devonport. However, there is no reliable evidence that any of the ships dismantled at Cairnryan were fouled with *S. clava*. Thus this method of distribution must remain an intriguing hypothesis. Minchin and Duggan (1988) reported *S. clava* attached to the hull of a vessel that had been moored in Cork Harbour for almost a year and suggested that transport as hull fouling could be a possible introduction method. This observation appears to support the hypothesis; however, it is possible that the animals had settled and grown on the hull after it was moored in the harbour.

Man-aided dispersal: fouling on pleasure craft

Pleasure boats have also been proposed as a possible vector. The majority of small boats are taken out of the water during the winter months for maintenance and storage, so any juveniles resulting from summer settlement that were not removed prior to treatment with anti-fouling paint would die before re-immersion. However, some boats are left on moorings throughout the year; if one of these boats is colonised and moved to another harbour without anti-fouling maintenance, it could provide an inoculum for the receiving harbour. We have found *S. clava* growing on un-maintained boats in harbours; but neither we, nor the boatyards that we have approached, have found *S. clava* attached to the hulls of well maintained small boats. In this respect, it is interesting that we found no specimens of *S. clava* on the Isles of Scilly (south-west UK), yet numerous small pleasure craft sail to the islands from a variety of infested European ports. Nevertheless, pleasure craft are a feasible vector. Furthermore, since *S. clava* can survive up to three days out of water, a population jump in excess of 1000 km could be possible if an infected boat was transported across land on a trailer. The associated ropes, fenders and anchors could also transport organisms to new sites.

Man-aided dispersal: ship ballast tanks

Operational commercial shipping could transport *S. clava* as eggs and larvae carried in ballast water on short duration voyages, or as established mature adults attached to the interior surfaces of bow-thruster tunnels and sea-chests. Transport in ballast water has been proposed as the main modern-day vector for introduced species (see, for example, Carlton 1985). During August and September, the water in a port that supports a population of *S. clava* will contain eggs and larvae of this ascidian. Any ship taking on ballast water in such a port will inevitably take up some eggs and larvae with the water. If the ship discharges the ballast water in another port while the eggs or larvae are still viable, a new population may develop in that port, provided that the conditions are suitable for growth and reproduction. Minchin and Duggan (1988) considered this method of dispersal unlikely for *S. clava*. However, it would be possible for voyages of less than about 24 hours duration, such as the continental car ferry routes. Using this dispersal mechanism, the original population in Plymouth could have initiated settlement in Roscoff; then, once established there, *S. clava* could have spread to Cork Harbour. Similarly, larvae from Portsmouth and Southampton could have colonised Cherbourg, St. Malo and Le Havre. Ferries were often exchanged between Portsmouth and Dover, allowing a population of *S. clava* to become established in the Port of Dover. Larvae from the Dover colony could then have been carried to Calais, Dunkerque and Ostend.

It is difficult to prove that larvae are transported in ballast water other than by filtering the contents of the ballast tanks, which would have safety implications for the vessel; moreover, it is not easy to obtain access to the ballast tanks of operating ferries. Furthermore, the presence of larvae in ferry ballast water today provides only circumstantial evidence for the source of established populations that may have arrived many years earlier; in addition, the ferries have hulls and sea-chests, so it is impossible to exclude adult transport as the vector. However, the main limitation of this dispersal method is that the voyage must be less than 24 h duration or the larvae would have to settle on the walls of the tank and metamorphose into juveniles that would not be able to reproduce for many months. Given the limited water exchange, it is extremely unlikely that there would be sufficient food and dissolved oxygen in the ballast water to sustain the developing animals. So it is probable that any settled animals would die in the ballast tank before reaching maturity.

Nevertheless, populations of *S. clava* have been found in most of the commercial ports surveyed although, since access to the dockside in ports is normally difficult, the search has often been restricted to adjacent marinas. Examples include Shoreham, Sheerness and Liverpool Docks, and Holyhead Harbour in the UK; Wilhelmshaven in Germany; Esbjerg in Denmark; the French ports of Le Havre, Calais, Cherbourg and La Rochelle, and the Spanish ports of Santander and La Coruña. This suggests that commercial shipping could be a vector. However, some of the ports are situated close to other populations of *S. clava*, so the presence of the ascidian could be explained by both natural and man-aided dispersal.

