

Reproductive behaviour of the Clubbed Tunicate, *Styela clava*, in northern New Zealand waters

BRP 186 / 2005

MAF Biosecurity New Zealand Technical Paper No: 2009/01

Prepared for BNZ Post-clearance Directorate
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ISBN 978-0-478-33838-6(Online)
ISSN 1177-6412 (Online)

December 2008



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Executive Summary

The ascidian *Styela clava* was first reported as an invasive species in New Zealand in Auckland in 2005. Several overseas studies have researched aspects of the reproductive behaviour of this species, but it is unknown whether the reproductive patterns described in these studies are applicable to *S. clava* in New Zealand. The scope of the current project was thus to study the seasonality of sexual maturity, timing of larval release, and timing of recruitment of *S. clava* in New Zealand waters.

Field sampling for the investigation of the reproductive behaviour of *S. clava* at Bayswater Marina commenced on 13 May 2006 and was completed on 17 April 2007, covering a total of 24 sampling events at fortnightly intervals. A total of 180 individuals were collected and gonad and body weight information was recorded. Gonad index values rose over the winter then declined sharply in the early spring, indicating a spawning event. A further two reductions in gonad indices were observed in mid-February and mid-March. Fertilised ova were first recorded in gonad smears beginning in late September 2006 through to the completion of sampling in April 2007.

Weekly plankton sampling began on 18 October 2006 and was completed on 24 April 2007. A total of 10 ascidian larvae were recovered from the samples during this time, but this did not include any larvae of *S. clava*. The first set of settlement collectors was deployed on 25 October 2006, with completion of the programme by 24 April 2007. Despite recruitment by a variety of biofouling taxa, no *S. clava* were detected on the plates at any of the three sampling sites. Environmental data was collected and recorded as required throughout the sampling period.

Overall the fluctuations observed in the gonad index and gonad histology are indicative of a species in which at least some members of the population are competent to reproduce at different times throughout the year. There does, however, appear to be a “lull” in reproductive potential over winter and in mid spring, with peaks in early spring and late summer.

In terms of managing this species, the following points require consideration:

- The presence of fertilised eggs in gonad smears of individual *S. clava* indicates that physical disruption of even a single mature *S. clava* can result in the release of viable gametes and a fertilisation event.
- Small scale eradication is theoretically possible, but given that this species is potentially self-fertile, all individuals would need to be removed from the target area.
- If required, control operations should be targeted to the period when fertile gametes are not present in the gonads, as physical disturbance could trigger the release of gametes. Mid winter or mid spring appears to be the most suitable periods for this type of control activity.
- Control measures should ideally be completed in mid-winter prior to the period when Sea surface temperature rises above 15 °C. After this time the potential for spawning and recruitment is high; in addition, there will likely be a number of potential cohorts present, many of which could be too small to detect or positively identify during a given removal operation, thus preventing effective control of the population.
- In-water removal, if attempted, must be effected by first isolating the individual or clump of individuals in a water-tight, sealable bag, then carefully removing the animal from the substrate at the base of the stalk and sealing the bag; care must be taken to ensure that the test is not damaged or otherwise subject to physical shock prior to encapsulation and complete integrity of the bags must be maintained throughout the disposal process to ensure that gametes and embryos are not released back into the water column.

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1. General Introduction

1.1. BACKGROUND

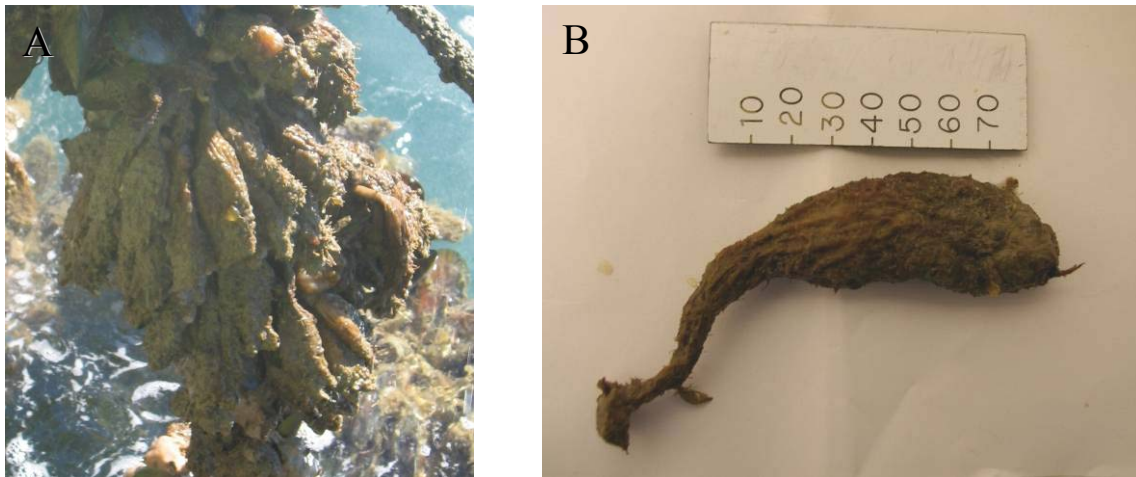
Styela clava (Herdman 1881) is a solitary, club-shaped, stalked ascidian which can reach 200 mm in length (Davis and Davis 2007). Test colours ranging from brownish-white, yellowish-brown to a reddish-brown (Fig. 1.1). It is commonly found in shallow depths but can occur as deep as 25 m (Sims 1984). *Styela clava* is frequently found fouling floating piers, wharf piles, rocks, vessel hulls, aquaculture equipment and other artificial substrata (Gust et al. 2005). This species has a lifespan of up to two to three years (Lambert & Lambert 1998).

Styela clava is native to the northwest Pacific, including Japan, Korea, Northern China and Russia and has readily adapted to sheltered coastal habitats in a variety of locations throughout the world (Gust et al. 2005; Lambert & Lambert 1998). *Styela clava* was first reported outside its native range in Plymouth (England) in 1952 and Devon in 1953. Circumstantial evidence suggests that it was transported on warships hulls at the end of the Korean War in 1951 (Eno et al. 1997). It has spread across the Channel to France by 1968 and was first recorded in Ireland in 1972 (Eno et al. 1997). On mainland Europe, *S. clava* has spread to Belgium, the Netherlands and Denmark (Davis & Davis 2007). In the last 20 years, it has also colonised parts of Spain and Portugal (Davis & Davis 2007). To date, no specimens have been found in the Mediterranean (Davis & Davis 2007). *Styela clava* is believed to have been introduced to the Atlantic Coast of North America via Europe sometime in the late 1960s (Berman et al. 1992). It was first reported on Long Island, New York in 1973 and by 1988, it was present from Connecticut to Maine (Berman et al. 1992). The western North American (California) population pre-dates that on the east coast and is believed to have established in the 1920s (Lambert & Lambert 1998). In the Southern Hemisphere, *S. clava* was first recorded in Australia from Hobson's Bay in Port Phillip Bay in 1976 (Holmes 1976).

Throughout parts of its introduced range, *S. clava* poses significant economic and ecological impacts through high-density fouling of commercial equipment, competition with native and farmed species for resources (i.e. food and space), overgrowth of shellfish and predation on plankton (Gust et al. 2005, Parker et al. 1999). On artificial substrate, *S. clava* often occurs in very dense assemblages of up to 500–1500 individuals/m² and has been identified as posing a serious threat to the sustainability of the long line shellfish aquaculture industry (Davidson et al. 2005, Lützen 1999).

The species was positively identified as present in New Zealand in Auckland in September 2005. Following identification, a delimitation survey of this species in Viaduct Harbour and Freeman's Bay in Auckland indicated densities ranged from 1–10 individuals/m² and specimens were most commonly observed at 0–1.5 m depth on fixed structures (Gust et al. 2005). This species is most abundant in low energy, sheltered environments and is not commonly found in exposed open coast habitats (Osman & Whitlatch 1999; Davis & Davis 2005). Adults of this species are considered resilient to fluctuations in both temperature and salinity (Eno et al. 1997). The larvae, however, are more sensitive to salinity variations and studies indicate that decreases in salinity to 18ppt and below usually result in larval mortality (Kashenko 1996).

Figure 1.1: Example images of *Styela clava*.



Note: A) A cluster of *Styela clava* fouling a mussel line of Waiheke Island, 2005. B) A large individual specimen collected from Bayswater Marina, April 2007.

1.2. ASCIDIAN REPRODUCTION

Ascidians are primitive members of the phylum Chordata (Brusca & Brusca 1990) and can be divided into colonial and solitary species. Colonial species are composed of few to hundreds of individuals called zooids (Ruppert 1994, Cloney 1992) that are enclosed within a common tunic (Barrington 1970). Colonial ascidians reproduce both sexually and asexually by budding (Cloney 1992). Solitary or simple ascidians, such as *Styela clava*, on the other hand, are often found in clumps, but reproduce only sexually (Cloney 1992). Ascidians are predominantly outcrossing hermaphrodites, with a few species being capable of self-fertilization (Gretchen 2006, Ruppert 1994). Most species contain a single ovary and a single testis that lies near the loop of the digestive tract (Brusca & Brusca 1990). Members of some families (e.g. Styelidae) have multiple gonads (Brusca & Brusca 1990).

Life cycles of ascidians range from oviparous, ovoviviparous to viviparous, with most solitary species, including *Styela clava*, being oviparous (Cloney 1992). Fertilization in the oviparous species is external, with eggs and sperm released from the atrial siphon to the sea (Brusca & Brusca 1990, Cloney 1992). Most ascidian larvae are first positively phototactic and swim to the surface, but soon become negatively phototactic and move downwards into shaded crevices and overhanging surfaces that are suitable for adult life (Barrington 1970). Planktonic stages of all ascidian larvae are short and most solitary ascidian larvae settle within 6-24 hours (Cloney 1992).

1.3. REPRODUCTION IN *STYELA CLAVA*

The reproductive periodicity of *Styela clava* has been studied at several locations throughout its introduced range (e.g. Lützen & Sørensen 1993; Parker et al. 1999; Davidson et al. 2005). This species appears to reproduce predominantly in the spring to late summer months and is thought to spawn in water temperatures of more than 15° C (Eno et al. 1997, Bourque et al. 2007). A study conducted in Prince Edward Island (Canada), revealed the presence of larvae in the water column from June 24 to October 29 2004 with a maximum density of 0.56 larvae L⁻¹ observed on August 19 (Bourque et al. 2007). Similarly in Cork Harbour in Ireland, spawning occurred predominantly in September and October 1997, which was several weeks after peak temperatures of 18° C were recorded (Parker et al. 1999). *Styela clava* is thought to

undergo short-range larval dispersal, which may explain why abundant populations tend to be localised (Osman & Whitlatch 1999).

The larvae are negatively geotactic and are approximately 0.85 mm in length (Bullard & Whitlatch 2004, Davis & Davis 2007). They can swim a few millimetres in short bursts of activity and settle on hard substrata after 12-24 hours (Davidson et al. 2005, Davis & Davis 2007). In a laboratory study conducted by Kashenko (1996) however, larvae survived in plankton for up to 3 days. At salinity ranges from 24 to 32ppt, *Styela clava* larvae were selective with respect to the surface type and preferred substrates covered by a bacterial film (Kashenko 1996). In natural conditions, larvae settle from late spring to early summer (Keough & Ross 1999). In Canada, however, recruitment occurred from the third week in June to the third week in October 2004, with a peak in settlement on September 23 2004, with larval production and recruitment initiating at temperatures between 10 and 15°C (Bourque et al. 2007). The age at reproductive maturity appears to be variable throughout different geographic ranges. In Denmark, reproductive maturity is attained after 10 months (Lützen 1999), while in Canada some individuals as small as 20 mm, and all individuals at 25 mm, have shown to be reproductively active (Bourque et al. 2007) and are therefore at least 10 months old (Parker et al. 1999).

The gonads of *S. clava* have been described extensively in previous works (e.g. Van Name 1946 in Lambert & Lambert 1998). Gonads (ovaries and testes) are separate within the body cavity and located between the body wall muscles and the atrial epithelium (Ermak 1976). Male and female gonadal development is also asynchronous indicating that for most of the time, this species is not capable of self-fertilisation. The gonads are located toward the upper left of the body cavity, in line with the atrial siphon (Fig. 1.2). The ovaries appear to be long tubes of orange-coloured smooth tissue running in a posterior direction from the atrial siphon towards the centre of the body cavity (Ermak 1976). Within the ovary, a single layer of germinal epithelial tissue coats the inner edge and is continuous with the hair-lined epithelium of the ovary and oviduct. Developing oocytes lie within the ovarian wall and as individual oocytes mature they are displaced further from the edge of the germinal epithelium, but remain connected via a follicular stalk. Numerous testicular follicles can be found immediately adjacent to the ovary and appear, to the naked eye, as whitish opaque “lumps” alongside the shiny, orange tubes of ovarian tissue. At any one time, the different cells within these follicles may be in different stages of spermatogenesis. Similarly to the ovary, the sperm ducts are lined by a ciliated epithelium (Ermak 1976).

