

Background Information to Support Management of the Clubbed Tunicate, Styela clava, in Picton

Top of the South Marine Biosecurity Partnership







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EXECUTIVE SUMMARY

The clubbed tunicate, *Styela clava* is a type of sea squirt, and in New Zealand is a marine pest formally designated as an unwanted organism under the Biosecurity Act. The species was first recorded in New Zealand in 2005 and was discovered in Port Nelson in 2010. In early June 2013, four *Styela* individuals were found in the inner part of Picton marina, representing the first reported range extension of *Styela* into Marlborough. In eastern Canada, biofouling by *Styela* has historically had a catastrophic effect on shellfish aquaculture.

In New Zealand to date, *Styela*'s adverse effects on mussel aquaculture (in the southern Hauraki Gulf) have been localised and episodic, and the species is considered a nuisance more than a major problem. However, as the impacts of marine pests can be highly variable among locations and over time, the possibility of significant impacts on aquaculture and other values in Marlborough cannot be discounted. Marlborough District Council (MDC) is therefore considering management options for *Styela*, as part of its role within the Top of the South (TOS) Marine Biosecurity Partnership.

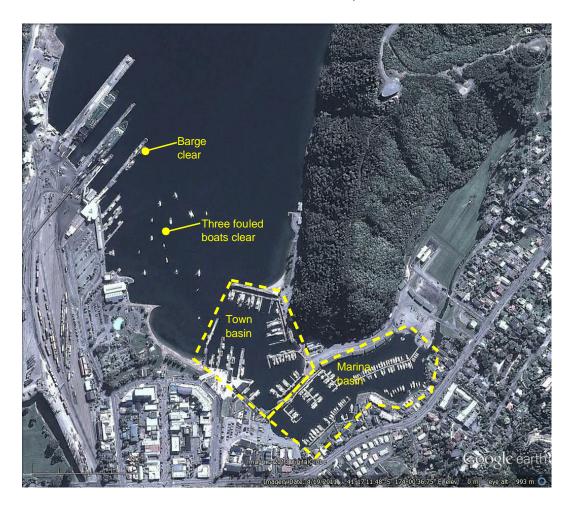
At the time of writing (October 2013), two dive surveys (24-25 June & 3-5 September) have been undertaken in Picton marina (see map below), to define the extent of the population, and remove those *Styela* found. Simultaneously, reports are being prepared on the impacts of the species, and the Ministry for Primary Industries is considering the costs and benefits of various management options. The purpose of this report is to: provide background technical information on the biology of *Styela*, summarise the results of the two *Styela* surveys, and discuss some of the key requirements and considerations for effective management.

Styela numbers, distribution and sizes have been detailed in separate reports accompanying the two dive surveys, and are only summarised in the present document. Key findings are as follows:

- In total, 118 Styela have been found and removed from the survey area: these were the four initial finds, 99 in June, and a further 15 in September.
- Two specimens were found in the outer marina (town basin), with remainder in a few "hot spots" in the inner marina basin. Two vessels were infected, but they were heavily fouled in general and not thought to have moved for some time (i.e. they were probably colonised by *Styela* while berthed).
- The size of the *Styela* specimens ranged from 20-120 mm. The reproductive state of 30 individuals was assessed, which revealed some *Styela* that had already spawned (i.e. released gametes into the water column, from which larvae develop and drift in water currents), and some that appeared ready to spawn.



Styela survey zone (yellow hatched areas) with vessels found to be clear at the time of the June 2013 survey. The marina basin configuration differs to that shown, because of recent construction.



Based on survey results and reproductive biology, it appears that *Styela* may have been present in Picton since late 2012. It has the potential to be reproductive throughout most of the year, albeit at a reduced level in winter. Studies of *Styela* from elsewhere in New Zealand and overseas suggest that spawning activity will be greater when the water temperature reaches approximately 15 °C, which in Picton is likely to occur in late October to early November. The higher density and relatively clumped distribution of *Styela* in the inner marina basin is consistent with the majority of spread by larval dispersal being over scales of tens of metres, with a larger "jump" reflected by the two specimens from the outer town basin. However, the species has a larval duration of approximately one day, meaning water currents could spread larvae outside Picton Harbour into Queen Charlotte Sound.

The reproductive seasonality and dispersal potential of *Styela*, as well as other characteristics of its biology, habitat, and present distribution in New Zealand, are important to take into account when considering different management options. The key issues are summarised in the Table below.



SUMMARY OF TECHNICAL ISSUES RELATING TO STYELA MANAGEMENT IN PICTON

Attributes of <i>Styela</i> and its environment	Management implications and other considerations
Impacts potentially significant, especially to mussel aquaculture, but cannot be reliably predicted	Difficult to assess the benefits of local eradication, population control or other management measures; however, a precautionary approach is advised given evidence of aquaculture impacts overseas.
Unmanaged <i>Styela</i> populations exist in Nelson and elsewhere in New Zealand	Increasing risk (over time) of reinvasion to Picton, or range extensions into other parts of the TOS region even if the Picton population is eradicated or contained.
Poor knowledge of TOS distribution	Absence of regional surveillance means that <i>Styela</i> may already be established elsewhere in the TOS region, undermining the benefits of response in Picton
Habitat generalist (can live in most artificial and natural habitats)	 Most coastal habitats across the TOS are probably suitable for Styela, from the low intertidal to as deep as about 40 m. Delimitation surveys and population response are relatively difficult in natural habitats.
Wide environmental tolerances (regarded as "hardy")	 Resilient to wide range of temperatures and salinities. Can probably persist at salinities as low as 20 ppt. Probably has some resistance to predators in natural habitats.
Reproductive biology poses management challenges	 May be reproductive for most of the year, meaning the frequency of population management may need to be every 1-2 months to facilitate detection of individuals before they mature. Generally thought not to be self-fertile, but this is uncertain; if self-fertile, every individual would need to be detected before maturity if local eradication was the goal.
Moderate natural dispersal potential	Larval dispersal may generally lead to localised spread (e.g. within tens to hundreds of metres of spawning adults), but a planktonic larval duration of around one day makes longer distance dispersal possible, and complicates the clear definition of a delimitation/control zone.
High potential for human- mediated dispersal	 Effective vector management is critical for the goal of reducing spread. Generic vector management measures (e.g. vessel antifouling) are effective against many species, hence have added benefits beyond Styela alone. Public awareness undertakings in the TOS do not appear to have been very effective at reducing vector risk to date, suggesting a need for improved approaches.
Restricted ability to detect all Styela in the Picton due to high existing fouling and multiple habitats available	 Local eradication will be difficult, but sustained control to low density may be achievable given sufficient effort. Generally good water in Picton clarity improves detection ability.