Populations of *S. clava* have also been found in isolated commercial harbours that are not served by ferries and have no non-native oyster fishery, for example Fenit (on the west coast of Ireland), Porto and Lisboa (Portugal). Until recently, the only population recorded on the west coast of Ireland was in Fenit, a small isolated harbour visited by commercial ships that transport locally manufactured cranes to other European ports. The Portuguese specimens of *S. clava* were found in the marinas at Leixões, Cascais and Bom Sucesso (Davis and Davis 2005); the marina at Leixões is adjacent to the commercial port of Porto, and the marinas at Cascais and Bom Sucesso are close to the commercial port of Lisboa. All these commercial ports are too far from the nearest populations of *S. clava* for planktonic larvae to have been carried there by tidal movement, or transported in ballast water.

Since larval dispersal is unrealistic, sessile adults must have established these populations of *S. clava*. There are no populations of *S. clava* close to Fenit, Porto and Lisboa that could provide flotsam with attached adults. In fact, there are four marinas with suitable conditions for *S. clava* colonisation in the 300 km between Porto and Lisboa; but, despite exhaustive searches, no specimens of the ascidian were found in these intervening marinas (Davis and Davis 2005), as might be expected if flotsam or sub-littoral 'stepping stone' populations were vectors for adult dispersal.

Ships could transport adults if larvae settle and grow on the protected surfaces of the ship's hull, for example inside the bow-thrusters tunnels and sea-chests. A sea-chest is a void built into the hull below the waterline with direct connection to the surrounding seawater (Davis and Davis 2004b). It is the source of the cooling water, fire-fighting water and ballast-water pumped aboard, and is covered with a grill (typically 25 mm apertures) to protect the pumps from damage by large organisms and flotsam. The number and size of sea-chests is in proportion to the size of the ship, e.g. a 3,500 ton destroyer has eight sea-chests, typically 1m x 0.5m in cross section and 2m high, providing approximately 50 m^2 of settlement surface; some frigates of similar displacement have horizontal sea-chests, often providing in excess of 200 m^2 of settlement surface. Since the sea-chests are located within the bottom of the hull, all will receive a flow of seawater during passage that will be considerably less than that experienced on the hull surface. For example, a 2,000 m^3h^{-1} ballast water pump (typical of the type fitted to very large bulk carriers) will generate a water velocity of 0.56 m s^{-1} at the surface of a 1 m^3 sea-chest, less for a more realistic 2 m^3 sea-chest; furthermore, to avoid erosion of the internals of the ballast pumping and piping systems, the maximum design water velocity inboard of the sea-chest is usually 3 m s^{-1} (Taylor and Rigby 2001). Thus sea-chests provide a sheltered environment that can readily be located by negatively geotactic larvae, with sufficient substratum for large populations to become established.

The continuous water exchange in the sea-chests allows the transported animals free access to food and dissolved oxygen and provides good conditions for growth and development prior to spawning. Furthermore, if the ship travels through cold deep water into the warm shallow water of a harbour, the temperature shock may trigger synchronised spawning which would provide the high-density inoculum necessary to give a high probability of successful establishment of a population in the new habitat. Populations of reproductively mature organisms attached to mobile substrata, which spawned when suitable water quality conditions were encountered, could explain the heterogeneous distribution of *S. clava* and other non-indigenous sessile species. As yet we have only found hydroids, barnacles, mussels and tubeworms growing in sea-chests, but a study by Coutts et al. (2003) reported numerous species living in the sea-chests of a ferry that travelled between Tasmania and Australia. Furthermore, following the discovery of *S. clava* in New Zealand (Davis and Davis 2006), a 120 mm long solitary ascidian previously found in the sea chest of a tug that was surveyed in Lyttleton Dry Dock, New Zealand was identified as *S. clava* (A. Coutts, personal communication). This indicates that sea-chests are a suitable vector for adult sessile organisms such as *S. clava*, and one that could explain the initial transfer of the species from Korean waters to the UK.

