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Short Communication

Managing invasive *Styela clava* populations: Inhibiting larval recruitment with medetomidine

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Editor's note:
This paper is a contribution to the proceedings of the 3rd International Invasive Sea Squirt Conference held in Woods Hole, Massachusetts, USA, on 26–28 April 2010. The conference provided a venue for the exchange of information on the biogeography, ecology, genetics, impacts, risk assessment and management of invasive tunicates worldwide.

Abstract

The toxicity of the synthetic catemine medetomidine to <12 h-old larval clubbed tunicate, *Styela clava*, was assessed in 2-h laboratory bioassays. Exposure to medetomidine resulted in increasing rates of larval immobility with increasing concentration. The 2-h EC50 was 3.8 mg/L medetomidine. Larval settlement was highest at a concentration of 0.1 mg/L medetomidine. At higher concentrations of medetomidine, metamorphosis was initiated but not all larvae settled. The ability of medetomidine to reduce *S. clava* larval mobility and interfere with settlement suggests that it has potential as a management tool for controlling subtidal invasive *S. clava* populations.

Key words: tunicate, population control, Selektope™, toxicity bioassays, settlement inhibition, metamorphosis, biofouling

Globally, invasive ascidians are a growing problem, with continuing range expansion of numerous species of solitary and colonial ascidians (Lambert 2007). The Asian clubbed tunicate, *Styela clava* (Herdman, 1881), is a large (up to 20 cm total length, Minchin et al. 2006), stalked, solitary ascidian that is native to the coastal waters of the northwest Pacific (Millar 1960). This ascidian is widespread on artificial substrata in harbours, marinas, and on aquaculture infrastructure, in both hemispheres (Clarke and Therriault 2007; Nunn and Minchin 2009). In some areas, *S. clava* is also colonizing natural hard surfaces, such as crevices on rocky intertidal platforms, and shell fragments on subtidal mud flats (Goldstien et al. 2010), posing a potential threat to valued marine environments and resources, as well as aquaculture industries.

Control of solitary ascidians has focused on removal of adults from artificial structures and mussel-farming seed stock using methods such as plucking by divers, high pressure water blasting of removable fouled structures on land, air drying, immersion in freshwater, acetic acid and chlorine exposure, encapsulation, and perforation (Carver et al. 2003; Coutts and Forrest 2005; Morrisey et al. 2008; C. Paetzold and J. Davidson pers. comm.). The development of control methods that focus on key stages in the ascidian life cycle, such as larval settlement and metamorphosis, offers alternative methods for management of invasive populations. Many natural and synthetic compounds inhibit settlement of larval ascidians (e.g. Pawlik and Hadfield 1990; Davis et al. 1991; Bellas 2006), and may provide tools that impede population growth. Medetomidine, which acts as a α_2-adrenoceptor agonist in vertebrates (Virtanen et al. 1988), inhibits barnacle cyprid settlement at non-lethal nanomolar concentrations (Dahlström et al. 2000). This compound is being developed as a novel efficacious and environmentally

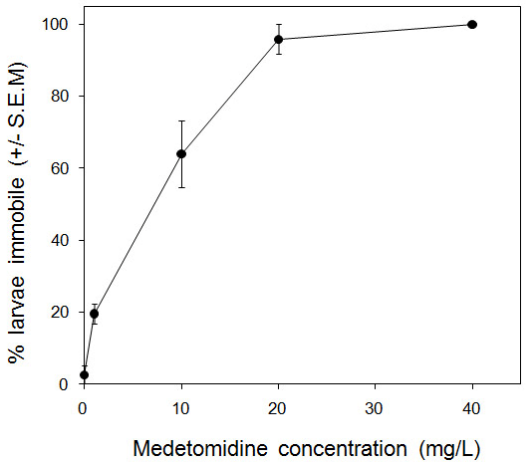

Figure 1. Mobility of *Styela clava* larvae following 2-h exposure to medetomidine in January.

sustainable antifouling agent with the brand name Selektope™, by I-Tech in Sweden (http://www.i-tech.se). We present the results of toxicity bioassays to determine whether medetomidine inhibits *S. clava* larval settlement, and assess the potential of this compound for management of invasive *S. clava* populations.

Adult *S. clava* were collected from a non-native population in the Port of Lyttelton (43° 36′S, 172°43′E), New Zealand, in January and March 2010 when seawater temperatures were ca. 18°C. In the laboratory, adults were maintained in large aquaria (80 L) with aerated seawater (salinity 32-34), at 18°C (±1°C), under dim fluorescent light (photoperiod 14 L: 12 D, 9.99 µmol m^{-2} s^{-1}). Within 48 h of collection, up to ten adults were dissected, and gametes obtained directly from the gonoducts with a Pasteur pipette. Oocyte and sperm suspensions were pooled, mixed, and diluted with filtered (38 µm) seawater, and incubated at 18°C (±1°C) overnight with constant aeration. The following morning, only larvae actively swimming close to the water surface were collected for use in toxicity bioassays.