Some of the features described in the Table make management efforts based on control of the established population particularly challenging, to the extent that vector management may be a more realistic approach (e.g. management of vessel hull fouling). Vector management has the benefit of focusing efforts on risk pathways and all associated species, rather than single species that are considered to be high risk. Clearly, however, there is a need for effective vector management approaches that are supported by the stakeholders that contribute to risk.

In the current context, probably the best outcome for the TOS is that population control, combined with effective vector management, may slow the spread of *Styela* to areas with values most at risk (e.g. the Pelorus Sound mussel growing region). However, as the species is not managed in Nelson or elsewhere in New Zealand, it is not possible to reliably estimate the extent to which its spread could be delayed. In fact, as there is no regional surveillance, it is possible that *Styela* is already present in the TOS in areas outside Nelson and Picton. The decision on whether and to what extent to respond to this particular range extension must be weighed against the uncertain, but potentially significant, adverse effects on aquaculture and other values. Clear goals for a response are needed (as this will drive the management approach and related information needs) along with criteria for termination of efforts. Decision makers should recognise that response efforts may need to be sustained (or even increased) over many years.



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1. INTRODUCTION

The clubbed tunicate, *Styela clava* (referred to hereafter as *Styela*) is a marine pest formally designated as an Unwanted Organism under the Biosecurity Act. The species was first recorded in New Zealand in 2005 (McFadden *et al.* 2007), and has been slowly spreading since then. In the Top of the South (TOS), a small *Styela* population was first recorded in Port Nelson in 2010. More recently (in early June 2013) it was discovered in Picton marina, during 6-monthly routine surveillance conducted by the National Institute of Water and Atmospheric Research (NIWA), which was funded by the Ministry for Primary Industries (MPI).

The Picton find is the first reported range extension of *Styela* into Marlborough. Because of the potential adverse impacts of *Styela*, the Marlborough District Council is leading a response to the range extension, as part of its role within the TOS Marine Biosecurity Partnership. As an initial step, MDC instigated a *Styela* population delimitation and removal survey in Picton, which was conducted on 24 and 25 June 2013. A second removal survey was then undertaken over 3-5 September 2013.

MDC and the TOS Partnership have sought guidance on longer term management options for *Styela*, and commissioned this report to:

- Provide background technical information on *Styela* that will assist those making decisions on the nature and extent of further management.
- Summarise the results of the Styela delimitation and removal operations.
- Discuss related implications for management.

The report has been produced by Marine Biologic Ltd, under contract to The Lawless Edge Ltd which leads a Co-ordination Team that is responsible for promoting and implementing marine biosecurity risk reduction practices in the TOS.

The intent of the report is to provide sufficient information to guide decision-making. It does not reflect a systematic or exhaustive review of available information on *Styela*, as there has been considerable overseas research on the species over several decades. The report identifies some aspects of *Styela*'s biology and impacts for which knowledge gaps and uncertainty are apparent, and where a precautionary approach to management is advised.



2. TECHNICAL INFORMATION ON STYELA

2.1. Background

This summary of technical information draws on New Zealand studies of *Styela* where possible, supported by overseas information where necessary. These information sources include various reviews and studies (e.g. Lützen 1999; Davidson *et al.* 2005; Clarke & Therriault 2007; McClary *et al.* 2008) which themselves cite literature that was not readily available (i.e. unpublished reports or old publications that were not available online). In such situations, it was necessary to accept at face value the accuracy of information provided.

The key New Zealand studies on the biology or management of *Styela* that are referred to throughout the document, are as follows:

- McClary *et al.* (2008): Reproductive behaviour of *Styela* in Waitemata Harbour.
- Wong *et al.* (2011): This work extended the time frame of the McClary *et al.* study.
- Webber (2010): Demography and population projections of *Styela* in Port Lyttelton.

Where the technical studies from New Zealand and overseas differed or conflicted in terms of the information they provided, greater weight was generally given to the information gained from New Zealand studies. Other New Zealand examples (e.g. the sea squirt *Didemnum vexillum* in the TOS) have shown that overseas information is not always directly transferrable to New Zealand (Fletcher 2013); there is no substitute for locally-collected knowledge and observations.

2.2. Styela description and habitats

Styela has an elongated, club-shaped body that attaches to hard surfaces by a stalk (Figure 1). The stalk may be less distinct in small specimens, with the body attached directly to the substrate. It has a tough, leathery and knobbly outer surface. Each individual is attached separately, although it can grow in dense clumps. It appears to commonly reach a length of around 150-160 mm, although a specimen as long as 220 mm has been reported (Davis & Davis 2009). Styela is reported to live for up to 3 years, but 1-2 years appears more common (Lambert & Lambert 1998; Lützen 1999). The Webber study in Lyttelton suggests most individuals in that locality live for about one year.







Figure 1. Images of Styela clava.

Most coastal habitats across the TOS are probably suitable for *Styela*. Globally, it has been found from the low intertidal zone (around neap low tide) to about 40 m depth, although it is generally reported as being most common at 25 m depth or less (Lützen 1999). *Styela* is described as having a preference for sheltered waters away from wave action; although overseas information suggests that it may inhabit semi-protected waters on more wave-exposed coasts (Clarke & Therriault 2007).

Styela can be considered as a habitat generalist: it occurs in a variety of habitats such as on marine farms and other artificial structures; and is found in natural habitats on rock, shell, and seaweed, including in areas of soft sediment where there is a hard surface for attachment. For example, in the southern Hauraki Gulf, it is common in low tide rocky areas adjacent to infected oyster farms (pers. obs.), and in subtidal soft-sediment habitats in parts of the Firth of Thames (Grange *et al.* 2011).

2.3. Distribution in New Zealand

Since being first discovered in New Zealand in Waitemata Harbour in 2005 (Gust *et al.* 2005), *Styela* has spread to a number of ports and harbours. The current distribution indicated in the Marine Biosecurity Porthole¹ website includes several locations between the northland east coast and Tauranga Harbour, as well as Wellington Harbour, Nelson, Lyttelton and Otago Harbours. Recently, *Styela* was also described from Pauatahanui Inlet near Wellington.

In Nelson, *Styela* has become widespread across the port and harbour since populations were first recorded in 2010, but it generally occurs at low densities. Although the incursion in Picton marina is the first recorded population in the TOS outside Nelson, it is of interest that specimens of *Styela* were removed from two

http://www.marinebiosecurity.org.nz



vessels in Nelson in 2006, and from a single vessel in Waikawa Bay in 2005 (Morrisey & Miller 2008). Such information, together with the fact that marine pest surveillance is not conducted outside of TOS ports and marinas, makes it conceivable that *Styela* may be more widespread in the region than is currently understood.