Understanding patterns of gametogenesis within a species is important for attempting to determine reproductive periodicity. Most studies of gametogenesis have revolved around simple histological techniques and the application of a stage or phase of gametogenesis observed at the time of sampling (e.g. Parker et al., 1999; Bourque et al. 2007). As gametogenesis is a continuous process, application of stages or phases of development is simply a means of better understanding potential synchrony within a population.

Gametogenesis in *S. clava* is asynchronous in the population and several stages of gonad maturity can be found at anyone time in different individuals (Parker et al. 1998, Bourque et al. 2007). A study on the reproductive cycle of *S. clava* in Cork Harbour, Ireland, revealed that gonad maturation began in February 1997, when a small number of ripe gametes was present once sea surface temperature was above 8 °C (Parker et al. 1998). Gametogenesis and spawning peaked during August to October 1997. Gonad maturation in Canada, on the other hand, was not reached until the middle of June from 2002 to 2004 (Bourque et al. 2007). Gonads remained mature until early December 2001, and until autumn in 2002 and 2004 (Bourque et al. 2007). In addition, lunar forcing of reproduction is a well studied phenomenon

Figure 1.2: General morphology of the reproductive system of *Styela clava* (from Ermak 1976)

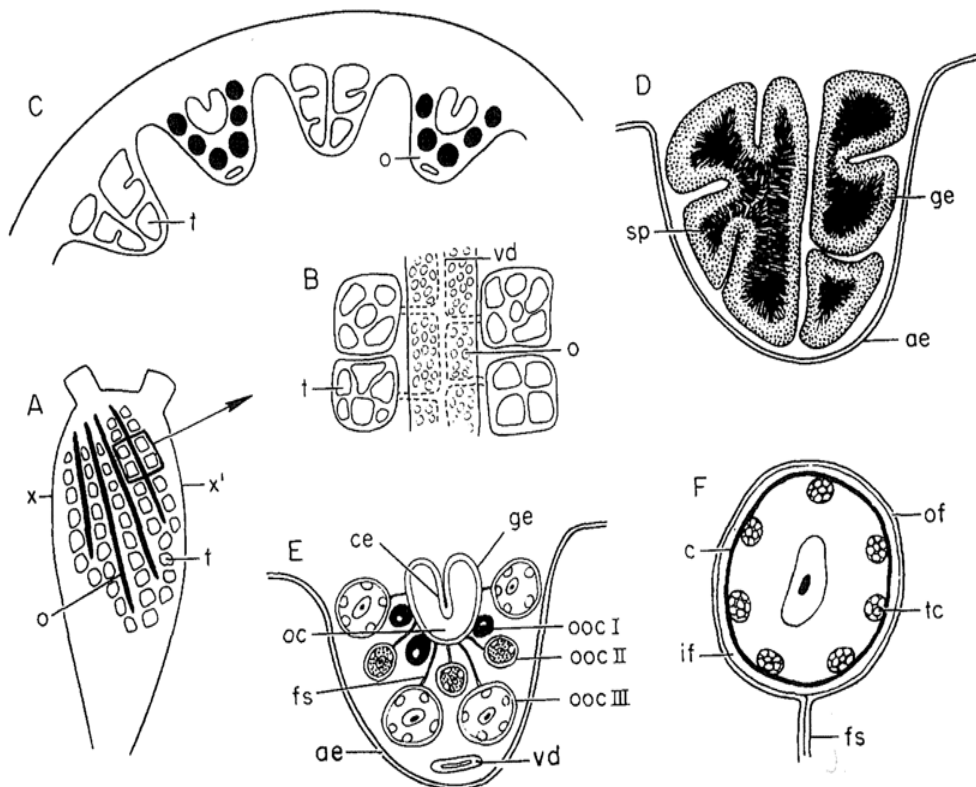


Fig. 1. (a) Gonads on left side of body of *Styela clava*. (b) Relationship between ovary (o), testis (t), and sperm ducts. (c) Cross section through part of body wall (x-x' in (a)) showing tubular ovaries and testis follicles. (d) Cross section through a testis in body wall. (e) Cross section through an ovary in body wall showing oocytes in progressive stages of growth. (f) Cross section through a stage III oocyte showing follicular envelope and follicular stalk (fs). ae, atrial epithelium; c, chorion; ce, ciliated epithelium; ge, germinal epithelium; if, inner follicle cells; oc, ovarian cavity; of, outer follicle cells; ooc I, stage I oocyte; ooc II, stage II oocyte; ooc III, stage III oocyte; sp, spermatozoa; tc, test cell; vd, vas deferens.

in marine taxa (e.g. Harrison et al. 1984; Berry 1986; Pearse et al. 1988; Pearse 1990; Lessios 1991; Kingsford & Finn 1997); sample collection at regular intervals in the lunar cycle can help to elucidate the factors affecting reproductive patterns.

1.4. SCOPE OF REPORT

1.4.1. Overall objective

1. The overall objective was to design and undertake a sampling regime to understand the season of sexual maturity, timing of larval release, and timing of recruitment of *Styela clava* in New Zealand waters.

1.4.2. Specific Deliverables

1. Determine the seasonal pattern of gonad development in *S. clava*. Undertake monthly sample collection and further histological analysis to determine the stage of gonad development on *S. clava* from a specified location.

2. Determine the timing and duration of *S. clava* larval presence in the water column. Throughout a specified study period undertake weekly sampling of seawater from a specified location.
3. Determine the time of onset and duration of *S. clava* larval recruitment onto settlement plates specified.
4. Record abiotic factors such as moon phase, day length, water temperature and time of sampling.

1.5. STUDY SITES

The first requirement for conducting a study of reproductive processes in a marine invertebrate is to locate a potentially interbreeding metapopulation. In the absence of accurate empirical information on the appropriate metapopulation size to choose, the approach was to sample from a geographically restricted location. Investigation of a variety of sites within Waitemata Harbour suggested that collection of sufficient *Styela clava* from such a location for the duration of the research project would be relatively easy to achieve.

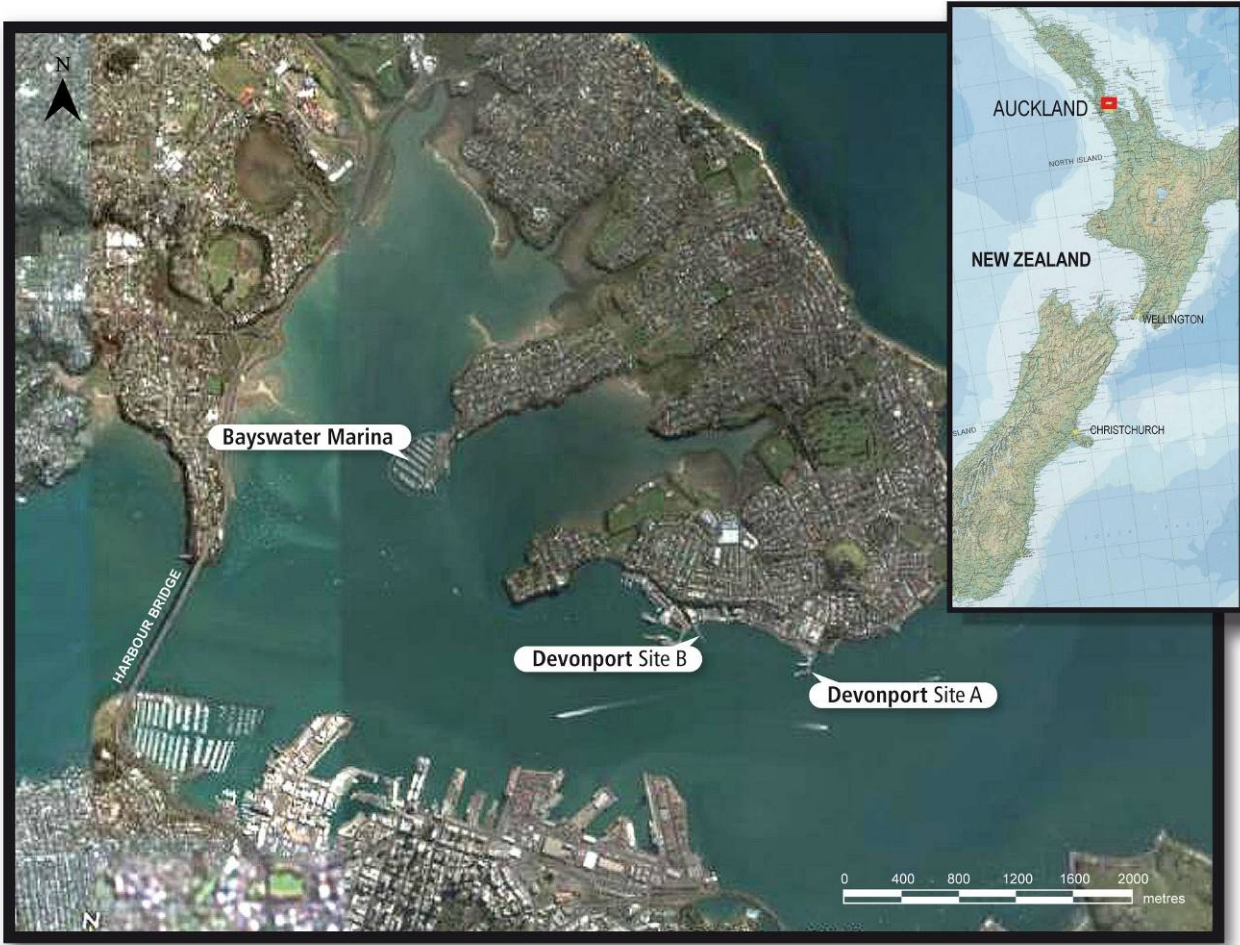
The floating pontoons at Bayswater and Westhaven Marinas, situated 2 km apart on the north and south sides of Waitemata Harbour, support extensive populations of *S. clava* (D. McClary pers.obs.) (Fig. 1.3). Bayswater Marina was chosen as the sampling point for studies of reproductive periodicity with Westhaven to be used as a contingency site.

Plankton samples were also collected from Bayswater Marina. Larval settlement equipment was deployed at three sites; at E-Pier (immediately adjacent to the sampled population), at the entrance to the marina (the “petrol” pier) and approximately 3 km east at Victoria Wharf, Devonport (Devonport Site A) (Fig. 1.3). The latter site had to be abandoned late in the study, after interference by unidentified members of the public which resulted in numerous losses of settlement plates on a number of occasions. The last set of settlement plates was moved approximately 0.5 km to the Royal New Zealand Navy (RNZN) training wharf (Devonport Site B) to ensure a final set of results could be obtained without further equipment losses (Fig. 1.3).

Bayswater Marina (36°49'20"S, 174°45'85"E) is located within Auckland's Waitemata Harbour and is accessible from Bayswater, North Shore City. The marina comprises 415 berths and a public ferry wharf. Vessels housed within include small day-sailors, sport-fishing boats, cuddy cabins, racing yachts, large cruiser yachts and launches. A sediment wall extends around the perimeter of the marina to reduce sediment deposition. Benthic sediments within the marina appear to be comprised of unconsolidated, highly organic mud-sludge. These soft sediments are dense in sheltered areas e.g. beneath the piers and within the sediment wall. A wall of basalt rock surrounds the wharf reclamation and supports typical hard substrate fauna e.g. *Crassostrea gigas* (Larcombe 1973).

While both the RNZN training wharf and Victoria Wharf sites are more exposed than Bayswater Marina, they are still relatively sheltered within the harbour. They are, however, subject to more wave action, swells and surges caused by heavy vessel traffic, operating in the immediate area. Encrusting fauna on both Victoria and Devonport Wharves are abundant and diverse, likely due to the presence of a strong, food-bearing current in this area (Larcombe 1973). East of the RNZN dockyards and the training wharf, the substrate comprises a mix of shell and basalt boulders, supporting mixed assemblages of fauna and algae found in both habitat types (Larcombe 1973).

Figure 1.3: Location of Bayswater Marina, Victoria Wharf (Devonport Site A) and the Royal New Zealand Navy training wharf (Devonport Site B).



2. Methods and Results

2.1. SEASONAL PATTERNS OF GONAD DEVELOPMENT

2.1.1. Methods

Whole, live *Styela clava* were collected from Bayswater Marina pontoons at Pier E. Individuals collected were attached to the underside of the pontoons on a floating concrete base, amongst other epibiota. No individuals sampled were removed from vessel hulls, rope, rip-rap walls or other structures nearby.

