1998

Microencapsulation As A Potential Control Technique Against Sabellid Worms In Abalone Culture

Jeffrey D. Shields
Virginia Institute of Marine Science

MA Buchal

CS Friedman

Journal of Shellfish Research, Vol. 17, No. 1, 79–83, 1998.

MICROENCAPSULATION AS A POTENTIAL CONTROL TECHNIQUE AGAINST SABELLID WORMS IN ABALONE CULTURE

JEFFREY D. SHIELDS,[1] MICHAEL A. BUCHAL,[2] AND CAROLYN S. FRIEDMAN[3]

[1]*Virginia Institute of Marine Science*
The College of William and Mary
Gloucester Point, VA 23062
[2]*P.O. Box 1335*
Bodega Bay, CA 94923
[3]*California Fish & Game*
Shellfish Pathology Lab
Bodega Marine Laboratory
Bodega Bay, CA 94923

ABSTRACT We have developed a novel application for lipid-walled microcapsules (LWMs) in the potential control of sabellid infestations in abalone aquaculture. The use of LWMs takes advantage of the filter-feeding nature of the worms, versus the herbivory of the host abalone. Initial observations indicated that the pest was capable of feeding on particles ranging from 3–30 μm in size. Lipid-walled microcapsules were prepared using different combinations of lipids (tristearin, tripalmitin, and fish oil) to encapsulate water-based solutions. Feeding experiments using worm-infested shells indicated that in a relatively short time (30–60 min) most of the worms (80–95%) fed on the LWMs and that large numbers of LWMs were ingested. Fecal pellets containing LWMs were observed in the rectums of worms within 15–30 min. Feeding efficiency was examined using different concentrations of LWMs. The sabellid worm was an efficient feeder. At low particle densities (2.6×10^4 particles/mL), 66.7% of the worms had eaten modest levels of LWMs. An asymptote in particle density in relation to feeding occurred at 2.6×10^5 particles/mL, with 83% of the worms feeding on large numbers of particles. In separate observations, LWMs composed of tripalmitin and fish oil were observed in various stages of digestion in the stomach, rectum, and fecal pellets of the worms. Microcapsules were also observed in the digestive tract of mud worms, *Polydora* spp. that were also inhabiting abalone shell. The utility of LWMs for delivery of toxins to the sabellid pest holds much promise in ridding the industry of this nuisance species.

KEY WORDS: Microcapsules, abalone, parasite, pest, sabellid, liposomes

INTRODUCTION

An introduced sabellid polychaete has become a serious pest of abalone (*Haliotis* spp.) in aquaculture facilities in California, USA. The worm causes shell deformations, stunting of young abalone in growout conditions, and detracts from the aesthetics of the live animals for export markets (Oakes and Fields 1996). The hermaphroditic nature of the worm, coupled with its short generation time, and rampant population growth, makes it a serious problem for the sale of contaminated seed stock, and parlays into a threat of introductions into foreign facilities, and habitats (Culver and Kuris in press). At present, two treatments have been used with little success in the control of infestations: lower water temperatures with resultant decreases in abalone production, and hot wax dips with considerable losses to stock (Oakes et al. 1995, Oakes and Fields 1996). Hot water treatment may, however, prove useful for treating the warm water species of abalone, *H. corrugata,* and *H. fulgens* (Leighton in press). Culturists currently harvest infested stock at suboptimal sizes, and retail infested product at low prices as an attempt to rid their facilities of the sabellid, and recoup losses (B. Hullbrock, pers. comm.). Aquaria are then disinfected with freshwater rinses and baths.

The sabellid worm has recently been found on native gastropods adjacent to certain aquaculture facilities (Culver and Kuris in press). The escape of the worm, and its potential for significant negative impact on native species, has led to the suggestion that the industry destroy all contaminated stocks, and sterilize infested facilities. This action may have dire consequences to the developing aquaculture industry. A control method is, therefore, required for immediate implementation against the worm.

A nondestructive treatment to eradicate the sabellid worms is a high priority to the nascent aquaculture industry. We have investigated a lipid encapsulation technique and a rapid assay that have great promise for use against the sabellid aquaculture pest. Our primary objective was to experimentally evaluate whether worms would feed on the LWMs. The secondary objective was to undertake efficiency trials to determine the relative number of LWMs eaten at different concentrations.

METHODS

Lipid-walled Microcapsule (LWM) Production

The method of Langdon (1983) was used to prepare LWMs composed of tristearin, or tripalmitin and fish oil. In brief, lipids were melted at 80 to 90°C and combined with aqueous core materials water and stains in a ratio of 2.86:1 (grams lipid: mL core) (after Villamar and Langdon 1993, Buchal and Langdon, 1998). The ratio of lipid to fish oil (Omega 3 fish oil concentrate, Dale Alexander) varied as a percentage as 100% lipid, 80:20, and 60:40 (w:w). A primary emulsion was formed by sonicating (50%, continuous, 4–5 sec, Fisher 50 Sonic Dismembranator, Pittsburg, PA) the mixture. Polyvinyl alcohol (2%, w:v) was added (80–90°C) to the primary emulsion, and a secondary emulsion was formed by homogenization (100%, 20 sec, Tissue Tearor, Biospec Products). The resulting suspension of LWMs was immediately poured into ice-cold polyvinyl alcohol (100 mL) and solidified. The chilled

LWM suspension was collected on a glass fiber filter (Whatman 934-AH), washed with ice-cold distilled water, and filtered to a wet paste. The LWMs were refrigerated and stored as a wet paste.