2.4. Impacts

Reliable knowledge of impacts is critical to understanding the benefits of management. However, as is the case for most marine pests, the level of *Styela*'s invasiveness, and its associated adverse effects, appear to vary considerably among locations and from one time to another. The highest reported densities cited from overseas literature are in the order of 500-1000 individuals/m² on artificial structures, with lower densities (50-100 individuals/m²) described in natural habitats (Lützen 1999; Clarke & Therriault 2007).

As *Styela* is a filter feeder, it is considered to have the potential to compete with native species for food and space. However, the species is most well-recognised for the devastating biofouling effect it had on the mussel farming industry in eastern Canada from about 2000-2007. The impact in Prince Edward Island led to considerable effort and cost for development and application of mitigation methods (Davidson *et al.* 2005; Bourque *et al.* 2007; LeBlanc *et al.* 2007).

If the level of infestation on TOS mussel farms mirrored the experience in eastern Canada, *Styela* would be one of the most significant threats to date to the mussel industry in the region. At present in New Zealand, *Styela* has caused problems for mussel farmers in the southern Hauraki Gulf (Figure 2), but not to the extent described in Canada. Personal communications with affected companies in the Firth of Thames (Sanford, Ted Culley; North Island Mussels Ltd, Steve Wells), indicate that *Styela* has reached problematic levels of approximately 20% of the weight on mussel long-lines as a worst-case; however, the effect is spatially patchy and has been less pronounced in the last couple of years. The high biomass increases the time/costs of harvesting, transporting and factory processing. However, the actual economic effects have not been quantified, and it may be difficult to discern the incremental effects of *Styela* from other factors that negatively affect mussel production.

An economic impact assessment conducted for MPI described a range of hypothetical impact scenarios on mussel farms, which highlighted some clear benefits in slowing *Styela* spread (Deloitte 2011). It would be useful to undertake further consultation with affected mussel companies to better understand actual effects in New Zealand, hence provide some context for the Deloitte projections.





Figure 2. *Styela* on cultured mussels in the Firth of Thames (image provided by Steve Wells, North Island Mussels Ltd).

In addition to effects on mussel culture, Nunn & Minchin (2009) suggest that biofouling by *Styela* may be problematic for other types of subtidal aquaculture systems, including fish culture cages, and elevated intertidal culture systems comparable to the rack system that is commonly used to grow Pacific oysters in New Zealand. To date in New Zealand, *Styela* does not appear to have caused problems for intertidal oyster culture. The species can be common on rack structures, but only occurs sporadically on the crop itself (pers. obs.); the tidal height at which the oysters are grown (i.e. on top of racks) is probably less than optimal for *Styela*. One of the unusual impacts of *Styela* cited in a number of overseas reports (e.g. Clarke & Therriault 2007) is an asthmatic condition that oyster shuckers have experienced when opening *Styela*-fouled oysters in poorly ventilated areas; however, I have not verified the source of this information.

Overall, *Styela* densities on artificial structures and natural habitats in New Zealand appear to have generally been quite low to date (e.g. a density of 1-10 individuals/m² in Lyttelton appears typical); with the exception of mussel farms in the Hauraki Gulf. However, even on those mussel farms, it is clear that the severity and spatial scale of *Styela*'s impacts have been considerably less than experienced in eastern Canada. Despite this situation, it would be unwise to assume that *Styela* would not result in significant adverse effects as it spreads in the TOS.

Further information on the impacts of *Styela* is contained in separate reports being prepared by MPI.



2.5. Environmental tolerances

Styela is described as having a "hardy" nature; being capable of withstanding salinity changes and temperature fluctuations, as follows:

- Temperature: Styela is reported overseas to occur in waters ranging from -2 to 24 °C.
- Salinity: Styela is not considered to be able to persist in localities with salinities < 20 ppt, but can withstand periods of salinity as low as 8 ppt due to its ability to close its siphons for "extended" periods (Sims 1984; Lützen 1999).

The TOS range is well within these temperature bounds, and *Styela*'s salinity tolerance suggests that estuarine as well as coastal habitats may be at risk.

2.6. Growth and reproduction

From a management perspective, information on reproductive strategies and seasonality, and size at (or time to) reproductive maturity, is particularly important. Such information helps to define the nature, timing and frequency of surveillance and response activities.

Immature *Styela* are reported from New Zealand and overseas studies to grow at around 10-15 mm per month, with growth apparently slowing during winter and as the animal reaches sexual maturity. The reported size and time to sexual maturity ranges widely among locations. The best available data for New Zealand populations suggests that sexual maturity may be reached at about 45 mm total body length (i.e. including stalk) (Webber 2010). Overseas, the reported range is from about 20 mm to 75 mm (Lützen 1999; Davidson *et al.* 2005). Reported times to sexual maturity range from about 2-10 months; Webber suggested that it could be as little as two months in Lyttelton.

Styela is a hermaphrodite (i.e. individuals have both male and female gonads) and spawns externally by releasing eggs and sperm into the water column, where eggs are fertilised and planktonic (drifting) larvae are produced. It is generally reported that the male and female gonads mature at different times to avoid self-fertilisation. Nonetheless, the McClary study in Waitemata Harbour suggested that single individuals may be able to self-fertilise, especially if physical disturbance (e.g. during removal) induces simultaneous spawning of both male and female gametes.

New Zealand and overseas studies indicate that developing or ripe gonads may be present at temperatures above 8 °C (Parker *et al.* 1999; McClary *et al.* 2008). As such, in New Zealand *Styela* populations there could be individuals with mature



gonads for much of the year (e.g. McClary study). Despite this situation, previous New Zealand studies are in agreement with overseas work in suggesting that 15 °C is a critical minimum temperature threshold for spawning (Parker *et al.* 1999; Bourque *et al.* 2007; Wong *et al.* 2011). However, it is important to consider that this threshold primarily relates to the onset of spawning as water temperatures warm moving from winter towards summer months. It is likely that in moving from summer into winter, the cessation of spawning will occur at a lower temperature. In their study in eastern Canada, Bourque *et al.* (2007) were perplexed by the fact that their research supported a 15 °C threshold for the summer onset of spawning, but they detected *Styela* larvae in autumn when water temperatures had declined to approximately 11 °C.

This same phenomenon was apparent from results of research in the TOS on the sea squirt *Didemnum* (Fletcher *et al.* 2013b). Whereas the onset of *Didemnum* spawning occurs in spring when water temperatures reach approximately 14 °C, there is a post-summer "trickle" of spawning that continues through to mid-winter when water temperatures have declined to 11-12 °C. Even when spawning was not detected, *Didemnum* tissue analyses revealed the presence of mature larvae (i.e. suggesting that spawning may have been occurring at a low level, even when it was not measured).