The Bayswater population of *S. clava* was sampled at fortnightly intervals, coinciding with the first daytime low tide following the new and full moon of each month (Table 2.1). In each sampling period, seven *S. clava* individuals were collected from pontoons by hand, to a maximum depth of 0.5 m, from a relatively restricted area. Individuals were bagged in seawater and transported to the laboratory for processing.

2.1.2. Assessing fertilisation

The gonads from two of the *S. clava* individuals collected in each period were dissected out for the purpose of determining gamete fertility and availability (Fig. 2.1). This was carried out by preparing a wet mount of a thin smear of the gonad contents on a glass slide, which will induce fertilisation of eggs, if male and female gametes are fertile. Upon examination under a microscope, ova were considered fertilised if the presence of a translucent envelope was observed surrounding individual ova. Germinal vesicles were noted in the smears. These normally break down prior to spawning while the oocyte is still in the oviduct (Strathmann 1987). As the method required forced removal of the oocytes for observation, the GV remained intact and obvious (see Fig. 2.2).

2.1.3. Gonad indices

The gonad index is a measure of the relative proportion of the body of an organism allocated to reproductive tissue. Changes in the gonad index over time provided an easy-to-interpret indicator of the reproductive periodicity of a population. The gonad index (GI) was determined for the remaining five *S. clava* collected in each sample using the following formula:

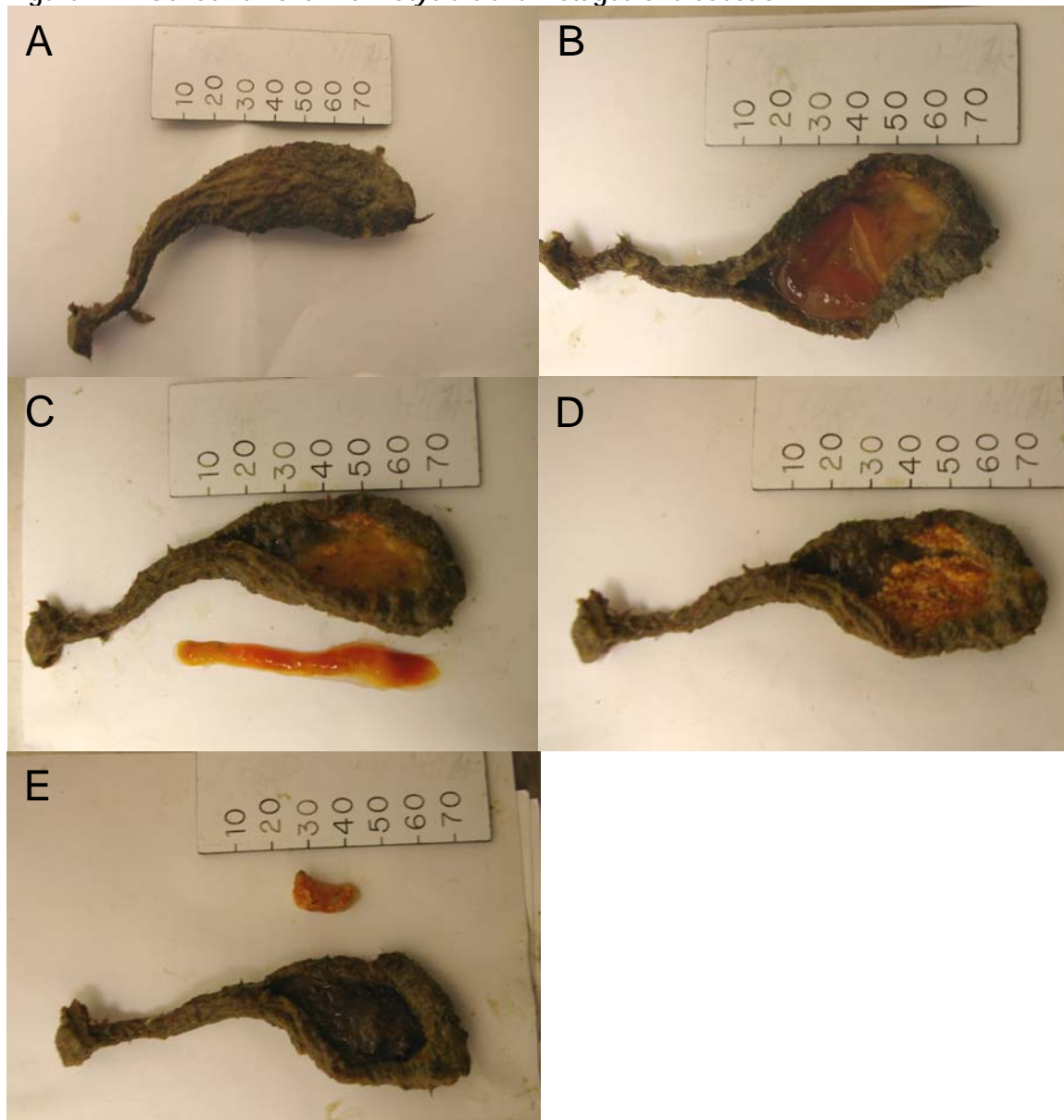
$$GI = \frac{\text{Gonad weight (g)}}{\text{Body weight (g)}} \times 100\%$$

Table 2.1: Lunar phases (24 h times are NZST) and projected dates for collecting *Styela clava* from Bayswater Marina.

Phase: Date / Time	Collection: Date / Time	Phase: Date / Time	Collection: Date / Time
F: 13.05.06 / 1853	13.05.06 / 1320	F: 06.11.06 / 0058	06.11.06 / 1326
N: 27.05.06 / 1727	28.05.06 / 1303	N: 21.11.06 / 1018	21.11.16 / 1325
F: 12.06.06 / 0605	12.06.06 / 1334	F: 05.12.06 / 1225	05.02.06 / 1308
N: 26.06.06 / 0406	26.06.06 / 1332	N: 21.12.06 / 0201	21.12.06 / 13:43
F: 11.07.06 / 1504	11.07.06 / 1309	F: 04.01.07 / 0157	04.01.07 / 1344
N: 25.07.06 / 1632	25.07.06 / 1315	N: 19.01.07 / 1601	19.01.07 / 1319
F: 09.08.06 / 2256	10.08.06 / 1339	F: 02.02.07 / 1745	02.02.07 / 1328
N: 24.08.06 / 0710	24.08.06 / 1336	N: 18.02.07 0415	18.02.07 / 1344
F: 08.09.06 / 0643	08.09.06 / 1318	F: 04.03.07 / 1117	04.03.07 / 1350
N: 22.09.06 / 2345	23.09.06 / 1346	N: 19.03.07 / 1443	19.03.07 / 1318
F: 07.10.06 / 1513	07.10.03 / 1256	F: 03.04.07 / 0515	03.04.07 / 1356
N: 22.10.06 / 1714	22.10.06 / 1316	N: 17.04.07 / 2337	18.04.07 / 1344

Note: New and full moons are indicated by "N" and "F" respectively

Figure 2.1: Gonad removal from *Styela clava* – stages of dissection.



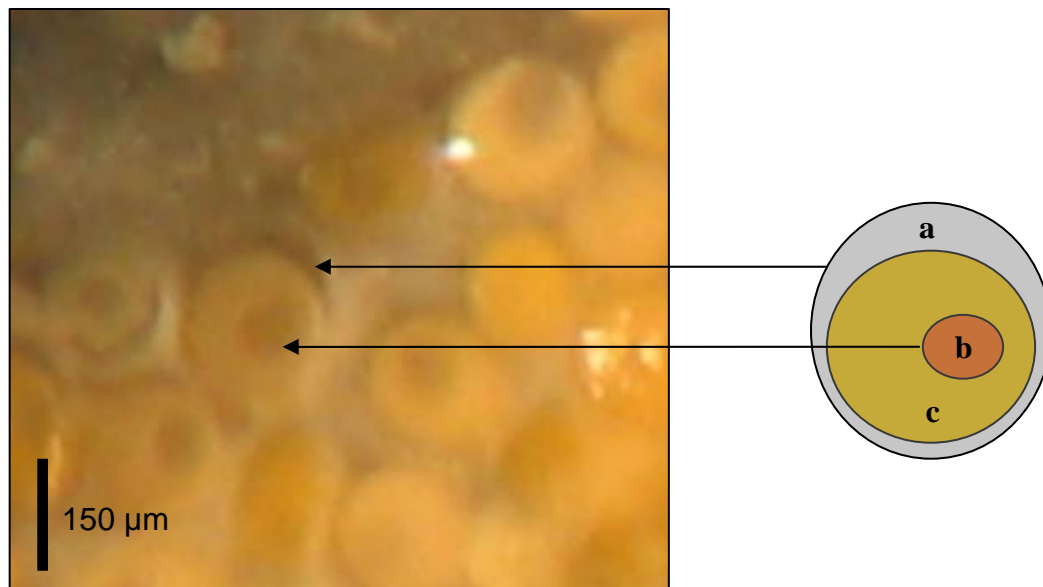
Note: A) Whole animal, prior to dissection; B) Anterior cut revealing gut and gonads; C) Removal of gut with gonads remaining intact; D) Gonad tissue exposed (orange tubes = ovaries; yellowish lumps = testes); E) Gonad tissue removed (alongside empty testis).

Each individual was “cleaned” (epibionts and excess sediment removed, excess fluid drained), blotted dry with paper towelling and the wet weight recorded. All samples were processed in a similar manner (exposure, cleaning and blotting time) to minimise variation. Gonads from each individual were removed, blotted dry for 30 seconds and weighed. Body and gonad weights were recorded and used to calculate gonad indices for each individual.

2.1.4. Gametogenic cycles

Each set of weighed gonads were preserved in seawater-buffered formalin (5% formalin). After remaining in the fixative for approximately one month, each gonad set was dehydrated gradually (to protect the integrity of the tissues) to 70% ethanol (after being passed through a mix of 5% formalin and 30% ethanol, 30% ethanol, 50% ethanol and finally 70% ethanol). The individual gonad samples were then completely dehydrated, infiltrated with paraffin

Figure 2.2: *Styela clava* ova.



Note: The top arrow points to a vitelline envelope (a) indicating fertilisation has occurred. The lower arrow indicates the germinal vesicle (b) indicative of the ripeness of the individual ovum (c).

and sectioned at 5 μm thickness. Sections were mounted on glass slides. After fully rehydrating the sections, they were stained with Lillie Mayer alum haematoxylin to demonstrate cell nuclei (Humason 1981). Rinsing the sections under running water was followed by differentiation in 0.3% acid alcohol to selectively remove excess colouration (Humason 1981). The sections were then rinsed again in tap water, Scott's tap water substitute and in tap water. The cytoplasm was stained with eosin to obtain full cellular detail (Humason 1981), dehydrated, cleaned and mounted (see Appendix 1 for detailed staining protocols and recipes of chemicals used).

The gametogenic cycle of *S. clava* has been subdivided into 4 arbitrary phases of development (Davidson et al. 2005), as follows:

- Stage I Inactive
- Stage II Developing
- Stage III Ripe
- Stage IV Spawned

Each gonad sample was observed and allocated a stage (or stages) according to the maturity of each of the ovarian and testicular tissue (see descriptions of each stage in Table 2.2)

2.1.5. Results

Sampling of the Bayswater Marina population of *Styela clava* commenced on 13 May 2006 and occurred at fortnightly intervals until April 17 2007. A total of 180 individuals were collected over 24 sampling events. Bayswater Marina was the sole collection site, as *S. clava* was abundant at this location. Inclement weather during one sampling event in June 2006 prevented complete collection at that time.

No fertilised ova were observed in the smear preparations until September 2006, indicating non-fertile gametes. Fertilised eggs were thereafter observed regularly until sampling ceased in April 2007 (Table 2.3).

Average gonad index (GI) values, i.e. the percentage of the total body weight made up by the gonads, ranged from a high of 9.2% in August 2006 to a low of 2.1% in September 2006 (Fig. 2.3) with significant differences present between all samples (non-parametric Dunn multiple comparisons test; $p < 0.0001$). Differences in GI values between September 2006 and January 2007 were not significant (Kruskal Wallis test: $H = 58.700$; $p > 0.05$). The average gonad index at the outset of sampling in May 2006 was ~6%. GIs then fell to just under 3% of the total body weight by mid June, rising to approximately 10% near the end of August. The largest decline in GI was observed between 24 August 06 and 8 September 06, suggesting the onset of a major spawning event at that time. Gonad indices remained (on average) relatively low for much of the rest of the spring and early summer 2006/07. In early 2007 (mid January to mid February) there was a relatively rapid increase in the proportion of body weight allocated to the gonads with the GI rising to approximately 8%. After this time there were a series of smaller declines in the level of the GI in late February and mid-March.