Most LWMs were stained to saturation with Sudan III to aid observations of feeding and digestion (Buchal and Langdon 1998). Nile Blue Sulfate (0.1% NBS in distilled water) was used to examine fecal pellets of worms fed LWMs. The NBS counter-stained Sudan III-labeled lipids that had been converted into free fatty acids, hence, indicating digestion of the contents (George, 1952). FITC-labeled (Anderson and Mora 1995) or Congo Red (0.4% in water)-labeled yeast cells were also used to observe feeding activity, but only LWMs containing 100% tristearin were quantified in experiments.

Feeding Assessments

Darkfield microscopy was superior to brightfield microscopy for viewing the stained LWMs inside worms. Only LWMs composed of 100% tristearin were used for the feeding experiments described below. To assess feeding, a semiquantitative log index was used for counts of relative numbers of LWMs eaten by individual worms. Because the sabellid worms were translucent, the stained LWMs were quantified from different regions of the digestive tract. The log index consisted of four steps: 0, no LWMs present; 1, 1–10 LWMs present; 2, 11–100 LWMs present; 3, 100+ LWMs present. Particles were quantified from the esophagus, the post-esophagus, the stomach, and the rectum. All experiments were done in a clean, unfiltered seawater at room temperature.

To conserve the laboratory stock of heavily infested abalone, only fragments of shells were used in initial trials. Abalone were maintained at 16–18°C, and were fed kelp regularly. The shells were prepared by removing the abalone with a spatula, and washing the shell twice in clean seawater to remove mucus. The shells were then gently broken into pieces with a hammer and a die stamp. After 30 min, each piece was assessed for the number of active crowns. Large (100–500 mm^2), heavily infested pieces (showing at least 75–100 active crowns) were used in each replicate.

The Feeding Experiment consisted of exposing worms to approximately 1.6×10^6 particles/ml (0.2 gm of LWMs composed of 100% tristearin in 50 mL seawater) in small (150 mL) plastic beakers. The beakers were then placed on a shaker (130 rpm) and timed for 15, 30, 60, 120, and 240 min. A hemocytometer was used to estimate densities of LWMs through time. An additional trial consisted of replicates of six small (18–25 mm shell length) abalone placed in suspensions of LWMs for 240 min. At the appropriate time, shell fragments were removed from the beaker, rinsed in clean seawater, and placed in a fingerbowl of clean seawater. Live abalone were processed as described above. Shell fragments were gently broken into smaller pieces, and whole worms were collected in wellslides of clean seawater. The worms were then pipetted onto a microslide, covered with a slip, and examined with a compound microscope. Whole worms were easily collected and examined in this fashion. The experiment was replicated three times with 20 whole worms examined per treatment per replicate.

For the Feeding Efficiency Experiment, worms were exposed to different concentrations of LWMs. A suspension of approximately 2.6×10^6 particles/mL (0.2 gm LWM composed of 100% tristearin in 50 mL seawater) was used. Two serial dilutions were made using 5.0 mL of the initial solution (to make estimated densities of 2.6×10^5 particles/mL) in 45 mL seawater, and 5.0 mL of the first dilution (to make estimated densities of 2.6×10^4 particles/mL) in 45 mL seawater. Shells were handled as described above, and pieces were placed in containers with the above concentrations of LWMs. After 60 min, the fragments were removed and processed as described above. A hemocytometer was used to estimate the number of LWMs present per mL of seawater. Some small discrepancies in counts between dilutions occurred as a result of the adherence of particles to the pipetters. Note that the LWMs also adhered to the plastic containers. The Feeding Efficiency Experiment was replicated four times with 20 whole worms examined per treatment per replicate.

RESULTS

Worms avidly ingested heat-killed yeast preparations (4–6 μm) and LWMs (mean = 15 ± 6 μm; range, 3–30 μm). LWMs constructed entirely of tristearin (mp 72°C on reheating) were not digestible. Hence, they were excellent markers for studying feeding and retention time in the digestive tract. LWMs constructed of tripalmitin (mp 66°C) mixed with fish oil (80:20, and 60:40) were completely or partly digested by the worms; fecal pellets contained digested matter and partially digested LWMs that stained positively with Nile Blue Sulfate. (Fish oil was used to lower the melting point of the tripalmitin to improve digestion.) The contents of the LWMs (fluorochromes and various stains) made of tripalmitin and fish oil were frequently observed staining the lining of the digestive tract of the worms. Feeding experiments using LWMs made of tripalmitin and fish oil (80:20 and 60:40) were not quantified. These LWMs were ingested, but they were digested and processed too quickly to be useful in our ingestion studies.