The seasonal disparity in temperatures for the onset and cessation of spawning conceivably explain why *Styela* specimens collected from Picton in June 2013 showed evidence of having spawned, or being ready to spawn, even though water temperatures were < 15 °C (see Section 3.2). The safest approach would be to assume that *Styela* in Picton has the potential to spawn year-round, albeit at very low levels in winter. However, increased spawning activity could be expected when a threshold temperature of 15 °C is reached, which in Picton occurs around late October to early November.

2.7. Natural and human-mediated pathways of spread

Knowledge of natural dispersal potential identifies situations where management of human-mediated pathways of spread may be worthwhile. Simultaneously, a knowledge of human vectors helps to define localities at risk from human-mediated spread, and the key vectors that should be targeted for management.

2.7.1. Natural dispersal

Being a species whose adult form is fixed to any suitable hard surface, *Styela*'s main mechanism of natural dispersal relies on its larvae being spread by water currents. Most estimates suggest that the maximum larval dispersal period is approximately one day (Davis *et al.* 2007), although larvae are reported to survive for three days under laboratory conditions (Kashenko 1996; cited in McClarv *et al.*



2008). After dispersal, the larvae "settle" to a suitable hard surface to then grow into the adult sea squirt. Most sea squirt larvae tend to settle within tens of metres of spawning adults (Clarke & Therriault 2007; Fletcher *et al.* 2013a); however, a one day larval duration in *Styela* means that longer distance dispersal is possible, depending on water currents.

Unpublished particle dispersion modelling for Queen Charlotte Sound (from Cawthron) suggests that larvae could be advected the length of the Sound in less than one week. A planktonic phase of even one day may therefore be sufficient to disperse larvae well outside of the marina area; however, the outer limits of dispersal are notoriously difficult to predict for marine species like *Styela* (Kinlan *et al.* 2005; Gaines *et al.* 2007). Factors such as larval and post-settlement mortality will greatly influence the realised distribution. Additionally, in the absence of self-fertilisation, larvae would need to settle very close to each other in order that successful spawning of the next generation occurred. Conversely, the occurrence of natural self-fertilisation is the worst-case scenario, as it raises the possibility that a single larva advected a few kilometres could give rise to a single reproductive adult and a new population.

Irrespective of the actual distance of larval-mediated dispersal, even where dispersal is limited, the fact that the species is a habitat generalist means that over time it is likely to gradually spread far and wide by natural mechanisms; i.e. it is unlikely to encounter unsuitable habitats that act as barriers to spread. An additional consideration is that *Styela* can attach to drift seaweed and flotsam (Lützen 1999; Davis *et al.* 2007), which could also be mechanisms of spread in the TOS.

2.7.2. Human-mediated spread

Biofouling on vessel hulls, or associated with aquaculture transfer pathways, is likely to be the primary mechanism for relatively rapid and longer distance human-mediated spread of *Styela* from existing populations in Nelson, and elsewhere in New Zealand (including Picton unless effective management measures are put in place). Over time, as *Styela* spreads nationally, populations elsewhere in New Zealand will likely become increasingly important as further infection sources to the TOS region. Entrainment of larvae in water (e.g. vessel bilge or ballast water) is also a potential risk mechanism, but probably not as important as biofouling (Clarke & Therriault 2007; Darbyson *et al.* 2009a; Darbyson *et al.* 2009b).

If Styela reaches TOS marine farms, intra- and inter-regional movements of fouled gear or seed-stock may rapidly exacerbate spread, as occurred during a TOS management programme for the sea squirt *Didemnum* during 2006-2008 (Forrest & Hopkins 2013). A key feature that makes aquaculture a particularly important pathway is that gear and stock transported from one place will often be redeployed



in another location for an extended period. As such, any *Styela* (even very small or microscopic stages) that survives the transport phase will have the opportunity to grow and reproduce. The same issue arises with any other vector (e.g. a vessel) that moves from an infected area and has a long "residence time" in a new location.



3. SUMMARY OF PICTON SURVEYS

3.1. Description of work undertaken

The NIWA survey in early June found four *Styela* individuals, but was estimated to cover only 10% or thereabouts of the habitat in the marina. The delimitation survey subsequently undertaken in Picton (24 and 25 June 2013) aimed to gain a better understanding of the extent of the population, and simultaneously remove any *Styela* that were found. The repeat survey conducted during 3-5 September aimed to contain the Picton population at a low level while longer term management options were considered. For delimitation purposes, two main zones were identified (see Figure 3):

- The marina basin (inner marina): this encompassed the area inside of the "Coathanger Bridge".
- The town basin: this encompassed the area seaward of the Coathanger Bridge, to a line extending from the rock wall enclosing the east side of the marina, across to the floating water taxi pontoons on the town side.

In these two zones, divers searched for *Styela* on: all vessels, pontoons (concrete and polyethylene), piles (concrete and wood), artificial walls (rock, concrete, wood), a small selection of seafloor habitats (e.g. shallow cobbles, deeper muddy areas) and associated debris (e.g. tyres), and other surfaces like hanging ropes and cables. All *Styela* found were encapsulated in plastic bags and then removed.

Water clarity ranged from about 2-3 m in the June survey, but was reduced in September due to dredging of the inner marina. In June, checks were made of a single barge outside of the main delimitation zones (the barge had been working inside the marina and was shifted to the ferry wharf area) and three moored boats that were conspicuously fouled. Neither the barge nor the boats had *Styela* on them.

Reports by Diving Services New Zealand Ltd (Lines 2013a, b) describe the survey work in detail, and show maps of the areas surveyed. Those reports also describe the location, numbers and sizes of the *Styela* found by divers. As such, only a summary is given below, along with data on the reproductive status of 30 of the larger specimens (23-93 mm length) found in the June survey. The reproductive assessment was undertaken by Mike Page from NIWA Nelson. Additionally, Cawthron sent samples to the University of Canterbury for genetic analysis, to try and ascertain the possible origin(s) of the Picton population.



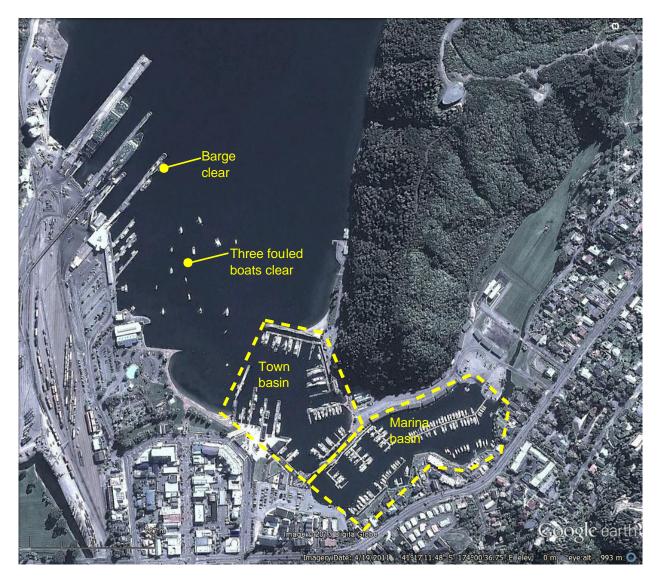


Figure 3. Overview map of *Styela* delimitation area, and vessels surveyed in June 2013. Actual configuration of inner marina basin differs to that shown, because of recent construction. Detailed maps with *Styela* distribution are reported by Lines (2013a,b).