The acute toxicity of medetomidine to larval *S. clava* was investigated in 2-h static toxicity bioassays using <12 h-old larvae. A 99% pure formulation of medetomidine hydrochloride (Tocris Bioscience, Bristol, UK) was used to prepare a stock solution (40 mg/L) in filtered (38 µm) seawater. Larvae produced by adults collected in January were exposed to four nominal toxicant concentrations (1, 10, 20 and 40 mg/L) and a seawater control, in 20 mL glass vials containing 15 mL of test solution. For each treatment there were three replicates of ca. 20 larvae. Bioassays were undertaken in a temperature-controlled room (18°C ±1°C) under dim fluorescent light. The test endpoint was larval immobility after 2 h, identified by a lack of movement when gently prodded. The median effective concentration (EC50) resulting in immobility of 50% of larvae was calculated according to the probit method (Finney 1971). Bioassays were considered successful if control survival was ≥90% (USEPA 1991). In the bioassay with larvae from adults collected in March, the number of immobile, metamorphosed (larvae without a tail), and settled larvae were recorded following 2-h exposure to five nominal toxicant concentrations (0.1, 1, 10, 20 and 40 mg/L) and a seawater control. Due to limited numbers of larvae there were only two replicates per treatment, giving totals of 44 to 85 larvae per concentration. The results of the March bioassay are presented as means of the two replicates and EC50 values could not be calculated.

In January, exposure of <12 h-old *S. clava* larvae to medetomidine for 2 h resulted in increasing rates of larval immobility with increasing concentration compared to the control, with 20% (S.E.: 2.9%) of larvae immobile at 1 mg/L and 96% (S.E.: 4.2%) immobile at 20 mg/L medetomidine (Figure 1). The 2-h EC50 was 3.8 (95% C.I.: 1.3, 10.6) mg/L medetomidine. In the March bioassay, larvae also underwent metamorphosis and settlement, consequently numbers of mobile and immobile larvae were lower than in January. The number of settled larvae was variable, with no apparent dose-dependent response and maximum settlement (80%) occurring at 0.1 mg/L medetomidine (Figure 2). At 20 mg/L, 30% of larvae settled, while metamorphosis was initiated in 65% of larvae, but they had not settled.

Short term exposure to medetomidine reduced mobility of *S. clava* larvae and interfered with settlement at concentrations similar to those causing mortality in the barnacle *Balanus improvisus* (Dahlström et al. 2000). Larval responses to medetomidine varied between bioassays, with higher rates of metamorphosis and settlement in bioassays undertaken in late summer (March). This may be due to seasonal variations in larval competency and their propensity to settle, possibly due to differences in adult fitness at the time of spawning. In March, settlement of *S. clava* larvae during the

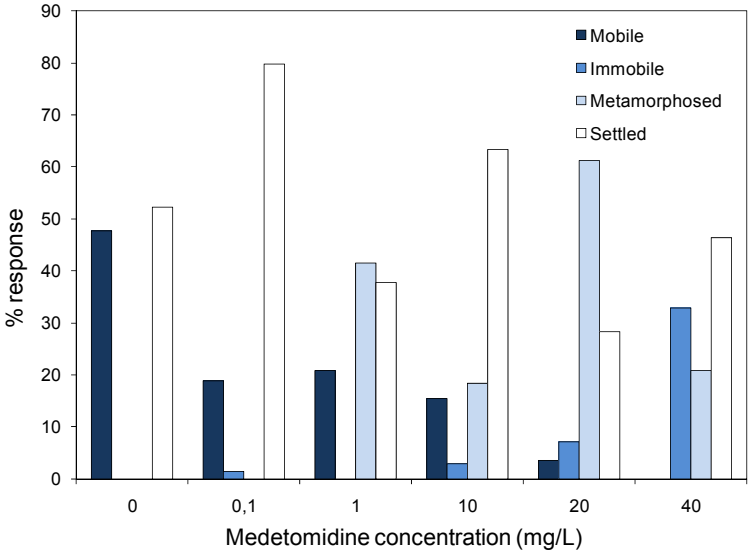

Figure 2. Mobility, metamorphosis, and settlement of *Styela clava* larvae following 2-h exposure to medetomidine in March.

2-h exposure was highest (80%) at 0.1 mg/L medetomidine. While metamorphosis was initiated at higher concentrations, not all larvae completed the settlement process. To determine the long-term viability of post-settlement stages, future bioassays should also monitor development through to complete metamorphosis. Medetomidine inhibits barnacle cyprid settlement by selectively binding to octopamine receptors, causing the legs to become hyperactive and unable to attach to surfaces (Lind et al. 2010). The mode of action of medetomidine is unknown in ascidians, but its ability to reduce *S. clava* larval mobility and interfere with settlement, suggests that it has potential as a management tool for controlling subtidal invasive populations by reducing recruitment rates, and ultimately population growth. Given a planktonic larval phase of <24 h (Davis 1997), and the difficulty of ensuring extended exposure in the marine environment, our 2 h exposures attempted to reproduce possible treatment scenarios. The application of mitigation compounds, such as medetomidine, that target ascidian early developmental stages necessitates knowledge of local reproductive and spawning behaviour, and development of underwater application techniques, such as antifouling coatings, slow-release pellets, gel adjuvents, and microencapsulated beads, to enable topical application and extended exposure during peak spawning activity.

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