The initial Feeding Experiment showed that 65% of the worms (n = 60) had ingested LWMs within 15 min of exposure (Fig. 1). By 60 min, 93% of the worms (n = 60) were actively feeding. After 240 min (4 h), 95% of the worms (n = 58) had fed. At 1.6×10^6 LWMs/mL, the asymptote in the percentage of worms feeding (95%) was reached between 60 and 120 min.

Worms actively processed LWMs through their digestive tract. Indeed, after 15 min, several worms (23.3%) had LWMs present in the rectum (Fig. 2 and 3). In general, though, LWMs were processed to the rectum more slowly. After 60 min, 65% of the worms had LWMs developing as fecal pellets in the rectum. After 240 min, 80% of the worms had large numbers of LWMs in the developing fecal pellet. Whereas the asymptote in the number of particles eaten was reached between 60 and 120 min, worms at later times (120 and 240 min) had continued to ingest large numbers of LWMs. Microcapsule densities in suspension were not significantly different through time (mean, $1.6 \pm 0.75 \times 10^6$ LWMs SD, n = 3 replicates/h, ANOVA).

Live abalone generated copious mucous strands that collected large quantities of LWMs. Changes in particle densities through time were not examined in the live abalone treatment. At the relatively high density of 1.6×10^6 particles/mL, the proportion of worms feeding, and the number of particles ingested in the live abalone treatment were not significantly different from those in the shell fragments (for 120 and 240 min) (Figure 4; Chi-square, d.f. = 5, n.s.; ANOVA, d.f. = 5,354, $p > 0.998$, Tukey's HSD).

The Feeding Efficiency Experiment indicated that the sabellid worms were efficient at ingesting LWMs (Fig. 5). At the lowest density examined in this study (2.6×10^4 LWMs/mL, a level barely quantifiable with a hemocytometer), 66.7% of the worms had ingested LWMs. The relative number of LWMs ingested was,