3.2. Key findings

Key survey findings are as follows:

- To date, 118 Styela have been found and removed from the survey area: these were the four initially found by NIWA, 99 in the June survey, and a further 15 in September.
- All but two specimens were found in the marina basin. Most of the *Styela* in the marina basin were found within 650 mm of the seafloor on relatively new concrete piles. Ten of the 15 specimens found in September were attached to clumps of mussel that fouled a wooden structure at the head of the basin.



- Specimens were also found on two vessels. Seven small individuals were collected from a boat propeller in the marina basin in June, and one specimen (85 mm length) was found on a vessel in the town basin in September. The vessels were both heavily fouled and not thought to have moved for some time (i.e. they were probably colonised by *Styela* while berthed).
- Total *Styela* body length (including the stalk) ranged from 20-120 mm in June and 35-95 mm in September.

The higher density and relatively clumped distribution in the inner basin is consistent with the majority of spread by larval dispersal being over scales of tens of metres, with a larger "jump" reflected by the two specimens from the outer town basin. The apparent distribution of *Styela* in the deeper habitats of the marina basin perhaps reflects the species' intolerance of reduced salinity in the surface water layer; during the delimitation surveys, divers noted that the surface waters were colder than deeper water, with the characteristic visual distortion that is often evident when the surface layer has reduced salinity.

The size distribution of the 99 *Styela* from the June survey is shown in Figure 4. Given the longish "tail" in the occurrence of larger individuals (> 70 mm), with a hint of a bimodal distribution, it is possible that the larger specimens spawned before summer (in late 2012), giving rise to a more abundant cohort growing through the first six months or thereabouts of 2013. Alternatively, there may have been more than one introduction event; however, this is not clear from the genetic analysis. Preliminary findings of that analysis are that the Picton *Styela* did not originate from Lyttelton, but could have come from either Nelson or the Hauraki Gulf (Jono Underwood, MDC, pers. comm.).

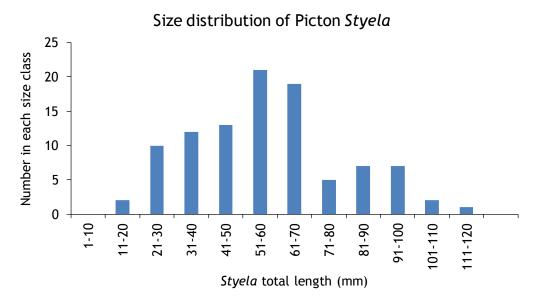


Figure 4. Size distribution of the *Styela* found in Picton during the June 2013 survey.



Of the 30 *Styela* collected in June that were assessed for reproductive state, 15 of 18 specimens less than 59 mm in length were immature. One specimen of 57 mm was classified as having spawned. A further four in the size range 65-93 mm had ripe female or male gonads. One specimen of 65 mm had ripe male (with motile sperm present) and female gonads present simultaneously, and appeared ready to spawn. Such results are consistent with the discussion is Section 2.6, and suggest a long seasonal reproductive window for *Styela* in Picton, with the possibility of year-round spawning.



4. CONSIDERATIONS FOR STYELA MANAGEMENT IN PICTON

Recent history in New Zealand, including efforts to manage *Didemnum* in the TOS, shows that effective management of marine pests after they have established is difficult and expensive. By far the best strategy is to prevent introduction, or slow the rate of spread where feasible. Hence, effective vector control is clearly paramount. If continued control of the *Styela* population in Picton is considered, a range of technical and other considerations must be evaluated, some of which are outlined in Appendices 7-9 of the TOS Operations Manual² (V2.5, September 2013). Some of the key management issues are outlined below.

4.1. Efforts to manage Styela in New Zealand

There is no national management programme for *Styela*. Analysis of management options for the species in Auckland and Lyttelton (by MPI), determined that eradication from those locations was not feasible. The absence of national management, combined with the occurrence of unmanaged *Styela* in Nelson, makes other range extensions in the TOS almost inevitable, along with increased likelihood of further introductions into Picton.

There appear to be no other regional management efforts for *Styela* that can serve as a model for the TOS. However, there are lessons that can be learned from previous or present regional attempts to manage the kelp *Undaria* (southern New Zealand, Nelson), the sea squirt *Didemnum* (TOS), and the fanworm *Sabella* (ongoing effort in Northland). These lessons are reflected in the sub-sections below; a key message is that success requires an intensive effort and a sustained commitment of resources.

4.2. Management techniques available

A range of possible management methods are outlined in Appendix 9 of the TOS Operations Manual. These and other methods are summarised below. Collectively they highlight that, while there are some control methods that are highly effective at small scales, none provide "silver bullets".

4.2.1. Measures to minimise human-mediated spread

Routine and effective antifouling (i.e. with a toxic paint coating) to minimise the risk of *Styela* settling on vessels is probably the best defence to slow spread via hull fouling. However, as *Styela* may be found in vessel "niche" areas (e.g. areas that are not antifouled), or may eventually settle and grow on compromised paint coatings, vessel cleaning may also be necessary. Hand removal by divers of individual *Styela* may be the best approach in some instances, or whole vessel

² The TOS Operations Manual is available at: http://www.marinebiosecurity.co.nz/downloads



cleaning may be necessary. If *Styela* became established on marine farms, effective vector control would become difficult, especially the control of mussel seed-stock transfer risk. Even though routine industry practices (e.g. mussel declumping and washing prior to transfer among regions) substantially reduce associating fouling, such methods may not be 100% effective (Forrest & Blakemore 2006).

4.2.2. Measures to control established populations

Diver hand picking will generally remain the best *Styela* control approach in Picton while the population remains at low density. However, there may be scope to also use in-water plastic encapsulation ("wrapping") of heavily infected structures. Wrapping has become a well-refined and widely used control method for vessels and marine structures in New Zealand, especially by Diving Services New Zealand who have undertaken the Picton surveys. In fact, they applied the technique to part of the wooden structure found to be infected during the September survey.

4.2.3. Other treatment methods

There are a range of relatively simple treatments that may be useful in certain circumstances. The efficacy of relatively eco-friendly chemicals such as bleach and acetic acid (the active ingredient in vinegar) has been well-researched, and has application in certain circumstances (e.g. by sprays and immersion dips); however, the use of such chemicals would require a consideration of consent requirements from both MDC and the Environmental Protection Authority.