Examinations of gonad histology revealed the presence of different gametogenic stages between individuals observed at the same time, indicating a pattern of marked asynchrony within the population (Fig. 2.4 and Fig. 2.5). The largest proportion of ripe or recently spawned ovaries was highest from mid-November 2006 to May 2007. From 16 September 2006 to 11 November 2006, no ripe or spawned ovaries were present. A similar pattern was observed with testicular tissue, where the highest proportion of ripe or spawned testicular tissue was observed from mid-November 2006 to May 2007. Ripe testicular tissue, however, was present throughout the entire sampling period.

Table 2.2: Histological assessment of the stages of gonad development in *Styela clava* (from Davidson et al. 2005)

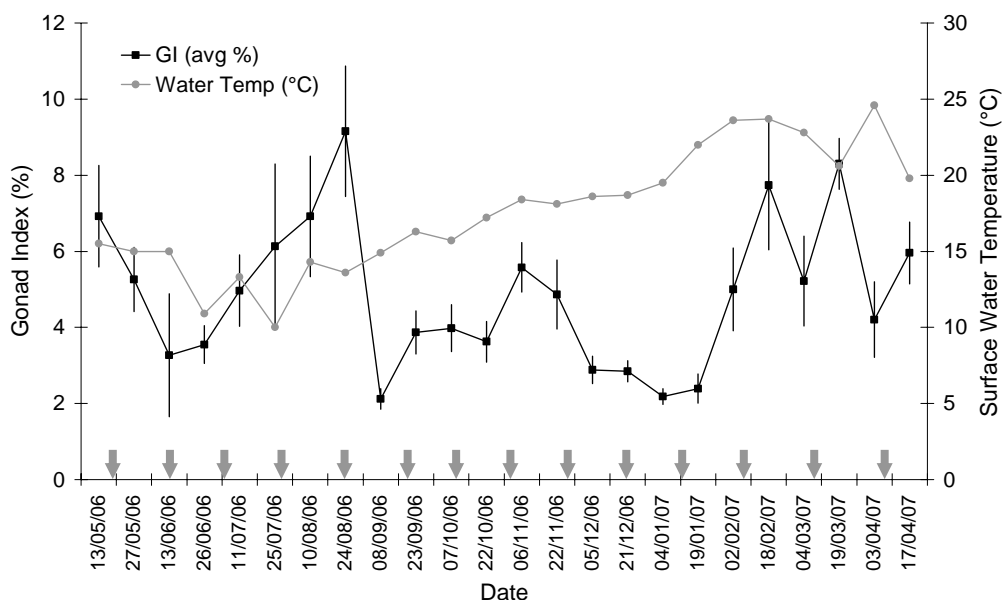
Stage	Description	Ovaries	Testes
1	Inactive	Ovaries small and compact, consisting only of germ cells. Central lumina absent	Testes small and compact consisting of only inactive germ cells.
2	Developing	Ovaries becoming larger with central lumina lined by germinal epithelia and containing ova in various maturational stages. A few ripe ova (150 μ m) may be present.	Testes becoming larger and possessing thick germinal epithelia composed of various maturational stages of sperm. Some small aggregates of ripe spermatozoa may be present.
3	Ripe	Ovaries large and extending in the direction of the body cavity, containing mainly ripe ova. May also contain some ova in various maturational stages. Germinal epithelia may form discontinuous layers or the occasional germ cell around the central lumina.	Testes large, containing medium to large aggregates of ripe spermatozoa.
4	Spawned	Ovaries ranging from large and slack with very few ova to small with remaining ova and oocytes in the process of breaking down.	Testes gradually reducing in size and containing few ripe sperm.

Table 2.3: Assessment of fertilisation of ova on gonad smears of *Styela clava* Bayswater, Auckland

Test Date	Individual		Test Date	Individual	
	1	2		1	2
13.05.06	-	-	27.05.06	-	-
13.06.06	-	-	26.06.06	-	-
11.07.06	-	-	25.07.06	-	-
10.08.06	-	-	24.08.06	-	-
08.09.06	-	-	23.09.06	-	+
07.10.06	+	+	22.10.06	-	+
06.11.06	+	+	22.11.06	+	+
05.12.06	+	+	21.12.06	+	+
04.01.07	+	+	19.01.07	+	+
02.02.07	+	+	18.02.07	+	+
04.03.07	-	+	19.03.07	+	+
03.04.07	+	+	17.04.07	+	-

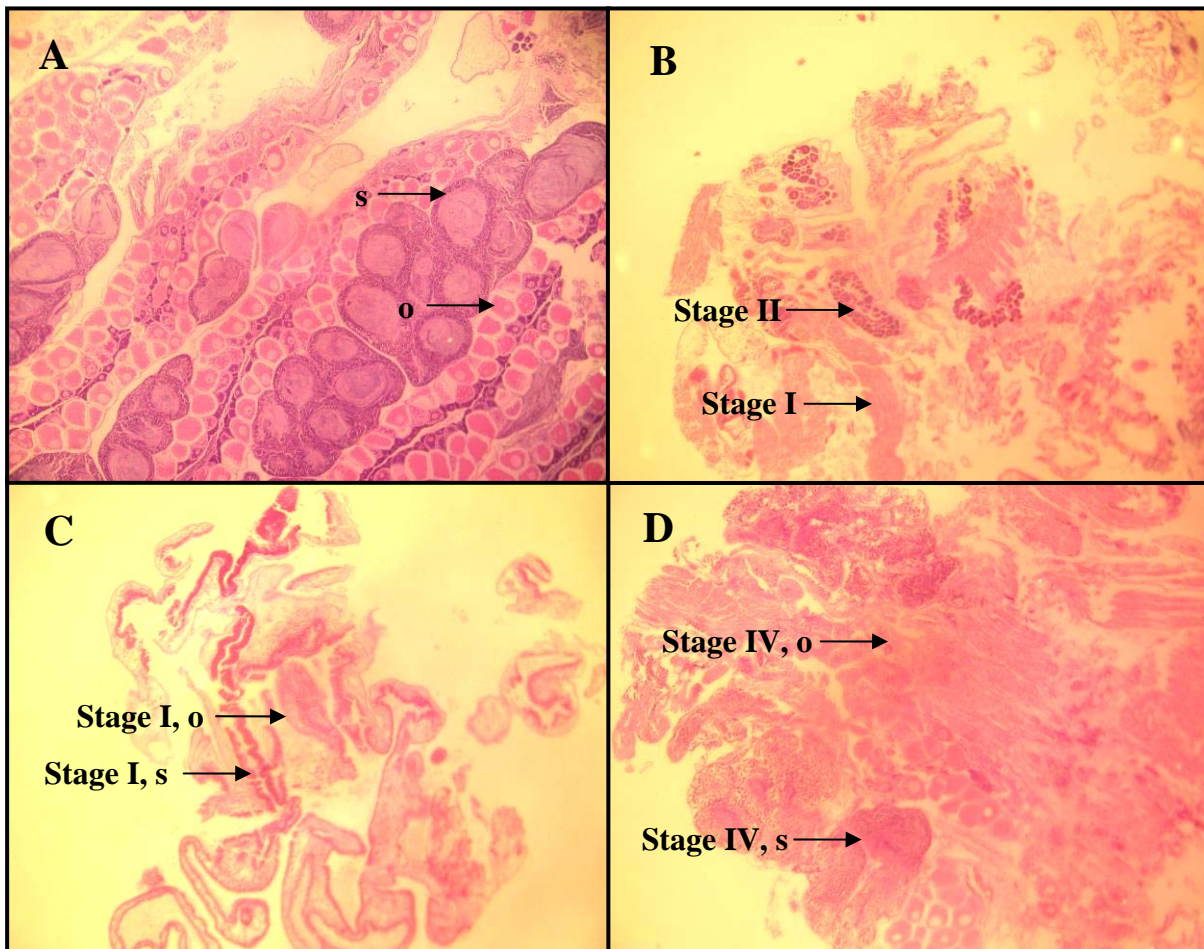
Note: - = no fertilisation; + = fertilisation observed

Figure 2.3: Mean % gonad index (\pm SE; n = 5) of *Styela clava* collected from Bayswater Marina, Auckland and mean sea surface temperatures ($^{\circ}$ C).



Note: The arrows on the x-axis refer to the dates of the new and full moons – coinciding with each sampling period

Figure 2.4: Examples of gametogenic stages observed in *Styela clava* individuals sampled in this study



Note: A) Stage III observed in ovarian (o) and spermatogenic (s) tissue, B) Stages I and II observed within the same individual; C) Stage I observed in both oogenic and spermatogenic tissue; D) Stage IV observed in both oogenic and spermatogenic tissue. Magnifications = x 45.

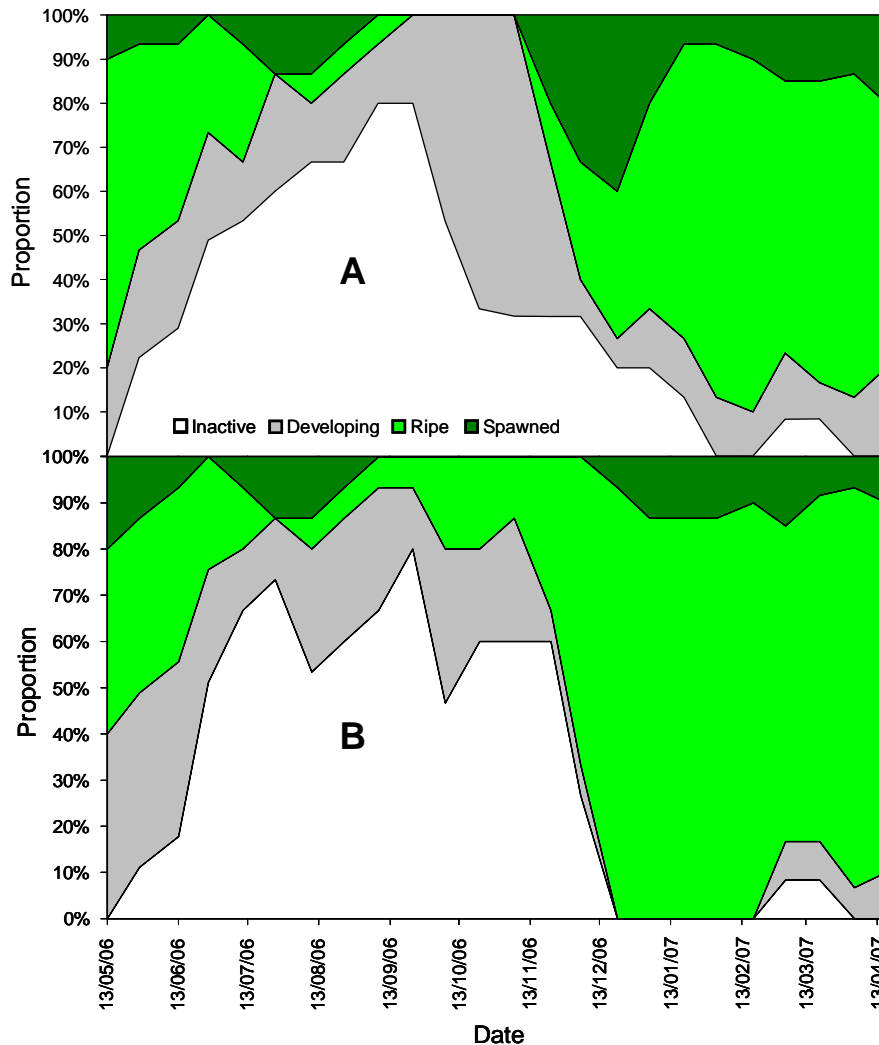
2.2. TIMING AND DURATION OF *STYELA CLAVA* LARVAE IN THE WATER COLUMN

2.2.1. Methods

Extant information suggests that the planktonic period of *Styela clava* is relatively short, approximately 1–3 days in total (Kashenko 1996). Despite this short larval period, discrete sampling can potentially detect the presence of *S. clava*, particularly if sampling can be targeted to the peak reproductive period. Discussions with aquaculturalists on Waiheke Island in 2005 (McFadden 2005; Kingett Mitchell 2005) suggest that *S. clava* larvae are present in the water column in the 6 weeks between mid February and late March.