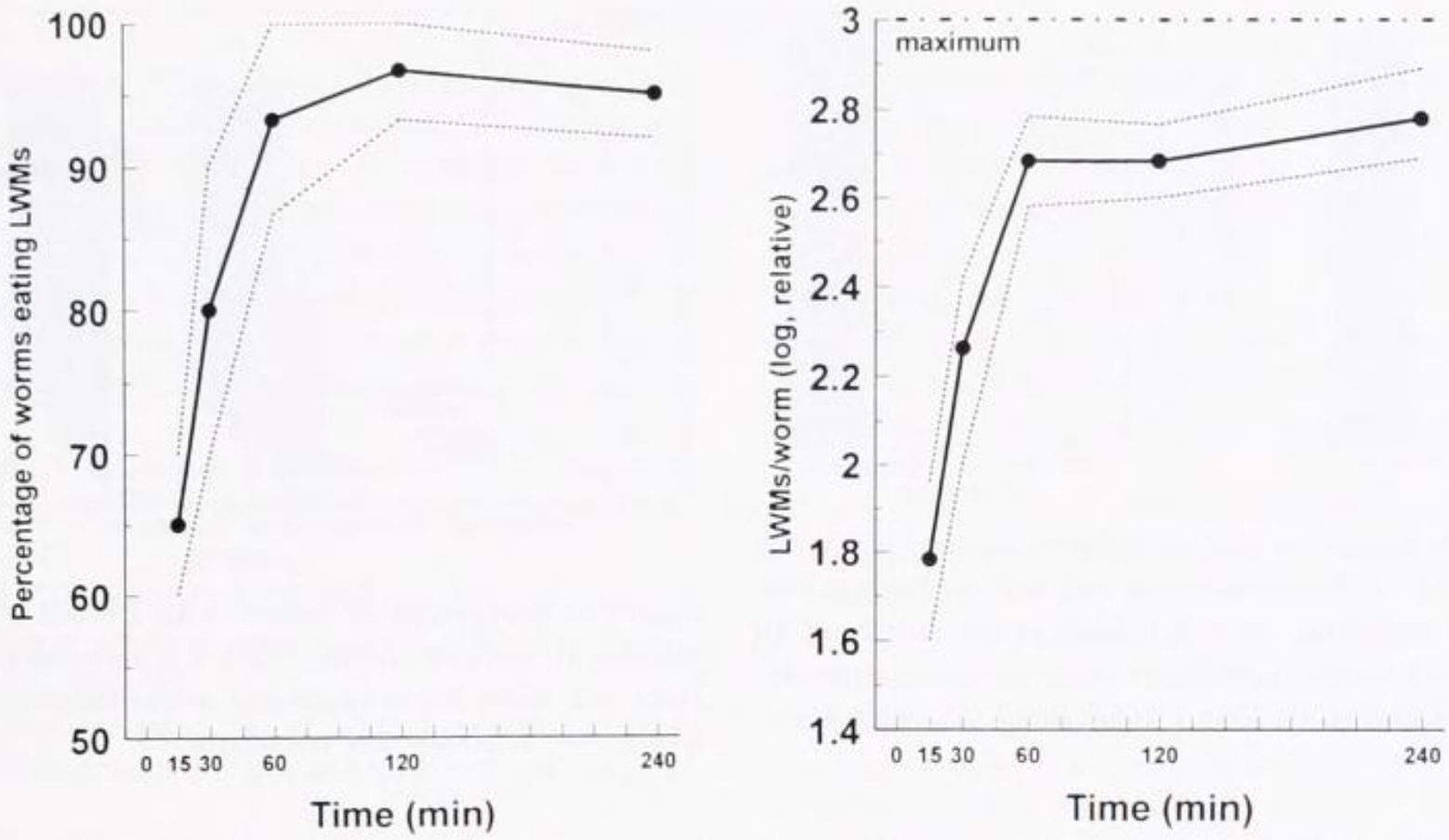

Figure 1. Percentage of worms feeding and relative number of LWMs ingested (log) over time (mean + SE). Data from three replicates, with independent assessment of 20 worms per replicate, per treatment. Worms were exposed to approximately 1.6 million particles/mL (0.2 gm LWMs in 50 mL seawater).

however, significantly smaller than that of the other densities (Figs. 6 and 7) (ANOVA, $p < 0.001$, d.f. = 2, 177, Tukey's HSD). The two higher concentrations showed no significant differences between percentage of worms feeding and relative number of particles ingested. There were, however, noticeable differences in the number of particles present in the different portions of the digestive tract (Fig. 6 and 7).

In the course of the different feeding experiments, several mud worms, *Polydora* spp. were observed feeding on LWMs. Mud worms had ingested a broad size range of microcapsules (mean = 29 ± 19 SD μm; range, 5–80 μm). LWMs made of tripalmitin and fish oil were observed breaking down into an oily bolus in the stomachs of several worms.

DISCUSSION

The sabellid worm has been a pest in abalone culture for at least 10 years (Oakes and Fields 1996). It does not occur as a native in coastal California. Indeed, it is a native of South Africa, and was presumably imported on infested shells of *Haliotis midae*, the South African abalone (Culver and Kuris in press). Unfortunately, the organism has yet to be described; hence, it remains unnamed. At present, there have been no recommended treatments against the worm. Various control strategies have been attempted including hot wax dips, and cold water culture (Oakes et al. 1995). Although both are moderately effective, they negatively affect production through mortality or slowed growth. Hot water treatment has shown promising results in killing the sabellid pest on warm water abalone (Leighton, in press). Unfortunately, there is no prescribed control of the worm for the red abalone, *H. rufescens*, other than destruction of contaminated stock.

Our data show that microencapsulated LWMs may be an excellent delivery device for toxins or chemical poisons to the pest. The sabellid worms ingested large quantities of LWMs over short time periods; and digested LWMs were present in the feces. LWMs or other microcapsules have several advantages over standard applications of toxicants. Primarily, the use of LWMs takes advantage of the filter-feeding nature of the worms, and should significantly reduce, if not nullify, chemical exposure to the herbivorous abalone. In addition, applications of LWMs may be re-

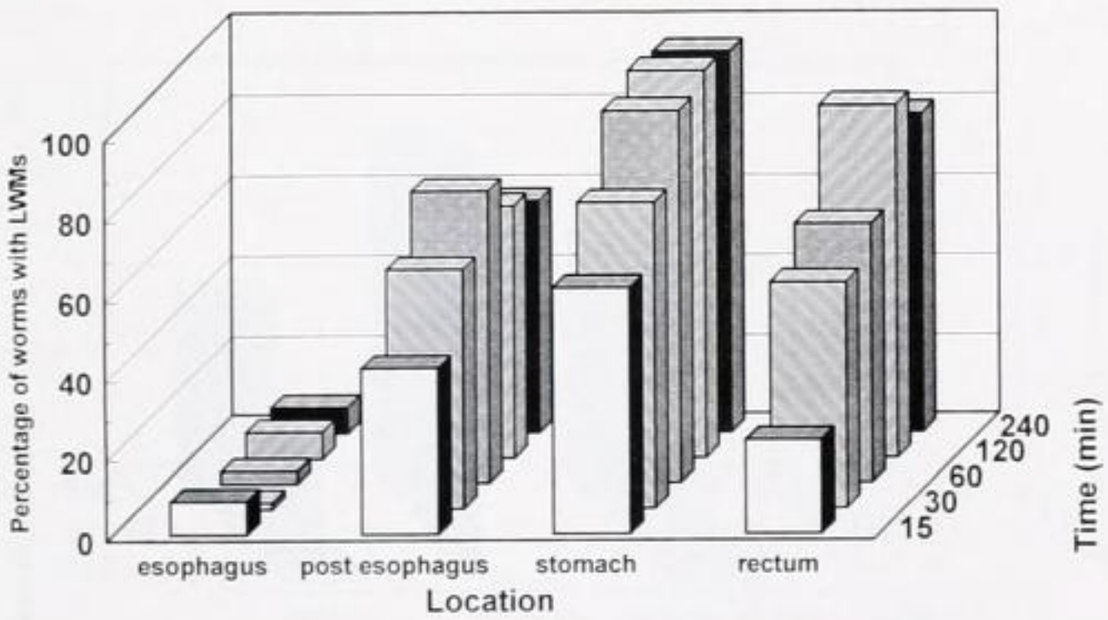

Figure 2. Percentage of worms and location of capsules in the digestive tract over time (means, error bars not shown). Data are from three replicates with independent assessment of 20 worms per replicate per treatment. Worms were exposed to approximately 1.6 million particles/mL (0.2 gms in 50 mL seawater).