The potential for further spread of Styela clava

The spread of *Styela clava* from one site to another can best be considered as an invasion. Biological invasions are composed of four stages; arrival, establishment, spread and persistence (Mollison 1986). Arrival and establishment may be considered as stochastic events; spread and persistence of a successfully established colony are deterministic processes. Successful establishment depends on the number of organisms arriving (inoculum) exceeding a viable minimum determined by demographic stochasticity, e.g. births and deaths, otherwise the founding population will die out. In addition, the receiving habitat must be suitable, a function of environmental stochasticity, with an available area exceeding the minimum area necessary to contain sufficient individuals to exceed the minimum inoculum or founder population size. Probabilistic modelling indicates that a translocated colony has a greater probability of establishing a new population than a single inoculum of larvae; thus man-aided dispersal of adults from a colonised site, for example by commercial shipping, is most likely to produce a successful invasion.

The donor and receiving ecosystems must be connected for any dispersal mechanisms to be successful, by currents for spread by natural dispersal or by transport routes for man-aided vectors. In addition, it appears that the salinity of the receiving environment should lie between 22 and 34.5 psu, and the summer water temperature should exceed 16°C for several weeks. Salinity, rather than temperature, will constrain the latitudinal dispersal of *S. clava*; the eastward spread through the Kattegat into the Baltic Sea is likely to be inhibited by the low salinity, and into the Mediterranean Sea by high salinity. Colonisation northwards is most likely to be restricted by the summer water temperature necessary for breeding; no populations have been found in Scotland above Ardrossan (18 sites examined), in North-East England above Grimsby (8 sites examined), on the Danish side of the North Kattegat (9 sites examined) or on the Swedish coast north of Gothenburg (13 sites examined). However, if *S. clava* adapts to colder waters, or global warming significantly increases seawater temperatures, it may colonise more northerly harbours during future years. The heated effluent from power stations could provide suitable water temperatures in the vicinity of the discharge point, but the enhanced temperature is rapidly dissipated and is usually less than 1°C within 500m of the outfall (Davis and Coughlan 1983); furthermore, the cooling water is treated to minimise the settlement of fouling organisms so the effluent near the discharge, although warm, is unlikely to stimulate the recruitment of *S. clava*. Colonisation does not appear to be restricted by the minimum winter water temperature experienced; populations in Prince Edward Island, Canada, survive under ice in winter (E. Darbyson, personal communication). Thus it is possible that *S. clava* could colonise some more northerly shallow harbours and bays if insolation raises the summer water temperature above 16°C for several weeks.

Given these constraints, we believe that there are several sites that *S. clava* could be expected to colonise in the near future. The species has not been found in Rosslare (Ireland) although this harbour has ferry connections with Cherbourg and Roscoff, and populations exist in Cork and Dun Laoghaire harbours. *S. clava* does not occur on Heligoland, but the harbour on this island is connected to mainland Germany by 13 shipping routes, including three harbours where *S. clava* is common (Wilhelmshaven and Hörnum) or present (Föhr), and the salinity and temperature conditions in the harbour are suitable for the establishment of colonies. No specimens have been recorded in Gothenburg, a busy North European commercial port, with shipping connections to many infected ports including Esbjerg and Aalborg (Denmark) and Harwich (UK); the absence of *S. clava* may be due to the frequent episodes of low salinity (there is large outflow of freshwater through the harbour from the River Göta Älv), which may restrict the survival of the species if introduced. However, the archipelago outside and immediately to the north of Gothenburg would satisfy the habitat requirements of *S. clava*. There are also some suitable sites along the west coast of Norway near Bergen where, once introduced, *S. clava* should thrive; many of the shallow, protected inlets, which contain oyster-polls, experience high summer temperatures and, thanks to the North Atlantic Drift, relative high winter temperatures. The species has barely entered the Kattegat, but there are a few sites in the northwest that would be suitable and could be reached by the spread of sub-littoral communities from the Limfjord. A similar bottom spread could produce populations in suitable harbours between Wilhelmshaven and Sylt on the German coast.

Conclusions

Since the immigrant ascidian *Styela clava* was first observed in British waters in 1953, it has spread along the coasts of the UK and Europe. The present distribution stretches from the Limfjord, Denmark, to Lisboa in Portugal. The distribution is patchy; no single dispersal mechanism can explain it. Of the dispersal methods proposed to explain the spread, natural dispersion of drifting larvae or adults attached to drifting flotsam has a limited range. Man-aided dispersal, as larvae carried in ships' ballast water on short-duration voyages and juveniles attached to oysters or to the hulls of ships, is feasible in a few situations. However, these dispersal vectors cannot readily explain the presence of many of the isolated populations. Transport of adults attached to the interior surfaces of ships' sea-chests is considered to be a more feasible mechanism. Sea-chests provide a relatively sheltered environment for the organisms to grow to maturity and spawn when suitable conditions are encountered.