Plankton samples were collected weekly over a period considered to include the likely peak reproductive period over the summer months from 18 October 2006 to 24 April 2007. Three replicate zooplankton samples (150 – 200 litres, each with a net of 64 µm mesh size and 250 mm mouth opening) were collected by vertical tow in the early afternoon from the upper 2 m of seawater in the area adjacent to the population of *S. clava* to be sampled. Collection sites were in close proximity to the parent population of *Styela clava* individuals sampled at

Figure 2.5: Gonad histology of *Styela clava* collected from Bayswater Marina, Auckland



Note: A) Ovarian tissue; and B) testicular tissue. Data is presented as the average stage of a 3-sample running mean.

Bayswater Marina (Fig. 2.6). When no ascidian larvae were detected in the samples for some time, however, additional plankton sampling was relocated to other piers in the vicinity as a verification process.

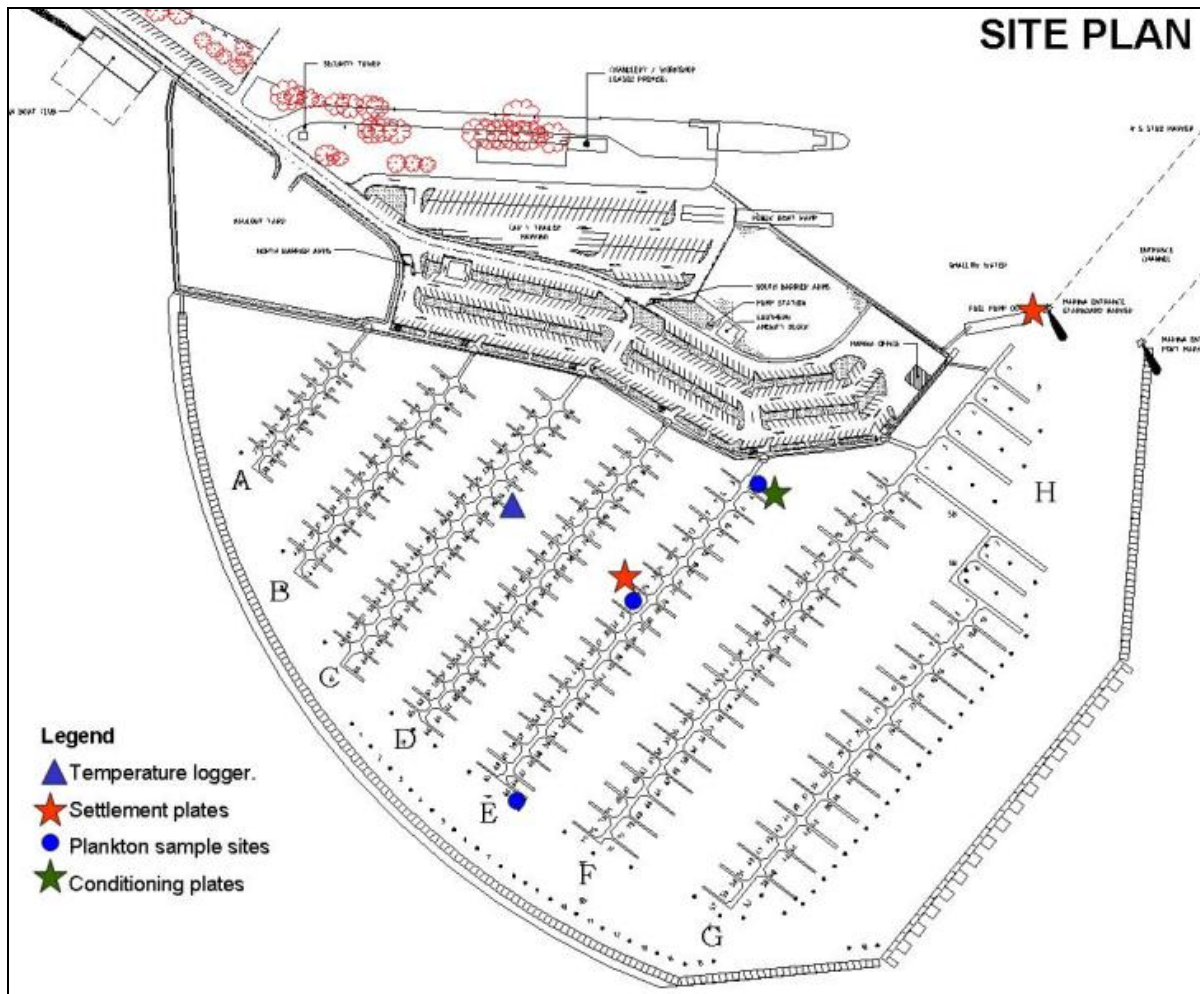
All plankton samples were immediately transferred from the net into sealed plastic jars, transported to the laboratory and analysed while fresh. All tunicate larvae present in the samples were isolated, identified and counted (as in Bullard & Whitlatch 2004; Bourque et al. 2005; Davidson et al. 2005).

2.2.2. Results

Throughout the entire sampling period, a total of ten ascidian larvae were collected. During October, one *Ascidiella*-like larvae was recorded in the samples. Plankton sampling in mid-November yielded two *Ascidiella*-like larvae and one more at the end of November.

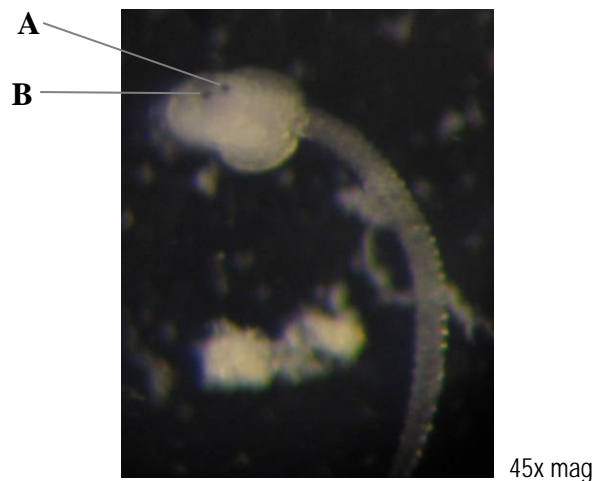
Ascidiella sp. larvae were observed in December, January, March and April (Fig 2.7). No *S. clava* larvae (e.g. Fig. 2.8) were observed in the plankton samples collected from Bayswater Marina.

Figure 2.6: Site plan of Bayswater Marina showing the location of the plankton sampling sites, seawater temperature datalogger, the marina settlement plates and conditioning plates



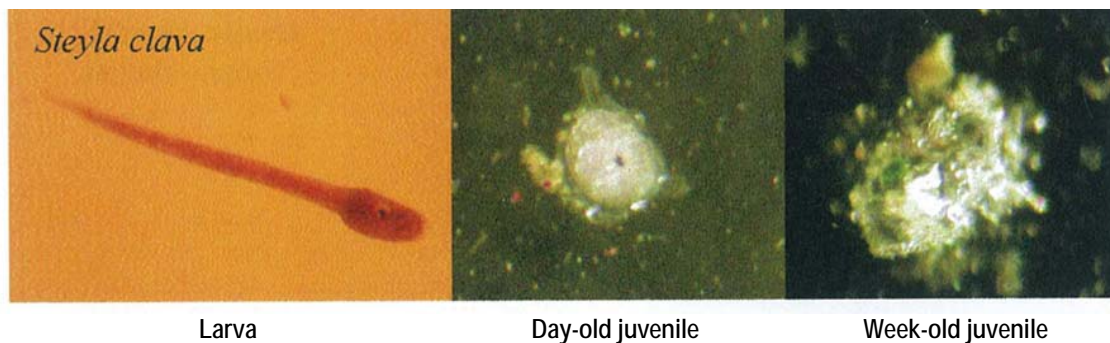
Source: Site plan reproduced with permission of Bayswater Marina Management.

Figure 2.7: Larva of *Ascidella* sp. observed during several sampling events over summer and autumn.



Note: the presence of an ocellus (A) (light sensing organ) and statolith (B) (gravity sensing organ), the latter is not observed in *Styela clava* but is prominent in *Ascidella* sp. larvae.

Figure 2.8: *Styela clava* larva, day-old juvenile and week-old juvenile (from Bullard & Whitlatch 2004).



Note: magnifications not specified in source text.

2.3. LARVAL SETTLEMENT AND RECRUITMENT OF *STYELA CLAVA*

2.3.1. Overview

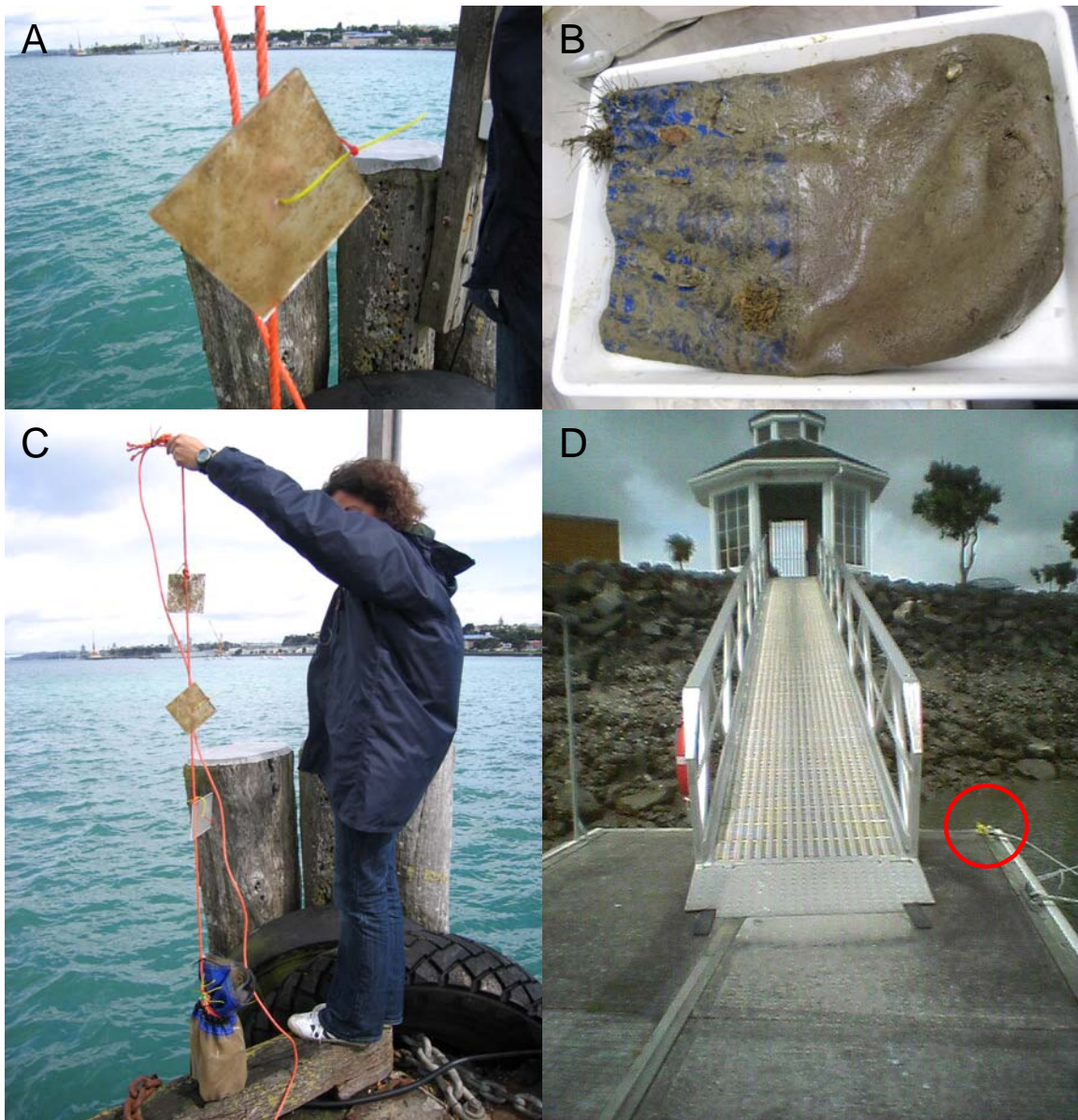
Larval settlement and recruitment is a well studied phenomenon in marine invertebrates. Ascidians in particular have been the focus of much research in this field (see references in Strathmann 1987). The use of artificial substrata as settlement collectors in the field has likewise been the focus of much work over the last few decades (see references in Pineda 2000). These studies have all indicated that a variety of factors (e.g. adult population density, larval supply, local hydrodynamics; sampling interval, collector design) affect the success of any given experiment designed to detect and measure settlement. The purpose of this component of the research programme is to determine the timing (onset and duration) of recruitment of *Styela clava* in the wild, as inferred from settlement onto artificial substrata.