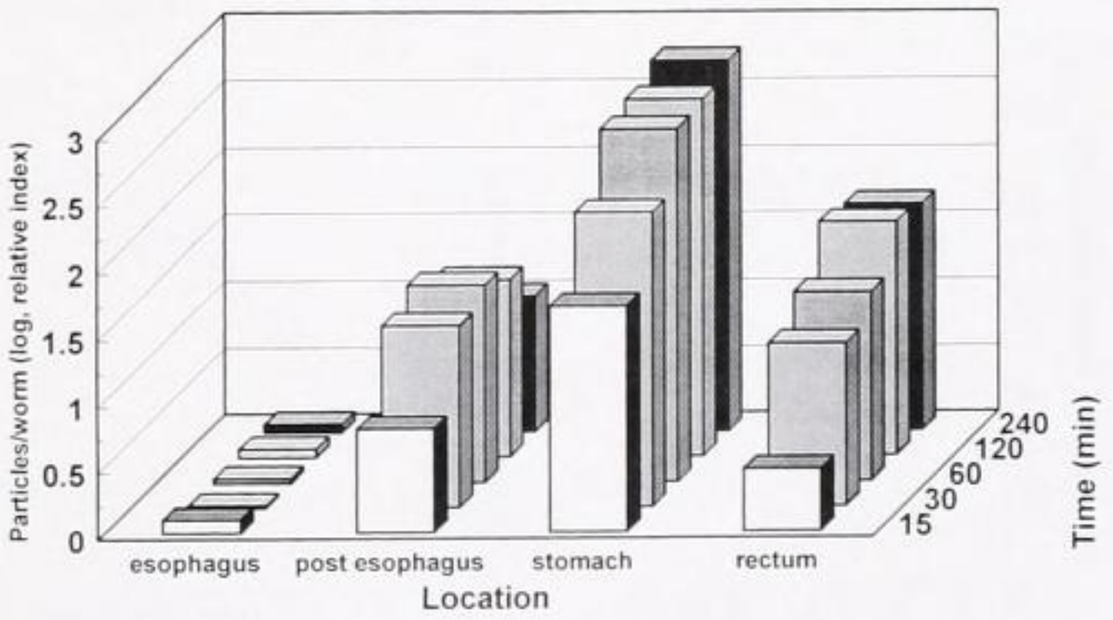

Figure 3. Relative number of LWMs per worm and their location in the digestive tract over time (means, error bars not shown). Data are from three replicates with independent assessment of 20 worms per replicate per treatment. Worms were exposed to approximately 1.6 million particles/mL (0.2 gms LWM in 50 mL seawater).

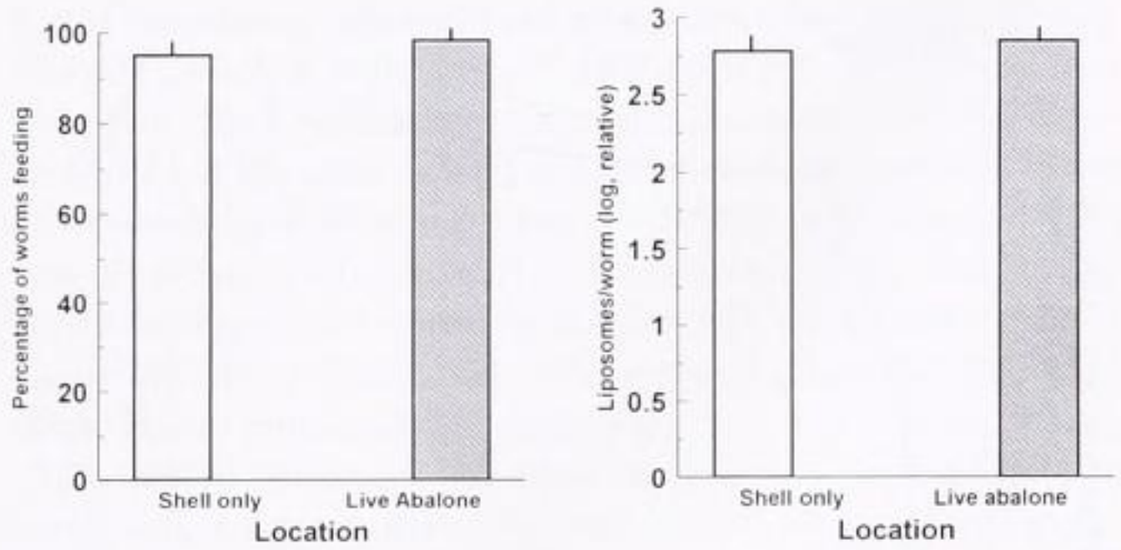

Figure 4. Percentage of worms feeding and relative number of LWMs ingested (log) (mean + SE) in treatments with and without live abalone (4 h). Data from three replicates, with independent assessment of 20 worms per replicate, per treatment. Animals were exposed to approximately 1.6 million particles/mL (0.2 gm LWMs in 50 mL seawater).