Sites that do not support populations of *S. clava* provide an indication of the necessary conditions for colonisation. The potential distribution of *S. clava* can be predicted from knowledge of its temperature and salinity tolerance, and the commercial shipping links to sites with established populations. It seems likely that the species has reached its northern-most limit unless summer water temperatures increase dramatically. The southward spread of *S. clava* may still be continuing. Monitoring of suitable non-colonised sites will, should they become colonised, provide a more precise chronology of the spread of *S. clava*.

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Annex

Records of *Styela clava* in European waters 1997-2005

Location	Geographic coordinates		Survey date	Species abundance (ACFOR)	Salinity (psu)	Initial record or observation
	Latitude	Longitude				
Plymouth, UK	50.3647°N	04.1308°W	30.05.2003	A	30.8	Carlisle (1954)
Southampton, UK	50.8953°N	01.4047°W	18.06.2000	A	28.5	Holmes (1968)
Fawley, UK	50.8175°N	01.3280°W	11.06.2000	C	33.5	Davis (1997)
Poole, UK	50.6942°N	01.9907°W	12.05.2001	F	31.3	Holmes (1968)
Portsmouth, UK	50.7947°N	01.1077°W	06.06.2001	F	32.5	(Wells 1987)
Langstone, UK	50.7908°N	01.0367°W	22.07.2001	O	31.3	Houghton and Millar (1960)
Chichester, UK	50.7858°N	00.9425°W	22.07.2001	F	32.5	Stubbings and Houghton (1964)
Alverstoke, UK	50.7887°N	01.1208°W	06.04.2002	A	32.4	Davis and Davis (2004)
Lymington, UK	50.7570°N	01.5317°W	04.06.2002	F	24.0	Davis and Davis (2004)
Gosport, UK	50.7975°N	01.1192°W	06.06.2002	F	32.4	Davis and Davis (2004)
Brighton, UK	50.8113°N	00.1025°W	22.06.2002	C	32.5	Davis and Davis (2004)
Shoreham, UK	50.8305°N	00.2403°W	22.06.2002	O	30.5	Holmes (1968)
Yarmouth, Isle of Wight, UK	50.7055°N	01.5023°W	27.06.2002	O	33.3	Davis and Davis (2004)
Cowes, (Isle of Wight, UK	50.7618°N	01.2963°W	27.06.2002	F	32.2	Davis and Davis (2004)
Wooton Creek, (Isle of Wight, UK	50.7343°N	01.2133°W	27.06.2002	O	32.9	Davis and Davis (2004)
Bembridge, Isle of Wight, UK	50.6934°N	01.1075°W	27.06.2002	O	31.6	Wells (1987).
Weymouth, UK	50.6075°N	02.4583°W	03.07.2002	F	26.6	Davis and Davis (2004)
Exmouth, UK	50.6180°N	03.4240°W	03.07.2002	F	28.5	Davis and Davis (2004)
Dartmouth, UK	50.3508°N	03.5708°W	03.07.2002	C	27.4	Davis and Davis (2004)
Queenborough, UK	51.3513°N	00.7363°W	07.07.2002	O	29.4	Davis and Davis (2004)
Sheerness, UK	51.4467°N	00.7450°E	07.07.2002	R	32.6	Davis and Davis (2004)
Ramsgate, UK	51.3313°N	01.4210°E	07.07.2002	O	33.1	Davis and Davis (2004)
Dover, UK	51.1250°N	01.3117°E	13.07.2002	O	31.4	Davis and Davis (2004)
West Mersea, UK	51.7783°N	00.8975°E	13.07.2002	F	33.3	Davis and Davis (2004)
Harwich, UK	51.9485°N	01.2853°E	13.07.2002	O	33.3	Davis and Davis (2004)
Lowerstoft, UK	52.4720°N	01.7508°E	13.07.2002	O	32.7	Davis and Davis (2004)
Salcombe, UK	~50.23°N	03.77°W	09.2002	O	ND	J. Lützen (unpublished)
Adrossan, UK	55.6400°N	04.8183°W	11.05.2003	F	30.7	Davis and Davis (2004)
Stranraer, UK	54.9062°N	05.0288°W	16.05.2003	O	32.8	Davis and Davis (2004)
Cairnryan, UK	54.9638°N	05.0167°W	17.05.2003	C	33.0	Davis and Davis (2004)
Heysham, UK	54.0337°N	02.9325°W	17.05.2003	O	31.2	Coughlan (1985)
Fleetwood, UK	53.9168°N	03.0108°W	18.05.2003	F	30.2	Davis and Davis (2004)
Swansea, UK	51.6105°N	03.9192°W	07.06.2003	O	26.5	J. Coughlan (Pers.Com.)
Holyhead, UK	53.3200°N	04.6425°W	08.06.2003	O	32.9	Davis and Davis (2004)
Torquay, UK	50.4510°N	03.5283°W	12.08.2003	F	32.1	Davis and Davis (2004)
Brixham, UK	50.3992°N	03.5067°W	12.08.2003	O	32.5	Davis and Davis (2004)