2.3.2. Methods and study sites

Methods as indicated elsewhere (Davidson et al. 2005) were used to sample recruitment in the Bayswater Marina population of *S. clava*. This section of the study was undertaken at three locations at Bayswater Marina; immediately adjacent to the sampled parent population (two off E-Pier) and also at the entrance to the marina (the “petrol” wharf) (Fig. 2.6). An additional site was later added and was located at Victoria Wharf (approximately 3 km from the marina). This site was abandoned later in the study due to apparent human interference and/or wave action and relocated to the training wharf at the Royal New Zealand Naval Base (Devonport) approximately 0.5 km from Victoria Wharf (see Fig. 1.3 for site locations). At each location, a settlement collector was suspended in the water column at a depth of one to two metres. Settlement collectors consisted of a single vertically weighted rope, to which three plates were attached with cable ties approximately 20 cm apart (Fig. 2.9 A, B, C). The plates used consisted of 10 cm x 10 cm clear, 5 mm thick, colourless roughened Perspex™ plates (Fig. 2.9A),

Deployment of settlement collectors occurred from 25 October 2006 to 24 April 2007. Each set of plates was removed fortnightly and replaced with a set of “new” pre-conditioned plates. The pre-conditioning of fresh plates involved immersion in seawater at the marina (Fig. 2.9D) for at least one week prior to replacing sampled plates on each of the three settlement lines. Each collected plate was individually bagged in seawater to keep them moist and transported unpreserved to the laboratory. Each plate was viewed under a stereomicroscope and analysed for the presence of recently settled ascidian individuals. Following visual inspection, each plate was photographed and fixed in 5% seawater-buffered formalin and retained for further examination if required.

Figure 2.9: Settlement plate equipment used to determine the seasonal recruitment patterns of *Styela clava*



Note: A) Close-up of a Perspex settlement plate prior to removal; B) an empty weight bag previously used for vertical anchoring of the plate lines in the water column; C) a single plate line at Devonport Site A; D) Plate conditioning site at Bayswater Marina. The conditioning line is suspended from the corner of the pontoon as depicted by the red circle.

2.3.3. Results

During the first deployment, three plates were lost, which is attributed to the prevailing stormy conditions during that period. Plates deployed at Victoria wharf (Devonport Site A) were subject to ongoing interference and likely removal by unidentified members of the public. In an attempt to gather information from this area plates were re-deployed for the final weeks of the sampling to the lay-by berth (training wharf) within the Devonport naval base (Devonport Site B). Most plates retrieved after deployment were covered in a dense layer of biofilm, algae and sediment (e.g. Fig. 2.10). One would expect newly settled *S. clava* to appear as demonstrated in Fig. 2.8. Nothing resembling these was detected on the plates.

One *S. clava* specimen was discovered on 14 March 2007 growing on the mesh part of the weight bag attached to the Bayswater Marina Petrol Wharf settlement collector (Fig. 2.11). This individual was approximately 30 mm long. When the weight bag was retrieved on 24 April 2007, the individual had grown to a size of 45 mm. Analysis of the gonad contents indicated that the gametes present in the tunic were fertile and the gonads comprised a relatively high proportion of the total body weight (9.4%).

2.4. COLLECTION OF ENVIRONMENTAL DATA

The reproductive periodicity (in terms of spawning or larval release) of marine invertebrates is often cued or entrained by predictable environmental variations. These variables can include lunar/tidal rhythms (e.g. Park et al. 2005), photoperiod (West & Lambert 1976 in *Styela plicata*), light intensity (Olsen 1983; Forward et al. 2000), sea temperature (Giese & Kanatani 1987) and levels of phytoplankton (Starr et al. 1990), or a combination of cues

Figure 2.10: Recruitment plate deployed in Bayswater Marina, Auckland

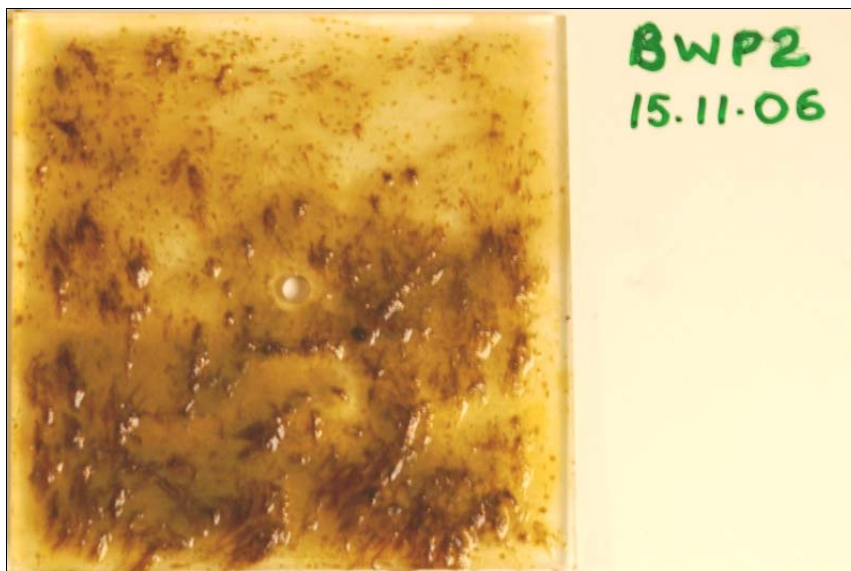


Figure 2.11: *Styela clava* individual detected on the weight bag at Bayswater Petrol Wharf



(Counihan et al. 2001), among others. For the duration of the study period, May 2006 to April 2007, environmental data was recorded in an attempt to infer the proximal cues for reproduction and settlement. Data collected/recorded included:

- Time of sunrise & sunset (from the Nautical Almanac).
- Moon phase (from the Nautical Almanac).
- Time/Depth/Location of sampling.
- Secchi disk reading (water clarity).
- Cloud cover (fortnightly).
- Daily rainfall (CliFlo – climate database)
- Rainfall surge events (storm related) preceding each *Styela clava* sampling
- Solar radiation/UV (CliFlo – climate database)

To record hourly surface water temperature, a TinyTag™ temperature datalogger was deployed at Bayswater Marina for the duration of the sampling period (see Fig. 2.6). Fortnightly environmental parameter measurements were made in conjunction with the collection of *S. clava* individuals. Dissolved oxygen, salinity and conductivity were measured *in situ* using a Schott Multimeter. Water clarity was estimated using a Secchi disk and cloud cover was qualitatively estimated upon arrival at the site.

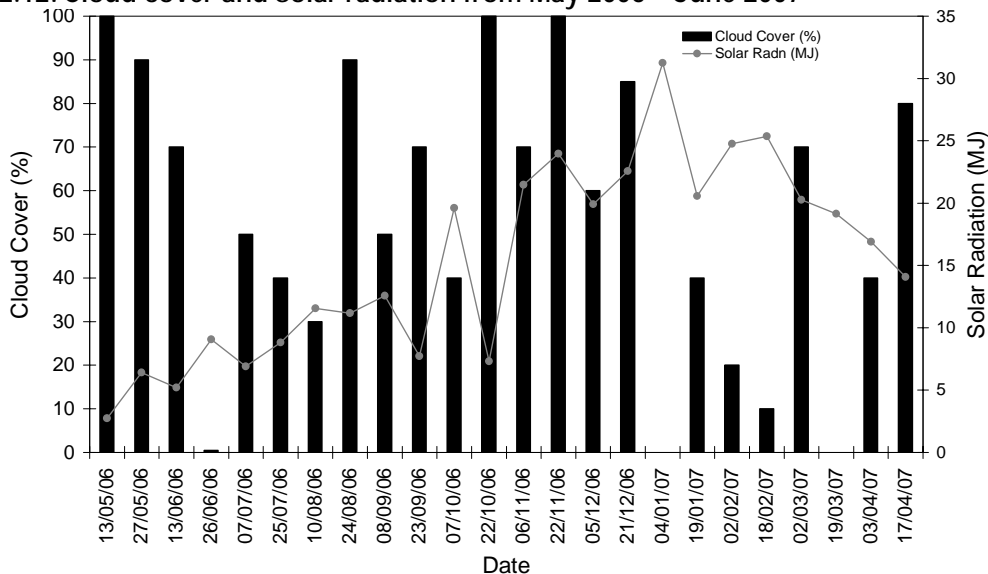
2.4.1. Results

Abiotic environmental data collected is summarised in Figs. 2.12 – 2.17. Solar radiation was highest on 4 January 2007 (Fig. 2.12). Dissolved oxygen recorded was quite variable throughout the sampling period, and highest levels of dissolved oxygen in the water column were reported on 5 December 2006 (Fig. 2.13). Water clarity throughout the sampling period ranged from 0.5 to 2.5 m (Fig. 2.14). Water clarity was highest in June 2006, January and March 2007. Salinities recorded ranged from 11.9 ppt to 35.8 ppt throughout the sampling period (Fig. 2.15). The low salinity reported on 7 October 2006 could be attributable to the concurrent high precipitation.

Information on average monthly rainfall records over the study period is displayed in Fig. 2.16. Average rainfall was lowest in February 2007, when 0.1 mm of rain was recorded. This dry period was followed by the highest amount of rainfall in March 2007 with an average of 6.5 mm. Solar radiation was lowest in the winter month of July 2006 and highest in summer, where 21 megajoules were recorded.

Photoperiod (number of daylight hours on the day of collection) and sea surface temperature (°C) over the sampling period is illustrated in Fig. 2.17. From June 2006, a gradual increase in sea surface temperature was recorded, until a high of 23.4 °C was reached on 18 February 2007. The 15 °C temperature threshold, above which spawning of *S. clava* has been observed overseas, was first reached after winter 2006 on 4 September 2006.

Figure 2.12: Cloud cover and solar radiation from May 2006 – June 2007



Note: MJ = Megajoules. Solar radiation data obtained from CliFlo (NIWA Climate Database) Stn. 12328 (North Shore, Auckland Regional Council, Agent # 141).

Figure 2.13: Percentage oxygen and dissolved oxygen (mg L⁻¹) measured in Bayswater Marina from May 2006 – April 2007

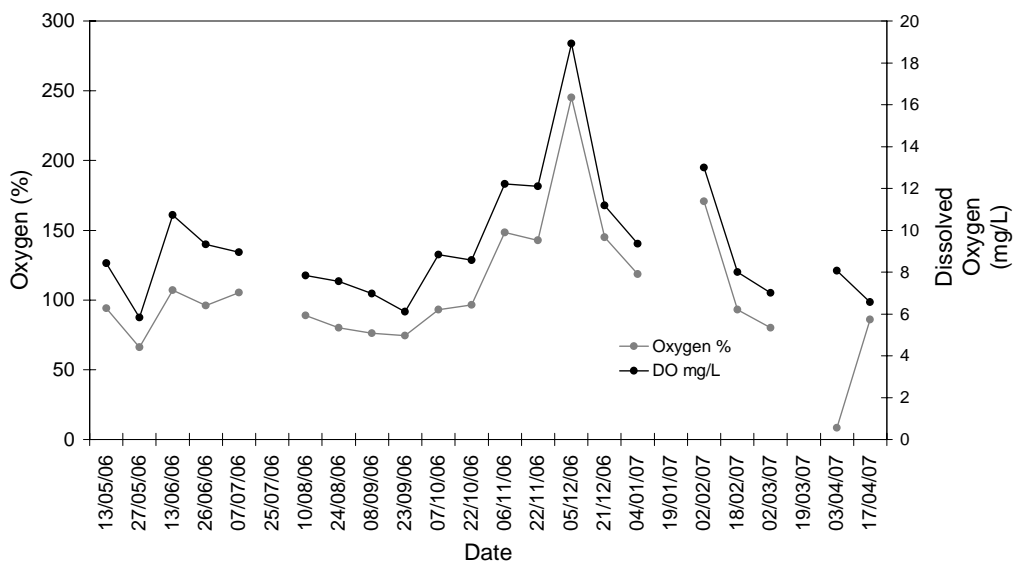


Figure 2.14: Water clarity in metres recorded in Bayswater Marina from May 2006 – April 2007