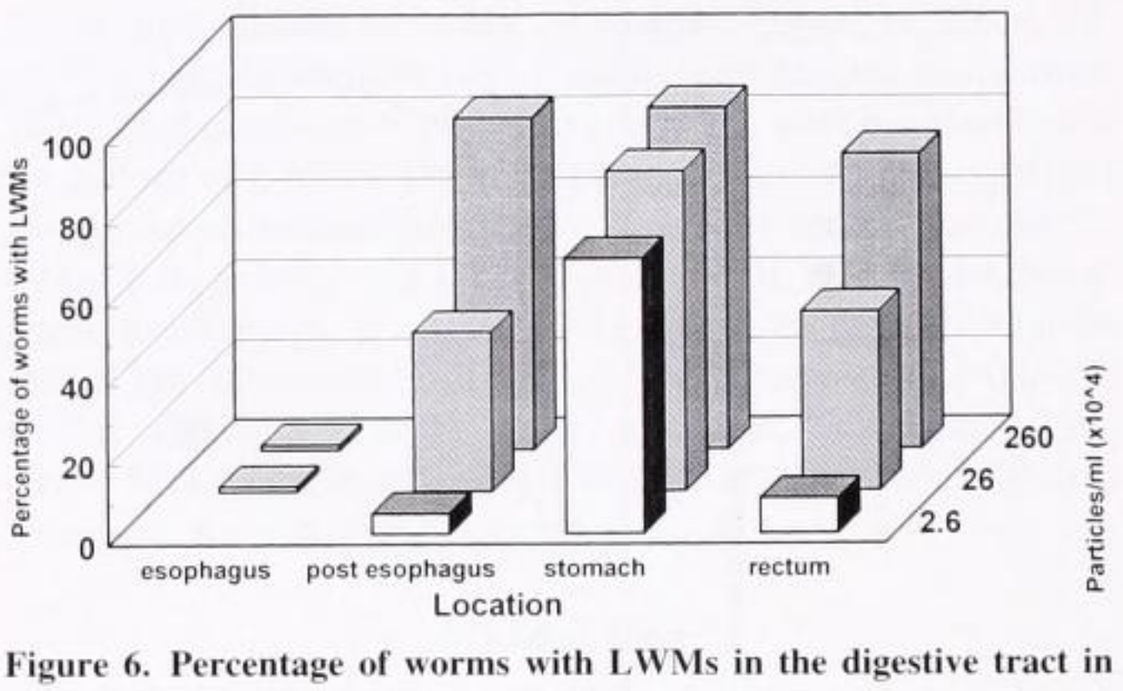

Figure 6. Percentage of worms with LWMs in the digestive tract in relation to particle density after 1 h (means, error bars not shown). Data are from three replicates with independent assessment of 20 worms per replicate per treatment.

moved from large raceways via large, relatively inexpensive, activated charcoal filters.

Microcapsules have been examined for use in the culture of several marine invertebrates. Most notably, various formulations have been investigated as a means to add nutrient supplements to artificial feeds for crustaceans (Jones 1974; Jones et al. 1979, 1987; Kanasawa et al. 1982; Levine et al. 1983), and bivalves (e.g., Gabbot et al. 1975; Chu et al. 1982, 1987; Kreeger and Landon 1993, 1994). Delivery of antibiotics has also been examined (Touraki et al. 1995; Langdon and Buchel, 1998). However, retention of low MW, water-soluble core materials has been an ongoing issue (Gabbott et al. 1975; Chu et al. 1987; Lopez-Alvarado et al. 1994). Indeed, in preliminary efficacy trials with encapsulated copper sulfate, sabellid mortalities were not significantly different between experimental and copper sulfate controls, indicating significant leakage of the core toxin (Shields et al. unpubl.). Hence, the technique requires some refinement.

Because LWMs can be constructed with a fluorescent core or other markers, they may be of considerable utility to ingestion and assimilation studies of invertebrates. For example, *Nereis diversicolor* has a high assimilation rate of algae at 1700 cells/mL, but has a low assimilation rate at higher cell densities (Vedel and Riisgaaard 1993). *Sabella penicillus* has a high ingestion (filtration) rate of 400 cells/mL (Riisgaard and Ivarsson 1990). With both worms, the gut capacity was probably exceeded at the higher cell densities, thus lowering the filtration rate (Vedel and Riisgard 1993). Our finding microcapsules in the digestive tract of *Polydora* spp. indicates that the LWMs may have broad applications to other polychaetes. Nondigestible LWMs could be used to investigate gut capacity and feeding, whereas digestible LWMs could be used to examine assimilation.

In conclusion, encapsulation of materials for use against aquaculture pests warrants further study. Although initial efficacy trials indicate problems with leakage of small molecules (i.e., CuSO4) (Shields et al. unpubl.), improvements should resolve the problem. Improvements will include the use of less toxic reagents, the use of large molecular weight, or lipid soluble compounds such as ivermectin, and the development of formulations to reduce leakage, yet retain digestibility. The utility of LWMs for delivery of toxins to the sabellid pest holds much promise in ridding the industry of this nuisance species.