Simple measures such as air drying, hot water, and freshwater immersion, can also be effective (Lützen 1999; LeBlanc *et al.* 2007). Rudimentary trials on basic treatment measures for *Styela* (e.g. air drying, bleach) were conducted when it was first discovered in Auckland, and provide some ballpark guidance (Coutts & Forrest 2005). In Canada, mitigation of *Styela* on mussel lines using lime immersion baths is the favoured method; however, various New Zealand studies indicate that lime is less effective than alternative chemicals like bleach and acetic acid (Forrest *et al.* 2007; Piola *et al.* 2010).

4.3. Reinvasion risk to Picton and range extensions elsewhere in the region

As already noted, in the absence of effective regional and national controls on movements of potentially infected vectors, the risk of further incursions to Picton is probably high and will increase over time as *Styela* continues to spread to new locations. Additionally, it is conceivable that points of *Styela* entry to the TOS may not be into busy vessel hubs such as ports and marinas. For example, infected recreational vessels from outside the TOS may travel directly to more remote parts of the Marlborough Sounds. An analogous example in New Zealand occurred with



the first discovery of the sea squirt *Pyura doppelgangera*³ in a remote stretch of the Northland east coast. The unpredictable nature of such events highlights the primary importance of effective border control combined with intra- and interregional vector management. The implication is that, even if *Styela* in Picton was completely eradicated, the species is still likely to spread to high value areas within the region; perhaps relatively rapidly by human vectors, and more slowly by natural dispersal.

4.4. Inadequate knowledge of Styela distribution in the TOS

The lack of good information on *Styela*'s distribution in the TOS relates to the previous section. A key reason for the failure of marine incursion responses in New Zealand to date is that unmanaged human-mediated spread has led to new populations appearing outside of known infestations, exemplified by the Asian kelp *Undaria pinnatifida* in Southland and *Didemnum* in the TOS (Hunt *et al.* 2009; Forrest & Hopkins 2013). Six-monthly surveillance funded by the Ministry for Primary Industries is limited to the main ports/marinas in the TOS. As there is no systematic regional surveillance programme, *Styela* may already be established in the TOS outside Nelson and Picton, which could clearly negate the benefits of any population control efforts in Picton.

4.5. Ability to reliably define the spatial limits of the Picton population

Adequate *Styela* population delimitation is a critical consideration. The *potential* larval dispersal of *Styela* of approximately one day (see Section 2.7) is sufficient to enable dispersal well beyond the marina and out into Queen Charlotte Sound. In that case, confining any ongoing response to the marina area has the potential to miss outlying *Styela*. These outlying areas were in part covered by NIWA surveillance in June, but have not yet been comprehensively checked. However, even though delimitation can be difficult, a sustained and intensive population control effort within the marina alone has the potential to greatly reduce the infection of berthed vessels, thus limit regional-scale spread. Nonetheless, a population control strategy that was undertaken for the purpose of reducing vector infection, would ideally also include surveys of vessels in the wider Picton port area and perhaps also Waikawa Bay.

4.6. Detection of Styela within defined population control areas

The entire area of the inner and outer Picton marina was not searched during the June and September survey, only the main habitats. Some areas of natural seabed that were not checked could contain *Styela* (especially where the presence of debris facilitates attachment). Additionally, in habitats that were checked by

³ This sea squirt is currently described in the MPI marine pest list as *Pyura praeputialis*.



divers, it is likely that some *Styela* were missed during delimitation. A recent Canadian study that used sea squirt surrogates (decoys) to evaluate surveillance efficacy found that divers detected approximately 80% of single surrogates and 94% of clusters (Kanary *et al.* 2010).

Surveillance is less likely to detect small individuals, and detection in Picton is made difficult by extensive fouling on the pontoons and piles, especially in the town basin. In the period from winter to early summer, the presence of an extensive growth of the seasonal kelp *Undaria* will greatly hinder the detection ability of divers. On the plus side, it helps that larger *Styela* are reasonably conspicuous and easy to identify. Additionally, water clarity in Picton is generally quite good (although was reduced in September because of dredging), but can decrease across the soft muddy sediments that characterise the deeper parts of the marina basin (e.g. where *Styela* could occur on debris).

4.7. Understanding the costs and benefits of management

High spatial and temporal variability in invasiveness means that any predictions of the adverse consequences of *Styela*'s spread in the TOS are uncertain, and may be unreliable. Where impacts cannot be reliably predicted, the costs and benefits of management are also difficult to reliably ascertain (Sikder *et al.* 2006), except when hypothetical scenarios of impact are considered. This situation was illustrated by the case of *Didemnum* in the Marlborough Sounds. Regional experience with this species provided an understanding of management efficacy and costs (Sinner & Coutts 2003; Coutts & Forrest 2007; Pannell & Coutts 2007), and related research filled critical knowledge gaps regarding aspects of *Didemnum*'s biology and impacts (Fletcher 2013). However, high variability in invasiveness and impact (e.g. on shellfish aquaculture) made the benefits of management, and the relative importance of different management scenarios, difficult to determine.

Additionally, even though efforts to manage *Didemnum* in Marlborough (especially intensive efforts over 2006-2008) had some successes, a lack of sufficient management of some risk pathways (especially mussel aquaculture) led to the need to control many populations across the Marlborough Sounds region. This situation meant that the funding and resource needs for effective management quickly escalated, to the point where long-term commitment was not forthcoming and efforts were abandoned. In part the reasons for lack of commitment reflected an emerging perception by some aquaculture industry operators that the level of risk did not justify the expenditure of their funds, and a belief that management should be a central government responsibility. Simultaneously, the invasiveness of *Didemnum* in the region declined for reasons that are unknown (Fletcher *et al.* 2013c). This situation led some industry operators to consider that the *Didemnum* efforts were a waste of money, and tainted their views regarding the value of marine pest management. Instead, they hold the view that the industry should "let



nature take its course", and develop management solutions to mitigate any direct adverse effects of marine pests if and when they arise.

4.8. Requirements for effective population control

If MDC and MPI embark on a local population elimination/control attempt, some additional issues that should be considered are outlined in the following sections.

4.8.1. Define management goals and level of commitment

Clear management goals should be set, accounting for the following:

- Given the possibility of self-fertilisation, a goal of local eradication may need to detect and remove every individual before reproductive maturity and spawning occurred.
- If more than one *Styela* is necessary for reproduction, an eradication strategy could be based on reducing densities to such an extent that reproduction is prevented. This was the approach taken in the apparently successful eradication of the brown mussel (*Perna perna*) from Tasman Bay (Hopkins *et al.* 2011).
- A more realistic approach than eradication may be sustained population control. A reasonable goal would be to suppress the population to very low densities, in order to: (i) reduce the infection of vessels in Picton to limit human-mediated spread; and (ii) reduce the natural spread potential from infected areas.