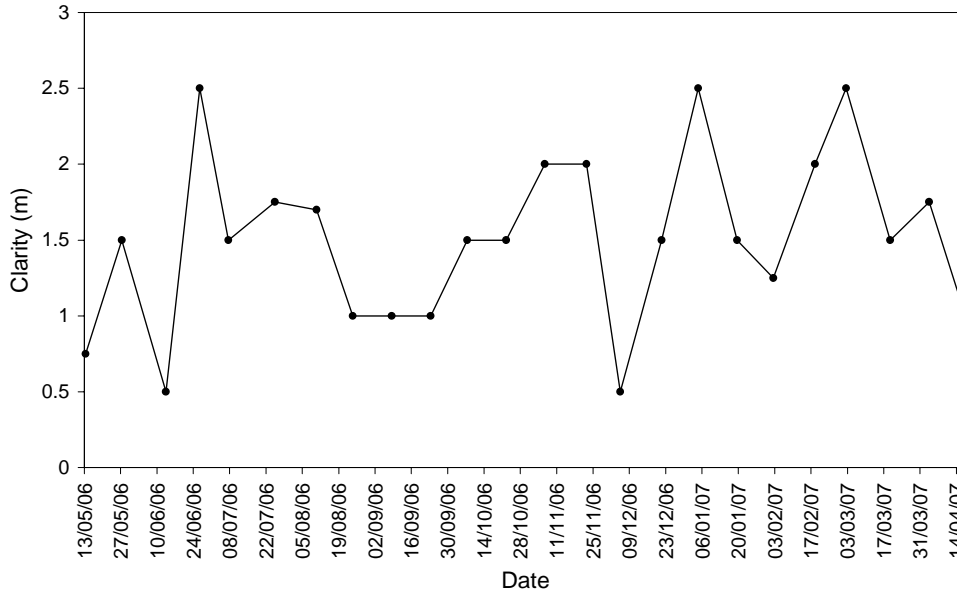


Figure 2.15: Surface water salinity (ppt) and conductivity (mS) recorded at Bayswater Marina from May 2006 – April 2007

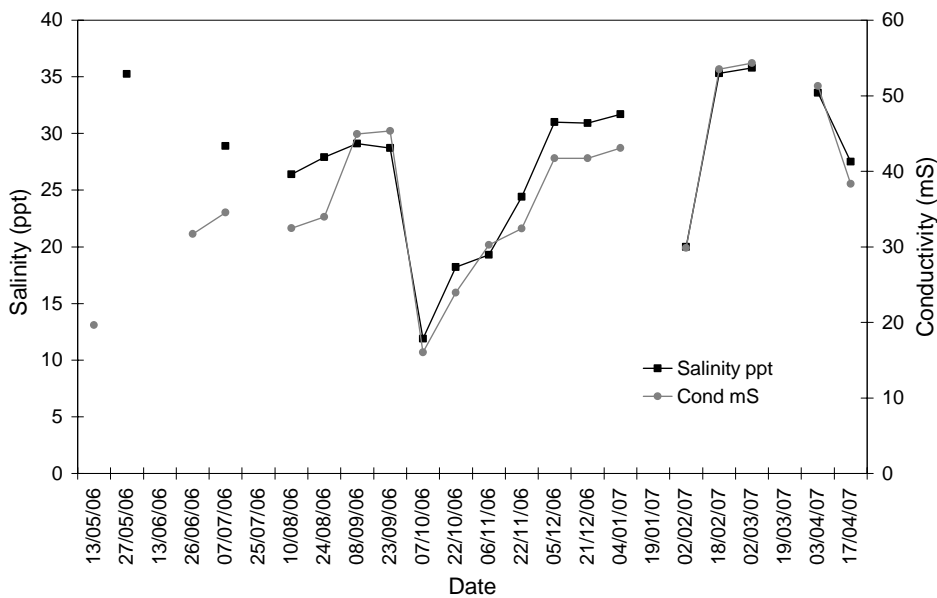
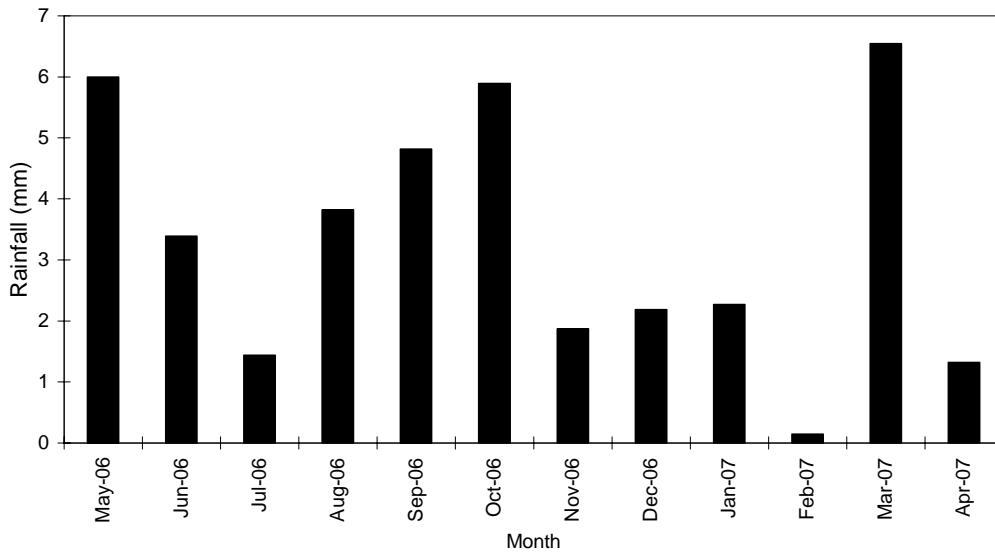
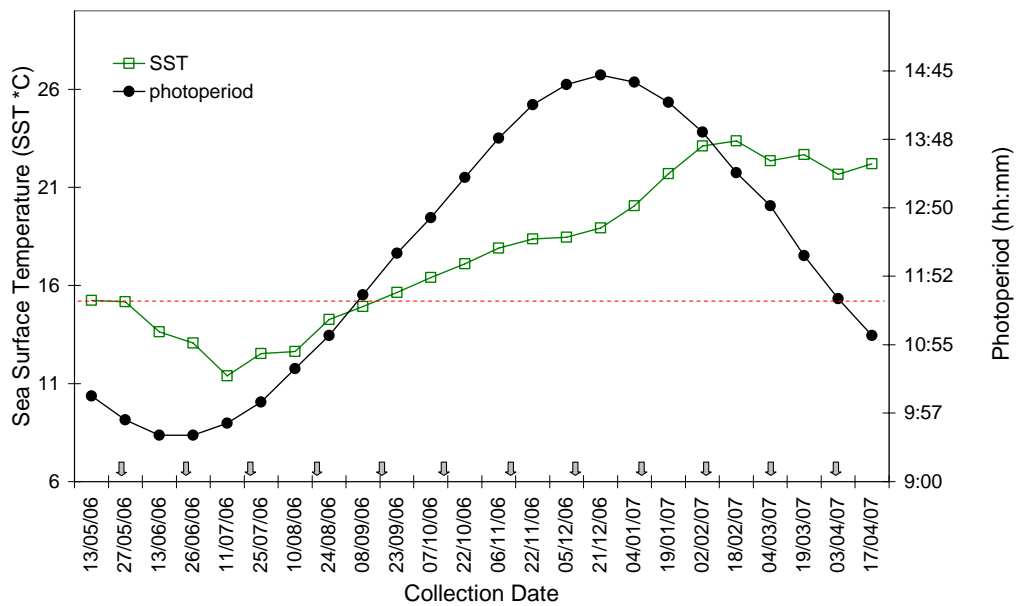


Figure 2.16: Average monthly rainfall from May 2006 to April 2007



Source: Data obtained from CliFlo (NIWA Climate Database) Stn. 12328 (North Shore, Auckland Regional Council, Agent #141).

Figure 2.17: Sea surface temperature (SST) (using smoothed data, recorded at hourly intervals) at Bayswater Marina, Auckland



Note: Grey arrows indicate dates of full moon. The red dashed line represents the 15°C temperature threshold, above which spawning of *Styela clava* has been observed overseas.

3. Discussion

3.1. REPRODUCTIVE PERIODICITY AND EARLY DEVELOPMENT

Many species of the ascidian family Styelidae are known to be either self fertile or partially self-fertile in the laboratory (Cloney 1987). Given the variable level of asynchrony observed in the development of male and female gametogenic tissue in this study, it is suspected that undisturbed populations of *Styela clava* in Waitemata Harbour would likely out-cross for the majority of the time. Examination of gamete smears in this study (wherein successful fertilization was noted) indicates that *S. clava* is at least partially self-fertile. It is therefore possible that when stressed (e.g. by mechanical disturbance such as being squeezed or abraded by vessel hulls or during removal) individual *S. clava* could release both male and female gametes which could potentially self fertilise and thus spread the population. This has implications for any efforts made to remove individuals from infested areas.

Average sea surface temperatures after winter reached 15°C for the first time on 4 September 2006, which coincided (approximately) with the first major spawning event between 24 August 2006 and 8 September 2006. This finding is consistent with overseas research, where spawning events were shown to be triggered by temperatures rising above this threshold (Eno et al. 1997, Bourque et al. 2007). Analysis of histological data collected immediately after this spawning event revealed a period of one month in September to November, when all female gonads were in the developing or inactive stage, and ripe or spawned gametes were absent.

Variability in the stages of gonad development among different individuals collected in any given sample was observed, which may be attributable to some extent to the relatively small sample size of 5. The presence of individuals at different stages of maturity at anyone time indicates asynchrony within the population at Bayswater Marina. These results concur with Parker et al. (1999), who have shown asynchrony within a population of *S. clava* in Cork Harbour, Ireland. Similarly, Davidson et al. (2005) observed individuals that were collected on the same day from Prince Edward Island from August 2001 to November 2004 at different stages of gonad maturity.

Gametogenesis in both sexes of *S. clava* in Waitemata Harbour appears to be continuous throughout the year, as indicated by the presence of developing or ripe gonads at any one time. In colder waters (3–4°C) when metabolic activity is very low obvious gametogenic activity in *S. clava* can stop completely (Parker et al 1999). The lowest sea surface temperature (SST) measured in Bayswater Marina was 9.9°C on 24 September 2006, which may be sufficiently warm for gametogenesis to occur for an extended period of the year. These findings, along with the results of the fertilization study showing that eggs are fertile from spring through to autumn, suggest that the population in Bayswater Marina appears to be competent to reproduce over an extended period of the year, from spring through to autumn. This is confirmed by the histological data, which suggested that the likelihood to spawn is highest between summer and autumn.

A comparison of the stages of gonadal maturation in *S. clava* in Northern Hemisphere populations and New Zealand is provided in Table 3.1

A total of ten ascidian larvae were identified in the plankton samples collected weekly from October 2006 to April 2007. Six of those were *Asciidiella* sp., but no *S. clava* larvae were detected. *Asciidiella* larvae are of similar size to *S. clava* larvae. *Asciidiella aspersa* (commonly found in New Zealand waters) and *S. clava* both produce tadpole larvae with a mean length of

0.85 mm (Bullard & Whitlatch, 2004). The sampling technique employed thus appears to be appropriate for the detection of ascidian larvae. One possible explanation for the non-detection of larvae may be the low adult population density in Bayswater Marina. Relatively high abundance of *S. clava* larvae were reported in areas of high adult population densities. For instance, a study conducted in Prince Edward Island, Canada, in 2003, observed a maximal density of 0.56 larvae L⁻¹ on 19 August 2003 (Bourque et al. 2007). Another likely explanation for the larval absence may be the short planktonic phase of 1–3 days reported overseas (Kashenko 1996, Davidson et al. 2005, Davis & Davis 2007), which minimizes the chances of detecting larvae in the plankton.

Most of the settlement plates deployed at Bayswater Marina and Devonport Wharf were covered in a dense layer of biofilm upon retrieval, but were devoid of *S. clava* and other ascidian juveniles. Possible reasons for the reported lack of recruitment are listed in Table 3.2. These include environmental conditions, such as water temperature, water flow and salinity being unsuitable, which are unlikely to be the case for this research. Competitive exclusion by other fouling organisms and unsuitable depth of the plates deployed were another two possible but unlikely explanations for the lack of settlement. More likely, however, could be the lack of a sufficiently darkened surface for settlement, which appears to be more suitable for settlement of *S. clava* larvae. Similarly, the relatively low adult population density at Bayswater Marina may be another possible reason why no recruitment was observed. Given the fact that only one of these usually gregarious individuals settled on the weight bag, which by that time was covered in a dense layer of biofilm, low population density and unsuitable settlement substrate appear to be the most probable explanations for lack of settlement on the Perspex plates.

Table 3.1: Comparison of maturation stages in *Styela clava* gonads studied in Northern Hemisphere populations and that which was sampled for this study.

Study	Spring	Summer	Autumn	Winter
Parker et al. 1999 (Cork, Ireland)	Inactive and early development of both male and female gonads.	Gametogenesis and spawning peaked	Spawning continued to peak in October	No gametogenesis in early February (water 3–4°C) Gonad maturation begins mid-February (water above 8°C). Regressing in December and inactive to early development observed in Feb-March.
Davidson et al. 2005 (Prince Edward Island, Canada)	Gametogenesis underway	Gametogenesis well advanced, gonads mostly mature and spawned	Mature and spawning individuals abundant in populations	Most individuals spawned out; little to no gametogenic activity
This study (Auckland, NZ)	Inactive and early development of both male and female gonads.	Spawning peaked in December. Gonads mostly universally ripe throughout summer months. Peak in gametogenesis.	Ripe gonads observed in most specimens	Gametogenesis occurs but at a low level until late August. Gonads mostly inactive.