ACKNOWLEDGMENTS

We thank Janet Moore and Thea Hibbard-Robbins for their technical assistance. Carolyn Vines, Brian Mulvey, Drs. Kristen Arkush, Mark Snyder, Ernie Chang, Robert Armstrong, and Gary Cherr graciously provided equipment, and/or critical discussion. This work was supported in part by a Saltonstall-Kennedy Grant NA76FDO052 to JDS and CSF.

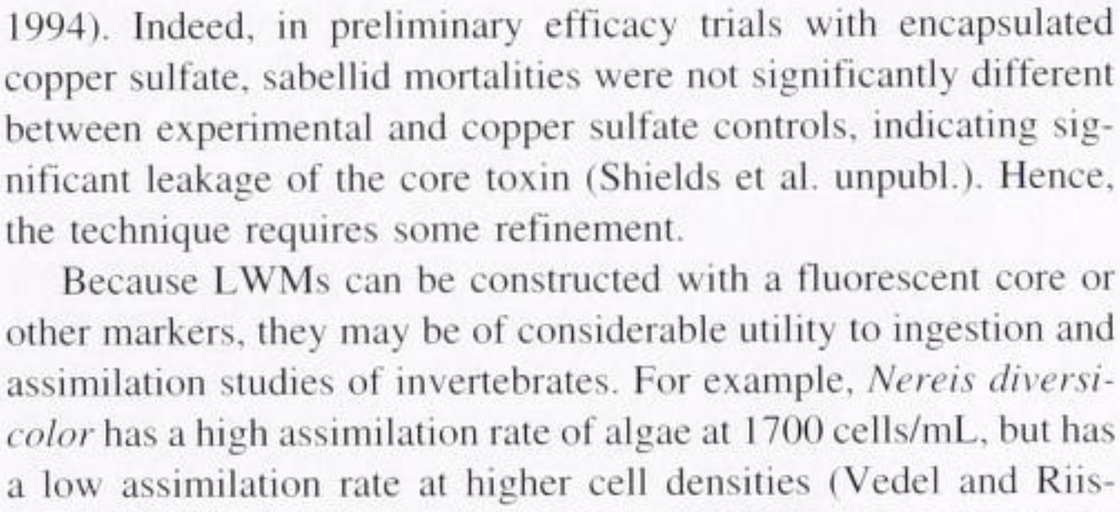

Figure 5. Percentage of worms feeding and relative number of particles eaten (log) after 1 h at different densities of LWMs (mean + SE). Data are from 4 replicates with independent assessment of 20 worms per replicate per treatment.

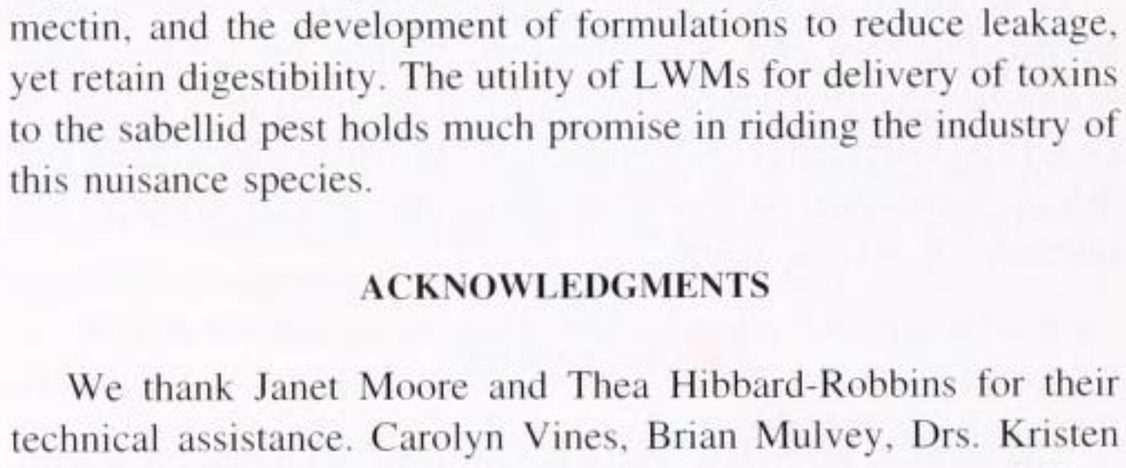

Figure 7. Relative number of LWMs in the digestive tract in relation to particle density after 1 h (means, error bars not shown). Data are from three replicates with independent assessment of 20 worms per replicate per treatment.

LITERATURE CITED

Anderson, R. S. & L. M. Mora. 1995. Phagocytosis: a microtiter plate assay. pp. 109–112. *In* J. S. Stolen, T. C. Fletcher, S. A. Smith, J. T. Zelikoff, S. L. Kaattari, R. S. Anderson, K. Soderhall, and B. A. Weeks-Perkins (eds.). Techniques in Fish Immunology 4: Immunology and Pathology of Aquatic Animals, SOS Publications, Fair Haven, NJ.