Annex (continued)
Records of *Styela clava* in European waters 1997-2005

Location	Geographic coordinates		Survey date	Species abundance (ACFOR)	Salinity (psu)	Initial record or observation
	Latitude	Longitude				
Looe UK	50.3505°N	04.4503°W	13.08.2003	R	31.3	Turk (1975)
Falmouth, UK; close to Helford	50.1542°N	05.0683°W	13.08.2003	F	30.6	Davis and Davis (2004); recorded in nearby Helford by Lewis (1971).
Grimsby, UK	53.5775°N	00.0700°W	19.08.2003	F	27.4	Davis and Davis (2004)
Portland Harbour, UK	50.5708°N	02.4392°W	11.09.2004	R	33.1	R. Bamber (Pers.Com.)
Milford Haven, UK	51.7113°N	05.0375°W	12.09.2004	R	28.4	Coughlan (1969)
Pwllheli, UK	52.8708°N	04.4320°W	12.09.2004	R	33.2	P. Brazier (Pers.Com.)
Liverpool, UK	53.3992°N	02.9915°W	13.09.2004	F	26.9	Davis and Davis, new record
St. Peter Port, Guernsey	49.4592°N	02.5308°W	23.04.2003	C	33.4	Davis and Davis (2004)
St. Helier, Jersey	49.1808°N	02.1167°W	24.04.2003	F	33.0	Davis and Davis (2004)
Fenit, Ireland	52.2708°N	09.8625°W	04.08.2003	R	30.2	Davis and Davis (2004)
Dun Laoghaire, Ireland	53.2970°N	06.1347°W	28.08.2004	R	31.8	Minchin et al. (2006)
Dingle Marina, Ireland	52.1382°N	10.2778°W	24.09.2004	R	29.8	Minchin et al. (2006)
Cork, Ireland	52.8085°N	08.2033°W	24.09.2004	O	29.4	Guiry and Guiry (1973)
Le Havre, France	49.4892°N	00.0958°E	01.06.2002	C	30.9	Breton and Dupont (1978)
Dieppe, France; close to Paluel, France	49.9292°N	01.0837°E	01.06.2002	C	30.3	Monniot (1970); recorded in Paluel by Davoult et al. (1993)
St. Malo, France; close to Dinard, France	48.6492°N	02.0222°W	02.06.2002	O	33.3	Davis and Davis (2004); found in Dinard by Huwae and Lavaleye (1975)
Cherbourg, France	49.6467°N	01.6225°W	03.06.2002	O	24.3	Davis and Davis (2004)
Calais, France	50.9617°N	01.8442°E	12.07.2002	F	29.1	Davis and Davis (2004)
Dunkerque, France	51.0470°N	02.3743°E	12.07.2002	F	25.9	Davoult et al. (1993)
Arcachon, France	44.6600°N	01.1500°W	25.10.2003	F	32.1	Bachelet et al. (1980)
La Rochelle, France	46.1450°N	01.0117°W	26.10.2003	F	34.0	Davis and Davis (2004)
Sables d'Olonne, France	46.5033°N	01.7933°W	26.10.2003	O	34.5	Davis and Davis (2004)
Pornic, France	47.1100°N	02.1145°W	27.10.2003	F	34.0	Davis and Davis (2004)
Pornichet, France	47.2587°N	02.3458°W	27.10.2003	F	34.0	Davis and Davis (2004)
Brest, France	48.3917°N	04.4303°W	27.10.2003	F	34.2	Minchin and Duggan (1988)
Lézardrieux, France	48.7897°N	03.0992°W	28.10.2003	F	33.5	Monniot et al. (1986)
Morlaix, France; close to Roscoff	48.5900°N	03.8367°W	28.10.2003	O	27.6	Dauvin et al. (1991)
Oostende, Belguim	51.2283°N	02.9243°E	14.06.2003	O	28.8	Eneman (1995).
Zeebrugge, Belgium	51.3167°N	03.2025°E	14.06.2003	O	29.2	Dumoulin (1987); and in nearby Knokke-Heist by d'Udekem d'Acoz (1986).
Breskens, Holland	51.3950°N	03.5725°E	14.06.2003	O	27.0	Davis and Davis (2004)

