BIOBULLETS FOR THE CONTROL OF MUSSEL FOULING IN SPANISH IRRIGATION SYSTEMS

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SUMMARY

Fouling of Spanish irrigation systems by invasive mussels - zebra mussels (*Dreissena polymorpha)* and Asian clams (*Corbicula fluminea*) - can compromise the supply of water to crops. Current solutions include the use of chlorine and the use of hydrogen peroxide-based products.

We tested the use of two formulations of microencapsulated active ingredients (BioBullets) on fouled pipes in an irrigation system in Mora La Nova, Spain. One product, SB1000, was dosed at a concentration of 150mg/l, while SB2000 was dosed at 30 mg/l. Both products have regulatory approval for use in UK drinking water supplies. Both products were dosed using a calibrated system for 8 hours on two successive days.

The number of live, freshly gaping, and freshly empty shells were monitored before dosing and over four weeks after dosing. Subsamples of mussels were also measured. Both products were highly effective at killing both species, with close to 100% of all shell material being collected at monitoring points being freshly dead. Mussels died more quickly using SB1000 than SB2000, but both products achieved the same ultimate result. There was no size selectivity in the mussels that were killed, showing that the products are suitable to control all population structures that might be encountered.

The BioBullet solution offers a viable tool for the control of fouling mussels in irrigation systems. There no impacts on the crops, it is safe to handle and dose and the products can be dosed for a short period (two or three days) at the end of each growing season, thus giving considerable convenience.

INTRODUCTION

Zebra mussels are recognised as one of the biggest economic and environmental pests in the world. Their invasion into western Europe (Aldridge et al., 2004) and the Laurentian Great Lakes (Hebert et al., 1989) has led to system-level changes (MacIsaac, 1996), the extirpation of some species of unionid mussels (Ricciardi et al., 1998), the fouling of raw water pipelines, such as those within power plants and drinking water facilities, and problems with irrigation systems, navigation and recreational activities ((Khalanski, 1997). In North America alone, zebra mussels are estimated to cost industry ca. U.S. $1-5 billion each year (Pimentel et al., 2005).

The arrival of zebra mussels within Spain's Ebro basin in the early 2000s has raised a number of economic and environmental concerns. Fouling of irrigation systems and reservoirs supplying industry is costly and will continue to increase, while ecosystem changes and fouling of threatened unionoid mussels, especially *Margaritifera auricularia*, represents a significant conservation worry. There is a clear economic and environmental case for identifying a control strategy for zebra mussels within Spain's freshwaters.

By far the most popular control strategy for industrial fouling by zebra mussels is chlorination. However, mussels will close and cease feeding when exposed to many toxins, including chlorine (Claudi & Mackie, 1994). They can survive for up to three weeks in this state, so chlorination must be continuous for this period. In addition, chlorine reacts with organic matter in water to produce carcinogens (trihalomethanes - THMs). For this reason chlorination is becoming less and less popular, especially where the treated water passes into the open environment.

Control of mussels in the open environment has been even more problematical because of the large volumes of water often involved, the risk posed to non-target organisms and the impacts on other services that the water body provides (e.g. irrigation, drinking water supply, fisheries, recreation). Numerous alternative chemical treatments to chlorination have been developed, but they often focus on the control of veliger larvae within industrial pipelines. In such situations, the control dose can be relatively low because veligers are more sensitive than adults, and it is necessary only to make the veligers close their valves so that they do not settle on the pipe walls.

The recent increase of fouling by both zebra mussels and Asian clams (*Corbicula fluminea*) within the irrigation systems of Spain has led to considerable limitations on the capacity to supply water. To date, the most favoured solutions have been the use of chlorine or products based on hydrogen peroxide.

The aim of this study was to test an emerging technology for zebra mussel control, the BioBullet (Aldridge et al., 2006). The product overcomes the limitations of chlorination and peroxide and has proven highly successful in treating mussel fouling of UK drinking water plants, for which it has been licensed by the UK drinking water regulators (DWI). The BioBullet uses the encapsulation of an active ingredient in microscopic particles of edible material. The mussels' natural filtering ability then removes and concentrates the

particles from the water, without stimulating the valve closing response. By using the mussels' filtering behaviour to concentrate BioBullets the absolute quantity of active ingredient added to the water can be reduced substantially. The approach enables the engineering of particles to break up and dissolve completely within a few hours, thus eliminating the risk of polluting the wider ecosystem.