Decision makers should also consider whether they are prepared to fund a response to the level required to be effective, noting that effort may need to be sustained (or even increased) over many years. Criteria for abandoning efforts should also be developed. For example, the detection of new populations of *Styela* in the region would logically trigger a re-evaluation of control efforts.

4.8.2. Styela surveillance and removal

Key considerations for surveys to find and remove Styela include the following:

• The frequency of surveillance and removal should be based around a goal of detecting any new (or missed) *Styela* before individuals reach reproductive maturity. Based on reproductive biology, and accounting for areas of uncertainty, surveillance and removal should probably be conducted at least every 1-2 months. This frequency assumes that: (i) reproductive maturity may be reached in as little as two months (see Section 2.6), and (ii) small individuals in a given month may be missed due to detection limitations (see Section 4.6).



- The spatial boundaries of a control zone need definition, as well as the target habitats, and consideration should be given to a periodic wider surveillance effort. These needs depend on the management goals as outlined above. One strategy could include intensive habitat and vessel surveys in Picton marina, with less frequent surveys of vessels in the wider harbour and Waikawa Bay.
- It may be appropriate to investigate whether regional surveillance could be improved by developing a semi-structured approach that enlisted the assistance of marine users. This approach would assist in determining the occurrence of further range extensions.
- In terms of removal operations, notice should be taken of the comment in the McClary report that physical disruption of mature *Styela* could result in a fertilisation event. The authors suggested that *Styela* individuals should be encapsulated in a sealed bag before removal. Although this practice is currently undertaken, it could become fairly onerous, depending on how the size and extent of the population changes over time.

4.8.3. Controls on vessels and other vectors

To reap the benefits of population control in Picton (e.g. for a goal of reducing spread), effective management of all risk vectors is clearly critical. Even if funds are insufficient for population control, effective vector management alone has the potential to slow the spread not only of *Styela*, but also of other actual or potential marine pests.

Of course, the key to vector management is putting in place measures that are *effective*. Approaches to vector management in the TOS to date have mainly been based on raising public awareness. In the case of recreational vessels, this approach appears to have had no measureable benefit in terms of risk reduction (Forrest 2013). Clearly, more effective management approaches are needed, which requires consideration of the intra-regional traffic within the TOS, as well as risk pathways into the region. Inter-regional pathway management for the entire country is currently being considered by MPI, but realistically it could be many years before effective solutions are in place.

One of the barriers to overcome in order to achieve effective management will be obtaining the support of exacerbators of risk (e.g. vessel operators). Although vector management by itself, or in combination with population control, may slow the spread of *Styela* to areas with values most at risk (e.g. the Pelorus Sound mussel growing region), the species in not managed in Nelson or elsewhere in New Zealand. As such, it is not possible to reliably estimate the extent to which its spread could be delayed. In fact, as noted above, it is possible that *Styela* is already present in the TOS in areas outside Nelson and Picton.



5. CONCLUDING REMARKS

Styela clearly has a number of biological features that create challenges for effective pest management. Simultaneously, the presence of unmanaged populations elsewhere in the TOS and in New Zealand will present an increasing risk over time of further range extensions into Marlborough and the TOS. On the other hand, Styela is known to have a clear potential to cause significant adverse effects, especially on mussel culture. The decision on whether and to what extent to respond to this particular range extension must be weighed against these uncertain, but potentially significant adverse effects on TOS aquaculture and other values.

If an effective approach to population and/or vector management can be developed, probably the best outcome for the TOS is slowing the spread of *Styela* to areas with values most at risk (e.g. the Pelorus Sound mussel growing region). However, as noted above, it is not possible to reliably predict the extent to which spread could be delayed, as the species could arrive from other parts of New Zealand, anywhere in the TOS at any time. Even with the best practical vector management measures, it may be a single unpredictable event that leads to further jump in *Styela*'s range. In the absence of regional surveillance, the only way of knowing whether *Styela* already occurs elsewhere in the region, or arrives in the near future, will be to rely on chance finds by the public.

As a final point, it is worthwhile considering what might happen if and when another marine pest arrives. For example, if the Mediterranean fanworm arrives next week, with its own biological traits that make it hard to manage (e.g. a lengthy larval dispersal period), will population eradication or control also be considered along with *Styela*? It is easy to appreciate that marine population control, with its poor track record of success and limited response tool kit, could fast become untenable in the event of multiple target species and/or multiple populations. Such situations provide a strong argument that the best use of limited funds may be to make a thorough job of vector management, aiming for effective generic measures (e.g. regular hull antifouling) that reduce the risk of marine pests in general.

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7. REFERENCES CITED

- Bourque D, Davidson J, MacNair NG, Arsenault G, LeBlanc AR, Landry T, Miron G. 2007. Reproduction and early life history of an invasive ascidian *Styela clava* Herdman in Prince Edward Island, Canada. Journal of Experimental Marine Biology and Ecology 342: 78-84.
- Clarke CL, Therriault TW. 2007. Biological synopsis of the invasive tunicate *Styela clava* (Herdman, 1881). Canadian Manuscript Report of Fisheries and Aquatic Sciences 2807. 23 p.
- Coutts ADM, Forrest BM. 2005. Evaluation of eradication tools for the clubbed tunicate *Styela clava*. Cawthron Report No. 1110, Cawthron Institute, Nelson, New Zealand. 28 p. plus appendices.
- Coutts ADM, Forrest BM. 2007. Development and application of tools for incursion response: lessons learned from the management of the fouling pest *Didemnum vexillum*. Journal of Experimental Marine Biology and Ecology 342: 154-162.
- Darbyson E, Locke A, Hanson JM, Willison JHM. 2009a. Marine boating habits and the potential for spread of invasive species in the Gulf of St. Lawrence. Aquatic Invasions 4: 87-94.
- Darbyson EA, Hanson JM, Locke A, Willison JHM. 2009b. Settlement and potential for transport of clubbed tunicate (*Styela clava*) on boat hulls. Aquatic Invasions 4: 95-103.
- Davidson J, Arsenault G, MacNair N, Landry T, Bourque D. 2005. Reproduction, epidemiology and control of the clubbed tunicate, *Styela clava*. Aquaculture and Fisheries Research Initiative, AFRI Project # 043AR15, July 2005. 33 p.
- Davis MH, Davis ME. 2009. *Styela clava* (Tunicata, Ascidiacea) a new threat to the Mediterranean shellfish industry? Aquatic Invasions 4: 283-289.
- Davis MH, Lützen J, Davis ME. 2007. The spread of *Styela clava* Herdman, 1882 (Tunicata, Ascidiacea) in European waters. Aquatic Invasions 2: 378-390.
- Deloitte. 2011. MAF Styela clava economic impact assessment. August 2011. 26 p.
- Fletcher LM. 2013. Ecology of biofouling and impacts on mussel aquaculture: a case study with *Didemnum vexillum*. PhD thesis, Victoria University of Wellington, New Zealand. 234 p.
- Fletcher LM, Forrest BM, Bell JJ. 2013a. Natural dispersal mechanisms and dispersal potential of the invasive ascidian *Didemnum vexillum*. Biological Invasions 15: 627-643.
- Fletcher LM, Forrest BM, Atalah J, Bell JJ. 2013b. Reproductive seasonality of the invasive ascidian *Didemnum vexillum* in New Zealand and implications for shellfish aquaculture. Aquaculture Environment Interactions 3: 197-211.