Buchal, M. A. & C. J. Langdon. 1998. Evaluation of lipid spray beads for the delivery of water-soluble materials to a marine suspension feeder, the Manila clam *Tapes philipinarum* (Deshayes, 1853). *J. Aquaculture Nutrition* (in press).

Chu, F. -L., K. L. Webb, D. Hepworth & B. B. Casey. 1987. Metamorphosis of larvae of *Crassostrea virginica* fed microencapsulated diets. *Aquaculture* 64:185–187.

Chu, F. -L., K. L. Webb, D. Hepworth & M. Roberts. 1982. The acceptability and digestibility of microcapsules by larvae of *Crassostrea virginica*. *J. Shellfish Res.* 2:29–34.

Culver, C. C. & A. M. Kuris. In press. An introduced polychaete pest of California cultured abalone: current status and environmental concerns. *J. Shellfish Res.*

Gabbott, P. A., D. A. Jones & D. H. Nichols. 1975. Studies on the design and acceptability of micro-encapsulated diets for marine particle feeders. 10th European Symposium on Marine Biology, Ostend, Belgium pp. 127–141.

George, W. C. 1952. The digestion and absorption of fat in lamellibranchs. *Biol. Bull.* 102:118–127.

Jones, D. A. 1974. Microcapsules as artificial food particles for aquatic filter feeders. *Nature* 247:233–235.

Jones, D. A., A. Kanazawa, & K. Ono. 1979. Studies on the nutritional requirements of the larval stages of *Penaeus japonicus* using microencapsulated diets. *Mar. Biol.* 54:261–267.

Jones, D. A., D. Kurmaly & A. Arshard. 1987. Penaeid shrimp hatchery trials using microencapsulated diets. *Aquaculture* 64:133–146.

Kanazawa, A., S. Teshima, H. Sasada & S. Rahman. 1982. Culture of the prawn larvae with micro-particulate diets. *Bull. Japan. Soc. Sci. Fish.* 48:195–199.

Kreeger, D. A. & C. J. Langdon. 1993. Effect of dietary protein content on growth of juvenile mussels, *Mytilus trossulus* (Gould, 1850). *Biol. Bull.* 185:123–139.

Kreeger, D. A. & C. J. Landon. 1994. Digestion and assimilation of protein by *Mytilus trossulus* (Bivalvia: Mollusca) fed mixed carbohydrates/protein microcapsules. *Mar. Biol.* 118:479–488.

Langdon, C. J. 1983. New techniques and their application to studies of bivalve nutrition. pp. 305–320. *In* G. D. Pruder, C. J. Langdon, and D. E. Conklin (eds.). Proc. 2d Intl. Conf. Aqua. Nutr. Louisiana State University, Baton Rouge, LA.

Langdon, C. J. & M. A. Buchel 1998. Comparison of lipid-walled microcapsules and lipid spray beads for the delivery of water-soluble, low molecular weight materials to aquatic animals. *J. Aquaculture Nutrition* (in press).

Leighton, D. L. In press. Control of sabellid infestation in green and pink abalones, *Haliotis fulgens* and *H. corrugata*, by exposure to elevated water temperatures. *J. Shellfish Res.*

Levine, D. M., S. D. Sulkin & L. V. Heukelem. 1983. The design and development of microencapsulated diets for the study of nutritional requirements of brachyuran crab larvae. *In* J. R. Berg (ed.). Culture of marine invertebrates selected readings. pp. 193–204. Stroudsberg, Hutchinson Ross Publishing Co. PA.

Lopez-Alvarado, J., C. J. Langdon, S. Teshima, A. Kanazawa. 1994. Effects of coating and encapsulation of crystalline amino acids on leaching in larval feeds. *Aquaculture* 122:335–346.

Oakes, F. R., R. C. Fields. 1996. Infestation of *Haliotis rufescens* shells by a sabellid polychaete. *Aquaculture* 140:139–143.

Oakes, F. R., R. C. Fields, P. F. Arthur, G. A. Trevelyan. 1995. The use of wax to control the shell parasites of red abalone, *Haliotis rufescens*. *J. Shellfish Res.* 14:273.

Riisgaard, H. U. & N. M. Ivarsson. 1990. The crown-filament pump of the suspension-feeding polychaete *Sabella penicillus:* Filtration, effects of temperature, and energy cost. *Mar. Ecol. Prog. Ser.* 62:249–257.

Touraki, M., P. Rigas, C. Kastritsis. 1995. Liposome mediated delivery of water soluble antibiotics to the larvae of aquatic animals. *Aquaculture* 136:1–10.

Vedel, A. & H. U. Riisgaard. 1993. Filter-feeding in the polychaete *Nereis diversicolor:* Growth and bioenergetics. *Mar. Ecol. Prog. Ser.* 100: 145–152.

Villamar, D. E. & C. J. Langdon. 1993. Delivery of dietary components to larval shrimp (*Penaeus vannamei*) by means of complex microcapsules. *Mar. Biol.* 115:635–642.