2. MATERIALS AND METHODS

Particle formulation

Controlled release particles were commercially manufactured using a modified spray drying process (TasteTech Ltd., Bristol, U.K.). A premix slurry was prepared containing the encapsulant and active ingredient under conditions of controlled shear. The premix was pumped into an ultrasonic atomising nozzle at the top of a cooling chamber. The atomized particles formed perfect spheres, cooling as they fell to the bottom of the chamber. Further cooling of the particles was achieved in an airconveying system before discharge via cyclone to a fluid bed processor. The encapsulated particles were then coated with nonionic surfactant to aid dispersion in water. Further cooling in the fluid bed removed all heat of crystallization from the product prior to packaging.

Two products were produced containing different active ingredients. SB1000 contained a quaternary ammonium compound. SB2000 contained a salt-based product known to be toxic to mussels. Both products have received approval for use in UK drinking waters and have proven successful in killing zebra mussels in laboratory and field trials.

Dosing

Products were dosed into irrigation systems in Mora la Nova. These irrigation systems provide water to 300 Ha of olives, peaches, cherries and almonds. In 2010 the main water supply pipe became blocked by mussels, and cost ca. 3,000€ to remove. Two fouling species of mussels are known from the system, the zebra mussel (*Dreissena polymorpha*)and the Asian clam (*Corbicula fluminea*).

Tappings were made into two supply pipes and product was delivered as a dry powder using a venturi system. The target flow rate along the pipes was 10,000 l/hr. Product doses were accurately measured using a vibratory feeder (Coote Vibratory, UK) that was calibrated in situ. SB1000 was dosed at 150 mg/l for 8h on two days (10 and 11 May, 2011); pipe length was 5km. SB2000 was dosed along a different supply pipe (2km length) at 30 mg/l for 8h on two days (17 and 18 May, 2011). An 8h period ensured that products were delivered along the entire length of pipe.

Monitoring

Monitoring was conducted by inspecting the material that became trapped within filters along the length of the dosed pipes. Three filters were selected along both pipes, and a seventh filter was located on an untreated pipeline. Filters were emptied before trials began and data were collected before dosing started to provide a baseline. To encourage the capture of mussel material, water was flushed through the filters whenever possible and onto the land. As the volume of water that passed through the filters each day varied considerably, in part because of the farmer's operational considerations, the most useful measure of success was to measure the quantity of live, gaping and freshly empty shells. Live mussels were those that closed their valves in response to a gentle tap to the shell. Gaping shells did not close valves in response to a tap, and represented mussels that had

recently died. Freshly empty shells were those from which flesh had very recently separated, but which retained a bright periostracum and iridescent nacre. Monitoring continued until 16 June 2011.

To investigate any size selectivity in mortality on each sampling occasion, a subsample of at least 30 live and at least 30 fresh shells were measured along their longest axis using vernier callipers. Where fewer than 30 mussels were present all individuals were measured.

After dosing had been completed (20 May 2011) an endoscopic camera was placed into both treated pipelines to image the presence of mussels along the first ca. 20m of the pipes.

Statistical Analysis

Mortality was calculated as a proportion of live mussels compared to gaping and freshly empty shells. A mean value ± standard error (SE) was calculated from the three replicate sampling points along each pipe.

The lengths of both zebra mussels and *Corbicula* were normally distributed (Anderson-Darling test $P>0.05$). Size-selectivity was therefore investigated by t-test to compare the mean lengths of at least 30 individuals within each category (i.e. live and freshly dead zebra mussels and *Corbicula*).

RESULTS

Fouling organisms

During sample collection it became clear that fouling was caused not only by the bivalves *C. fluminea* and *D.polymorpha*, but also by filamentous algae and considerable growths of the invasive hydrozoan, *Cordylophora caspia* (Fig. 1). In addition, a small proportion of the *Corbicula* specimens collected were morphologically distinct and are likely to represent a new record of invasive species for the Iberian Peninsula – *Corbicula* fluminalis (Fig. 2).

Figure 1. Massive growth of the fouling hydrozoan, *Cordylophora caspia*, removed from a filter trap.

Figure 2. *Corbicula fluminalis* (?) – upper line, compared with *C. fluminea* – lower line. Possibly a new invasive species record for the Iberian Peninsula.