Annex (continued)

Records of *Styela clava* in European waters 1997-2005

Location	Geographic coordinates		Survey date	Species abundance (ACFOR)	Salinity (psu)	Initial record or observation
	Latitude	Longitude				
Terneuzen, Holland; close to the Oosterschelde	51.3405°N	03.8275°E	14.06.2003	R	22.1	Davis and Davis (2004); recorded in Oosterschelde by Westerweel (1975)
Den Helder, Holland	51.9627°N	03.7827°E	15.06.2003	F	28.1	Huwae (1974)
Oost-Vlieland, Holland; close to the island of Texel	53.2232°N	05.0769°E	04.05.2004	F	ND	J. Lützen (new record); recorded on Texel by Huwae and Lavaleye (1975)
W. Terschelling, Holland	53.3889°N	05.2154°E	04.05.2004	F	ND	J. Lützen (new record)
Santander, Spain	43.4283°N	03.8100°W	23.10.2003	O	32.2	Davis and Davis (2004)
Gijon, Spain	43.5458°N	05.6642°W	23.10.2003	O	33.7	Davis and Davis (2004)
Muros (Galicia), Spain;	42.7773°N	09.0575°W	19.06.2005	O	31.2	Davis and Davis, new record;
close to Cambados, Spain	~42.50 °N	~08.83°W	06.1978	ND	ND	recorded in Cambados by Ortea and Vizcaino (1981)
La Coruña, Spain;	43.3667°N	08.3983°W	23.06.2005	O	31.5	Davis and Davis, new record
Sada (Galicia), Spain close to	43.3577°N	08.2467°W	23.06.2005	O	31.0	Davis and Davis, new record;
Figueras, Galicia, Spain	~43.50 °N	~07.00°W	05.1978	ND	ND	recorded in Figueras by Ortea and Vizcaino (1981)
Cascais, Portugal	38.6917°N	09.4155°W	06.09.2003	R	34.8	Davis and Davis (2005)
Lisboa, Portugal	38.6933°N	09.2100°W	06.09.2003	O	32.3	Davis and Davis (2005)
Porto, Portugal	41.1875°N	08.7033°W	07.09.2003	F	34.3	Davis and Davis (2005)
Wilhelmshaven, Germany	53.5045°N	08.1333°E	06.1998	F	ND	Lützen (1999)
List (Island of Sylt), Germany	55.0170°N	08.4330°E	09.1997	F	ND	Lützen (1999)
Hörnum (Island of Sylt), Germany	54.7500°N	08.3000°E	09.06.2004	F	ND	J. Lützen (new record)
Wyk (Island of Föhr), Germany	54.7000°N	08.5758°E	10.06.2004	F	ND	J. Lützen (new record)
From Thyborøn to Hals in the Limfjord, Denmark (72 sites)	56.7000°N to 56.9833°N	08.2167°W to 10.3000°W	1991-2005	F	>20	Christiansen and Thomsen (1981); Lützen and Sørensen (1993); Lützen (1999)
Havneby (Island of Rømø), Denmark	55.0720°N	08.5873°W	06.1998	F	ND	Lützen (1999)
Esbjerg, Denmark	55.4667°N	08.4333°W	03.1995	F	ND	Lützen (1999)

ND = Not Determined