- Forrest B. 2013. Vessel hull fouling as a marine biosecurity indicator in the Top of the South: 2013 survey. Top of the South Marine Biosecurity Partnership, Tecnical Report 2013/01. 26 p.
- Forrest BM, Blakemore KA. 2006. Evaluation of treatments to reduce the spread of a marine plant pest with aquaculture transfers. Aquaculture 257: 333-345.
- Forrest BM, Hopkins GA. 2013. Population control to mitigate the spread of marine pests: insights from management of the Asian kelp *Undaria pinnatifida* and colonial ascidian *Didemnum vexillum*. Management of Biological Invasions (accepted for publication, October 2013).
- Forrest BM, Hopkins GA, Dodgshun TJ, Gardner JPA. 2007. Efficacy of acetic acid treatments in the management of marine biofouling. Aquaculture 262: 319-332.
- Gaines SD, Gaylord B, Gerber LR, Hastings A, Kinlan BP. 2007. Connecting places: the ecological consequences of dispersal in the sea. Oceanography 20: 90-99.
- Grange K, Carney D, Carter M. 2011. Waikato marine finfish farming: site investigation. Draft NIWA client report no. NEL2011-004. 17 p.
- Gust N, Floerl O, Inglis G, Miller S, Fitridge I, Hurren H. 2005. Rapid delimitation survey of *Styela clava* in the Viaduct Harbour and Freemans Bay, Auckland. Biosecurity New Zealand Project ZBS 2005-32. NIWA Client Report CHC2005-147, National Institute of Water and Atmospheric Research Ltd, Christchurch.
- Hopkins GA, Forrest BM, Jiang W, Gardner JPA. 2011. Successful eradication of a non-indigenous marine bivalve from a subtidal soft-sediment environment. Journal of Applied Ecology 48: 424-431.
- Hunt L, Chadderton L, Stuart M, Cooper S, Carruthers M. 2009. Results of an attempt to control and eradicate *Undaria pinnatifida* in Southland, New Zealand, April 1997 November 2004. Department of Conservation, Invercargill, New Zealand. 48 p.
- Kanary L, Locke A, Watmough J. 2010. Evaluating the effectiveness of SCUBA-based visual searches for an invasive tunicate, *Ciona intestinalis*, in a Prince Edward Island estuary. Aquatic Invasions 5: 41-47.
- Kinlan BP, Gaines SD, Lester SE. 2005. Propagule dispersal and the scales of marine community processes. Diversity and Distributions 11: 139-148.
- Lambert CC, Lambert G. 1998. Non-indigenous ascidians in southern California harbors and marinas. Marine Biology 130: 675-688.
- LeBlanc N, Davidson J, Tremblay R, McNiven M, Thomas L. 2007. The effect of antifouling treatments for the clubbed tunicate on the blue mussel, *Mytilus edulis*. Aquaculture 264: 205-213.
- Lines B. 2013a. Picton marina June 2013 *Styela* pest management work: delimitation survey and removal. Diving Services New Zealand Ltd, June 26, 2013. 13 p.



- Lines B. 2013b. Picton marina September 2013 *Styela* pest management work: second round delimitation survey and removal work. Diving Services New Zealand Ltd, September 6, 2013. 10 p.
- Lützen J. 1999. *Styela clava* Herdman (Urochordata, Ascidiacaea) a successful immigrant to northwest Europe: Ecology, propagation and chronology of spread. Helgoländer Meeresunters 52: 383-391.
- McClary D, Phipps C, Hinni S. 2008. Reproductive behaviour of the clubbed tunicate, *Styela clava*, in northern New Zealand waters. MAF Biosecurity New Zealand Technical Paper No: 2009/01. 34 p.
- McFadden A, Rawdon T, Gould B. 2007. Response to a marine incursion of *Styela clava*. Surveillance 24: 4-8.
- Morrisey D, Miller S. 2008. Review of existing information on biosecurity in the top of the South Island. MAF Biosecurity New Zealand Technical Paper No. 2009/02. 87 p.
- Nunn JD, Minchin D. 2009. Further expansions of the Asian tunicate *Styela clava* Herdman 1882 in Ireland. Aquatic Invasions 4: 591-596.
- Pannell A, Coutts ADM. 2007. Treatment methods used to manage *Didemnum vexillum* in New Zealand. Report prepared for Biosecurity New Zealand. New Zealand Marine Farming Association. 29 p. plus appendices.
- Parker LE, Culloty S, O'Riordan RM, Kelleher B, Steele S, Van der Velde G. 1999. Preliminary study on the gonad development of the exotic ascidian *Styela clava* in Cork Harbour, Ireland. Journal of the Marine Biological Association of the United Kingdom 79: 1141-1142.
- Piola RF, Dunmore RA, Forrest BM. 2010. Assessing the efficacy of spray-delivered "eco-friendly" chemicals for the control and eradication of marine fouling pests. Biofouling 26: 187-203.
- Sikder IU, Mal-Sarkar S, Mal TK. 2006. Knowledge-based risk assessment under uncertainty for species invasion. Risk Analysis 26: 239-252.
- Sims LL. 1984. Osmoregulatory capabilities of three macrosympatric stolidobranch ascidians, *Styela clava* Herdman, *S. plicata* (Lesueur), and *S. montereyensis* (Dall). Journal of Experimental Marine Biology and Ecology 82: 117-129.
- Sinner J, Coutts ADM. 2003. Benefit-cost analysis of management options for *Didemnum vexillum* in Shakespeare Bay. Prepared for Port Marlborough New Zealand Limited. Cawthron Report No. 924. 5 p.
- Webber DA. 2010. Demography and population projections of the invasive tunicate *Styela clava* in southern New Zealand. MSc thesis, University of Canterbury, New Zealand. 145 p.
- Wong NA, McClary D, Sewell M. 2011. The reproductive ecology of the invasive ascidian, *Styela clava*, in Auckland Harbour, New Zealand. Marine Biology 158: 2775-2785.