Mortality

SB1000 and SB2000 were effective at killing both *D. polymorpha* and *C. fluminea* (Fig. 3). For both species, gaping individuals were observed within a few days of dosing and continued to be collected over approximately one week, after which fresh shell material became dominant. In both cases, there was considerable variation between the three monitoring points, which yielded some fluctuations over time, as revealed by the relatively large standard errors. It is important to note that interpretation of these figures may underestimate mortality because animals may have washed into the pipes during sampling that had originated in points that were not exposed to the BioBullet products.

For the two products (SB1000 and SB2000), mortality in zebra mussels appeared to occur faster than that of *Corbicula. SB1000 yielded a more variable pattern, with 100% mortality in zebra mussels being found four days after dosing ceased, but data stabilising at 95% mortality after approximately four weeks*. SB1000 produced a stabilised *Corbicula* mortality of approximately 80% after approximately two weeks. With SB2000 all zebra mussels collected were dead three days after dosing. With Corbicula, there was a general trend towards increasing mortality, with 100% being reached after approximateky four weeks.

Inspections of the control filter yielded no mussel material whatsoever. This matched the observations in the experimental filters prior to dosing of BioBullets and suggested that the mortalities observed were a true reflection of the effects of the BioBullets products.

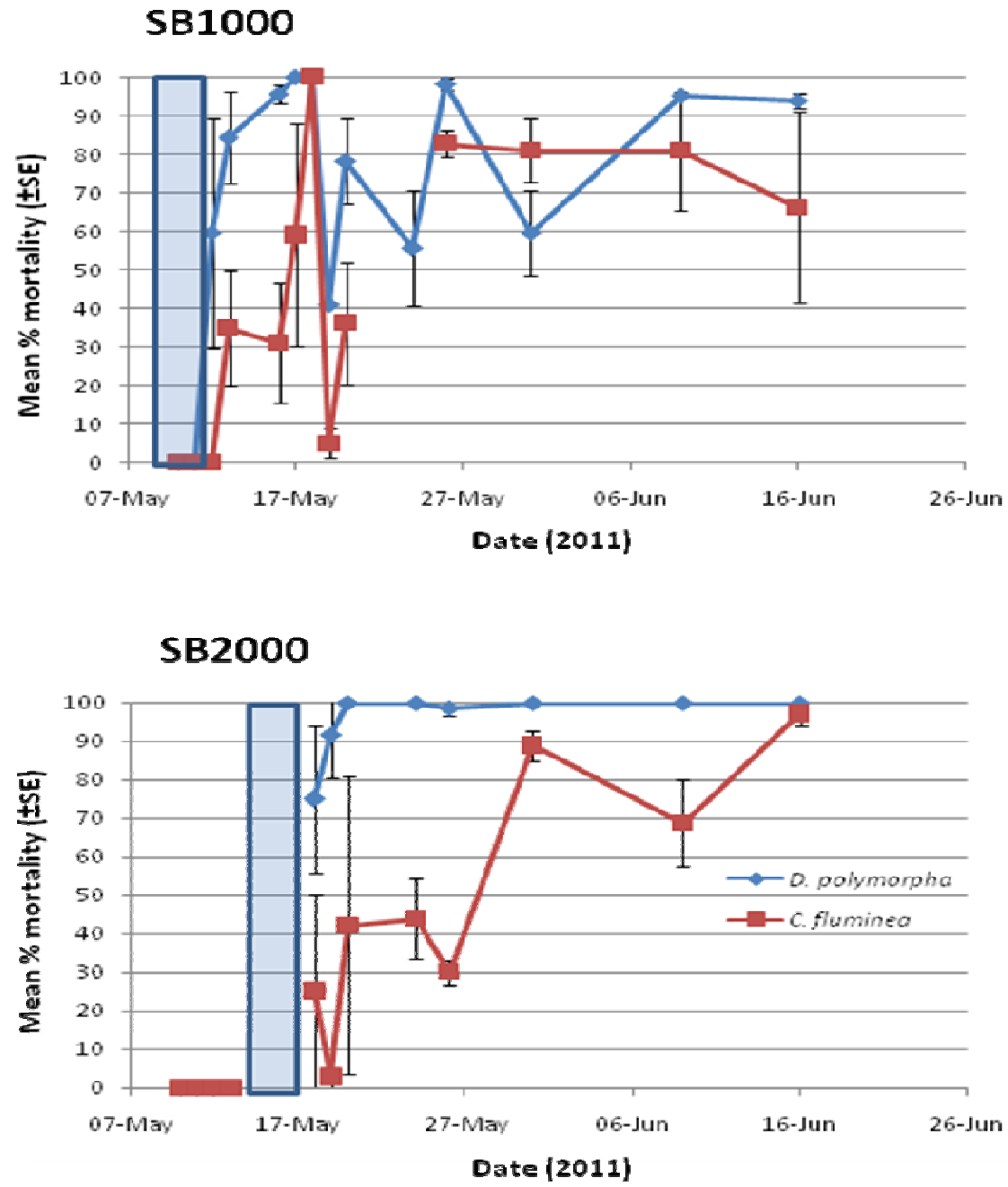

Figure 3. Mean mortality (live compared with gaping and fresh shells) of zebra mussels and *Corbicula* dosed with two BioBullet formulations (SB1000 and SB2000. Blue bars mark the dosing period for the two treatments.

Size selectivity

Corbicula specimens ranged in length from 7 to 28mm. Zebra mussels ranged in length from 8 to 27mm. The *Corbicula* in the pipe dosed with SB1000 were relatively small (7 to 18mm) and reflected two cohorts with modal peaks at 10 and 15mm. The zebra

mussels within the pipe dosed with SB1000 ranged in length from 8 to 27mm, but with no discernible discrete cohorts. Corbicula within the pipe dosed with SB2000 showed a wider range of sizes (8 to 28mm), but with the population dominated by the smaller sizes found in the SB1000 pipe. Interestingly, zebra mussels were relatively uncommon and of a small size in the SB2000 pipe.

The size-frequency distributions of live and dead (i.e. fresh and gaping individuals) was remarkably similar. There was no size difference between the live and dead zebra mussels (t=0.567, df=59, P=0.576) or *Corbicula* (t=0.360, df=65, P=0.720) exposed to SB1000 (Fig. 4). Similarly, there was no difference in the live and dead zebra mussels (t=0.05, df=2, P=0.966) or *Corbicula* (t=0.56, df=77, P=0.576) exposed to SB2000 (Fig. 5). This confirms that both SB1000 and SB2000 were capable of killing both *Corbicula* and zebra mussels of all sizes encountered, with no size selectivity.