The *S. clava* individual on the weight bag was approximately 30 mm long when initially detected on 14 March and grew to a length of 45 mm over the following 41 days. Assuming a relatively constant growth rate of 15 mm per 41 days (or 11 mm per month), then settlement of this individual was likely to have occurred in mid to late December 2006. This growth rate is similar to those reported in Prince Edward Island in Canada, 12.5 mm per month (Bourque et al. 2007), and 11–14mm per month in Denmark (Lützen 1999).

3.2. THE EFFECTS OF PHYSICAL VARIABLES ON *STYELA CLAVA* GONAD MATURITY

Styela clava are reportedly tolerant to fluctuations in both salinity and water temperature (Eno et al. 1997). The effects of other variables such as dissolved oxygen, water clarity, solar radiation and cloud cover have not been reported in previous studies. There are no obvious correlations between the reproductive cycle of *S. clava* and the different environmental variables recorded during the course of this study. As found in other studies, it is possible that a SST of 15°C may be a minimum threshold for the initiation of first spawning of *S. clava* in the Waitemata Harbour. Davidson et al (2005) found that once initiated in a given reproductive “season,” spawning could occur at temperatures as low as 10°C. Thus although experimental studies are required to determine the precise nature of this relationship, the fact that SSTs are greater than 10°C for much of the year in the Waitemata Harbour suggests that temperature will not be a major factor limiting reproductive activity of *S. clava* in the area.

Table 3.2: Factors which may account for the absence of *Styela clava* recruits on the settlement plates

Potential Factor	Possible degree of effect
Low adult population densities (spatial variation in larval supply).	No <i>S. clava</i> larvae were detected in the water column during the present study. Planktonic larval absence may explain, in part, the dearth of obvious recruitment on the settlement plates.
Larval reaction to settlement plate colour/surface texture (Wisely 1959, Roberts et al. 1991).	The plate type was selected according to results discussed in Wisely (1959). Wisely (1959) established that colour had little bearing on settlement and that settlement was heavier on roughened plates than on smooth ones. Conversely, Roberts et al. (1991) stated that colour can affect settlement but Wisely stated that colour is not an issue in muddy water or when biofilm obscures the plate colour. Both of these factors were observed in this study.
Larval light/dark preferences (Wisely 1959).	Previous studies on other species showed that most organisms prefer the shaded surface of the plate, indicating sciaphilic larval tendencies. While the plates used in the present study were clear and colourless, the formation of biofilm rapidly increased the opacity of the plates. However most plates in the present study sat fairly vertically in the water column, which may have influenced settlement by not providing a sufficiently darkened side.
Water temperature (Wisely 1959).	Settlement of most species is affected by water temperature with most settlement occurring in the warmer months. Despite the plates being deployed in the warmer months, no recruitment was observed suggesting other factors may be responsible for the absence of settlement.
Salinity levels (Kashenko 1996)	<i>Styela clava</i> larvae are more sensitive to salinity variations, with studies indicating that decreases in salinity to 18 ppt and below usually result in larval mortality (Kashenko 1996). During the recruitment trial in the present study, surface water salinity ranged from 18 – 35.8 ppt (average salinity = 27 ppt). Salinity remained well above 18 ppt for the majority of the settlement study and thus should not have had a bearing on the absence of settled individuals on the plates.
Depth of plate deployment (Davidson et al. 2005, Wisely 1959)	Plates were deployed to a depth of 2 m (after Davidson et al. 2005). While depth is an important factor in settlement in many species (Wisely 1959), 2 m proved suitable for <i>S. clava</i> in previous studies (Davidson et al. 2005) so was unlikely to have had adverse effects on settlement in the present study.
Water flow (Roberts et al. 1991)	This factor does have a significant bearing on settlement. However in the present study, no settlement was detected in either the low flow (E-Pier) or high flow areas (Devonport and the "petrol wharf") where the plates were deployed.
Substratum surface chemistry (Roberts et al. 1991, Wisely 1959).	This factor can also affect larval settlement by affecting larval behaviour, larval adhesive strength, permanent attachment and metamorphosis (Roberts et al. 1991). Perspex™, an acrylic thermoplastic, may leach substances which could impinge on settlement but this was not determined in the present study. Other studies have used this material with success, which suggests any chemical leaching which may occur may not be significant enough to adversely affect larval settlement (e.g. Wisely 1959).
Competitive exclusion	Following the rapid colonisation of the plates by biofilm, macroalgal propagules were observed shortly after. The density of algae and the rapid consumption of available space may have had an effect on settlement. However as settlement was not observed on the plates devoid of algae (e.g. the cleaner plates at the "petrol wharf") then this factor may not have influenced settlement.
Environmental interference	Plates deployed off E-Pier were found, on each occasion, to be coated in a heavy layer of sediment. This may have hindered larval settlement to some degree. Plates deployed off the marina's floating "petrol wharf" were surrounded by dense thickets of kelp (<i>Ecklonia radiata</i>) growing on the sides of the pontoon. This, combined with the increased flow rates at this more exposed site may have accounted for the fact these plates were often clean, devoid of sediment and any obvious biofilm. Larvae may have attempted settlement but abrasion by the algae may have hindered this process.

3.3. IMPLICATIONS FOR MANAGEMENT

The life history and reproductive behaviour of *Styela clava* highlights this species as a prime candidate for successful invasion and settlement in non-native areas, including New Zealand. Artificial surfaces provided by aquaculture facilities and the growing number of marinas provide suitable settlement substrate for this species, as indicated by the high densities reported in such environments (up to 1500 individuals/m²), compared to the considerably lower densities on natural substrates (50-100 individuals/m²) (Lützen & Sørensen 1993). *Styela clava* is tolerant of large fluctuations in salinity and temperature and is rarely food limited as a filter-feeder. While larvae and juveniles are preyed on by snails and some fish, adults have no known predators (Osman & Whitlatch 1999).

As a potentially self-fertilising (when subject to mechanical disturbance) hermaphrodite capable of reproducing over an extended period of the year, successful reproduction can easily be achieved at even very low population densities. This species becomes reproductively mature at a tunic size of approximately 20 mm in length (Davidson et al 2005). In mid summer in northern New Zealand observations made in this study suggest that this size may be reached within about 50 days of settlement. If spawning can begin in early September of the year (when SST rises above 15 °C) and continue on until the SST drops to 10 °C (Davidson et al 2005), it is thus possible that five to six generations of this species can occur within a single year in the Waitemata Harbour. Given this short generation time and therefore high potential for rapid population growth, any efforts at managing population sizes should be targeted on the period of time prior to the initial spring-time spawning event (i.e. when the SST rises above 15 °C for the first time)

Removal activities would ideally be restricted to a period when no potentially fertile gametes were present within the population. Examinations of gonad histology suggest that potentially fertile gametes (gametogenic stages III and IV) are, however, present throughout much of the year with the exception of the period following the first spring-time spawning in late August-early September of the year. Conversely, gamete fertility assessments suggest that the best period for removal activities would be between May and August (inclusive) when no fertilisation was noted in the gamete smear preparations.

Given the potentially large number of generations that can occur within a given year, if small-scale population control is to be successful, it is highly recommended that operations occur when the SST is less than 15 °C (prior to the first spring-time spawning). It is considered that control operations conducted in warmer water are less likely to be successful due to non-detection of small individuals of recently settled generations.

This study represents the data from a single year of sampling in the Waitemata Harbour. Although the results are similar to that found in other parts of the introduced range of *S. clava*, there is the possibility that there is considerable inter-annual variability in the periodicity of reproduction (Giese & Kanatani 1987). Studies of the reproductive periodicity of this species should be extended for at least an additional year in order to add some certainty to estimates. In addition, it is recommended that studies of recruitment be repeated adjacent to a high-density populations of *S. clava* (such as was present on drop lines in the marine farms in Man O' War Bay, Waiheke Island) in order to refine estimates of the timing of recruitment.

In summary, some of the points to consider when attempting to manage the potential effects of this species include:

- The presence of fertilised eggs in gonad smears of individual *S. clava* indicates that physical disruption of even a single mature *S. clava* can result in the release of viable gametes and a fertilisation event.
- Small scale eradication is theoretically possible, but given that this species is potentially self-fertile, all individuals would need to be removed from the target area.
- If required, control operations should be targeted to the period when fertile gametes are not present in the gonads, as physical disturbance could trigger the release of gametes. Mid winter or mid spring appears to be the most suitable periods for this type of control activity.
- Control measures should ideally be completed in mid-winter prior to the period when SST rises above 15 °C. After this time the potential for spawning and recruitment is high; in addition, there will likely be a number of potential cohorts present, many of which could be too small to detect or positively identify during a given removal operation, thus preventing effective control of the population.
- In-water removal, if attempted, must be effected by first isolating the individual or clump of individuals in a water-tight, sealable bag, then carefully removing the animal from the substrate at the base of the stalk and sealing the bag; care must be taken to ensure that the test is not damaged or otherwise subject to physical shock prior to encapsulation and complete integrity of the bags must be maintained throughout the disposal process to ensure that gametes and embryos are not released back into the water column.

4. References

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Appendix 1: Protocols for Examination of Gonad Histology

Table 1: Protocol for preparing tissues for sectioning

Solution	Time	Comment
5% formalin:30% etoh 1:1	30 min	Dehydrate tissues to 70% alcohol; tissues
30% etoh	30 min	may be stored in this solution for an extended period prior to processing
50% etoh	30 min	
70% etoh	30 min	
80% etoh	30 min	Fully dehydrate tissues, replacing water
90% etoh	30 min	with a solvent that is miscible with paraffin or other resin
95% etoh	30 min	
100% etoh	30 min	
100% etoh	30 min	
100% etoh: xylene 1:1	30 min	Histoclear™ can be used instead of xylene;
100% etoh: xylene 1:3	30 min	
Xylene	30 min	
Xylene	30 min	Maintain temperature at 56° -60 ° C
Xylene : paraffin 1:1	30 min	dependent upon the melting point of the resin or wax to be used
Xylene : paraffin 1:1	30 min	
Xylene : paraffin 1:3	30 min	
Paraffin	30 min	
Paraffin	30 min	

Transfer tissues to a cassette or mould to cool, noting orientation, for trimming and sectioning; mount on glass slides

Table 2: Protocol for staining sections with Haematoxylin & Eosin

Solution	Time
Xylene : paraffin 1:1	30 min
Xylene : paraffin 3:1	30 min
Xylene	30 min
Xylene	30 min
Xylene: 100% eth 1:1	30 min
Xylene : 100% etoh 1:3	30 min
100% etoh	30 min
100% etoh	30 min
95% etoh	30 min
80% etoh	30 min
70% etoh	30 min
50% etoh	30 min
30% etoh	30 min
dH ₂ O	30 min
dH ₂ O	10
Gills haematoxylin	4 min
Rinse running tapwater	20 min
Differentiate in 0.3% acid etoh	dip x2
Rinse running tapwater	5 min
Rinse Scotts tapwater substitute	2 min (varies)
Rinse running tapwater	5 min
Stain 1% eosin Y in alcohol	2 min
Tapwater	dip x2
30% etoh	30 min
50% etoh	30 min
70% etoh	30 min
80% etoh	30 min
95% etoh	30 min
100% etoh	30 min
100% etoh	30 min
100% etoh: xylene 1:1	30 min
100% etoh: xylene 1:3	30 min
Xylene	30 min
Xylene	30 min
Mount slide with glass coverslips on Permount™	

Table 3: Recipes for the chemical solutions:

Acid alcohol 0.3% Acid Alcohol	
Commercial grade ethanol	2800 ml
Distilled water	1200 ml
Concentrated hydrochloric acid	12 ml

Scott's tap water substitute

Sodium hydrogen carbonate 10 gm Magnesium sulphate 100 gm Distilled water 5 L

Eosin Alcohol

1% eosin Y (CI 45380) 400 ml 1% aq phloxine (CI 45405) 40 ml 95% alcohol 3100 ml gl
acetic acid 16 ml

NOTES

Differentiation with acid alcohol required can be variable depending upon the strength of the haematoxylin (which stain strengthens with age) and experience of the histologist (the acid solution alters the colour of the tissue). Two quick dips (blotting the slide dry briefly between dips) in 0.3% acid alcohol is generally all that is required for some tissues.

Eosin is highly soluble in water. Over-staining is removed by washing in running water.