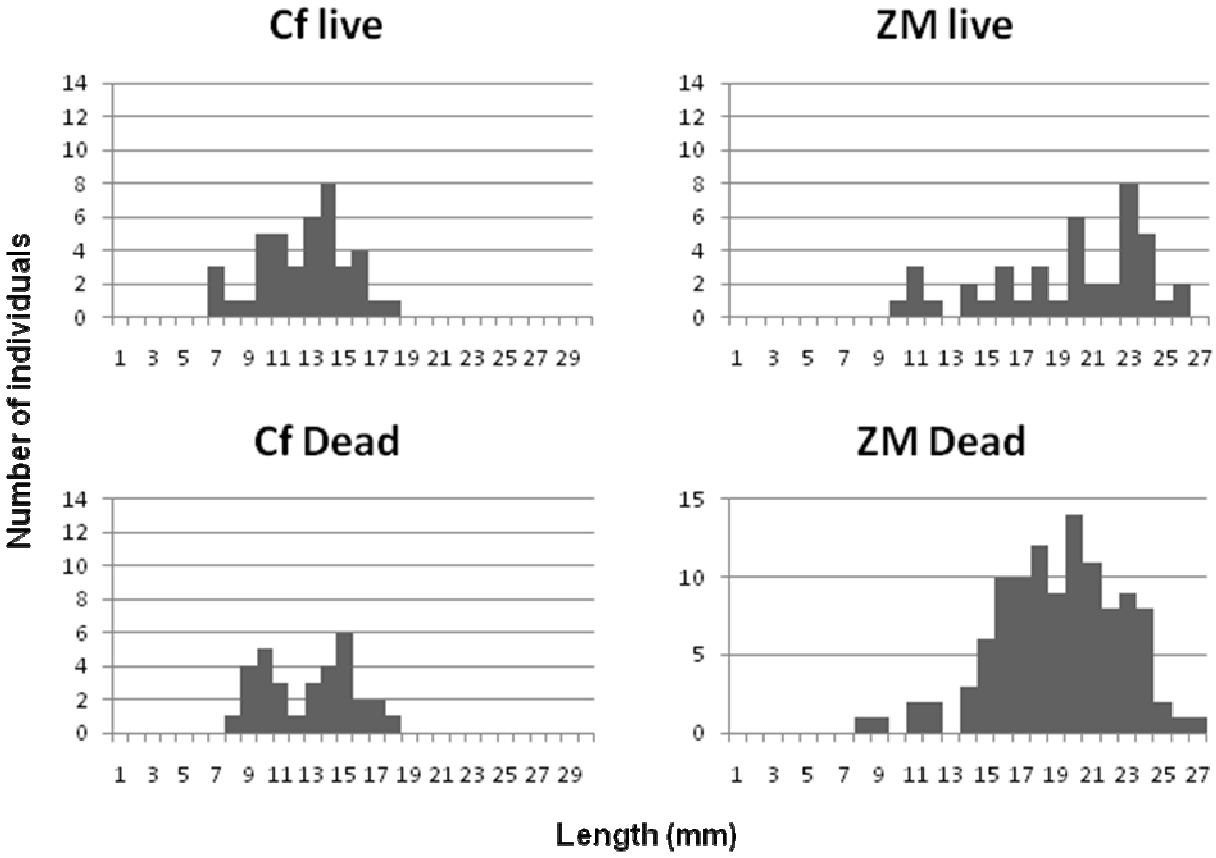

Figure 4. Size-frequency distributions for live and freshly dead zebra mussels (ZM) and *Corbicula* (Cf) exposed to SB1000.

Figure 5. Size-frequency distributions for live and freshly dead zebra mussels (ZM) and *Corbicula* (Cf) exposed to SB2000.

Camera inspection

Camera inspection showed both pipes to contain small numbers of zebra mussels, but no *Corbicula*. Most of the zebra mussels were on the upper quadrant on the pipes. As there were no baseline data on levels of fouling it was not possible to place these observations into context.

DISCUSSION

The trials were highly successful and showed that both SB1000 and SB2000 could kill zebra mussels and *Corbicula* in the irrigation pipelines. While BioBullets have been tested successfully against zebra mussels in the past, this is the first time *Corbicula* has been tested. The finding that neither product was size-selective for either zebra mussels or *Corbicula* shows that the products can be used to kill all individuals, ranging from the very smallest to the very largest. If the products were dosed annually, the size of mussels in the pipes would be reduced and so would the biomass of fouling material.

Dosing was achieved in a very straightforward manner, with no delays and no unexpected complications. Monitoring was constrained by the limitations of working within an area operated by *ca.* 300 farmers who each wished to irrigate their land to different degrees. This sometimes resulted in data being difficult to collect.

The presence of a low density of live zebra mussels remaining within the pipelines after treatment had finished is likely to reflect a number of factors. First, the reduced flow used during dosing of products will have resulted in the pipes being on partly filled with water. Zebra mussels attach to the pipe walls with their byssus threads and so are able to occupy surfaces around the entire pipe circumference. *Corbicula*, on the other hand, has no such byssus and there accumulates along the base of the pipe. This would have resulted in only the zebra mussels lowest in the pipe being exposed to BioBullets, and would explain the distribution of live zebra mussels in the upper pipe surfaces, but the absence of any *Corbicula*. Second, we have observed in UK water treatment works that mortality is lower in the 20-30m immediately downstream of the dosing point because the product takes some distance to disperse fully in the water. Third, treatment was conducted for only 8h on two days. During this time, some mussels will have naturally been closed and so not exposed to especially high levels of product. This issue could be overcome by longer dosing periods.

The continued presence of live *Corbiucla* in the filter points, but not zebra mussels, is likely to reflect that fresh material was being flushed into the pipes at all times from downstream of the dosing point. We were therefore not recording entirely mussels that had been exposed to our product. *Corbicula* is likely to be flushed into the pipes much more readily in this way than zebra mussels, because the zebra mussels remain firmly attached to the pipe wall.

The BioBullet treatment has considerable benefits over existing methodologies (e.g. chlorination and hydrogen-peroxide based treatments):

1. It is safe to handle and store.
2. It does not cause damage to crops.
3. It can be used as a short end-of-season treatment, this saving on operational and staffing costs.

This study provides compelling evidence that both SB1000 and SB2000 could be used as a viable control treatment of mussel fouling in Spanish irrigation systems.

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