Remote setting and nursing trials of *C. virginica* in bouncing buckets

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ABSTRACT

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This study explored the possibility of using a new technology, called "bouncing buckets," for *Crassostrea virginica* larvae remote setting and juvenile nursing in coastal waters. Very low recruitment (< 1%) was quantified through direct deposit of eyed larvae into bucket long lines and downwellers on a floating platform. However, in terms of nursing capabilities, the daily mean growth of juvenile oysters with an initial size of 1,300 µm was very good (~ 120 µm*day^{-1}). Furthermore, the density of 4,000 larvae (of 1,000 µm) per bucket with a 700 µm bottom screen mesh seemed to be the most profitable approach. Lessons learned in the course of these experiments will be valuable for the process of remote larval setting and nursing in coastal waters or up-welling systems, using a recent technology that to date has not been used in Canadian Atlantic coastal waters.

RÉSUMÉ

R. Sonier, L.A. Comeau and L. Lanteigne. 2008. Remote setting and nursing trials of *C. virginica* in bouncing buckets. Can. Tech. Rep. Fish. Aquat. Sci. 2789 :vii + 15p.

L'objectif premier de cette étude fut d'explorer l'utilisation d'une méthode innovatrice appelée «bouncing buckets» pour de fins de télécaptage de larves et nurserie pour les juvéniles de l'huître *Crassostrea virginica*. Les données de recrutement récoltées furent très minime (< 1%) dans les « buckets » ainsi que dans les systèmes de «downwellers» installés sur une plateforme flottante. Toutefois, les résultats de croissances dans ce système utilisé comme nurserie sont très intéressants. La croissance journalière moyenne d'une huître d'une taille moyenne de 1300 µm fut d'environ 120 µm*jour $^{-1}$. De plus, le traitement utilisant une densité de 4000 juvéniles (de 1000 µm) par « bucket » muni d'un filet de 700µm semble être le traitement le plus efficace. Les essais lors de ces expériences nous ont permis d'étendre nos connaissances sur cette nouvelle technologie jamais encore utilisée au Canada Atlantique.

INTRODUCTION

On Canada's Atlantic coast, particularly in New Brunswick, American oyster (*Crassostrea virginica*) aquaculture relies almost entirely on the settlement of wild larvae for spat supply. However, this supply of oyster's seed is vulnerable, not only to broodstock declines, but also to regulatory transfer restrictions. These transfer restrictions can occur unexpectedly, because of closures of areas due to health issues (e.g., MSX) or invasive species spread (e.g., tunicates). Such transfer restrictions can have devastating effects on the aquaculture sector, especially if they are applied to reliable and productive spat collection areas.

In theory, hatcheries are an effective way of ensuring a reliable spat supply, even though their economic feasibility remains a major obstacle. Hatchery-made spat is typically produced in five steps: (1) broodstock conditioning, (2) spawning, (3) larval rearing, (4) fixation of larvae (i.e., spat setting), and (5) spat rearing in upwelling or downwelling nurseries. While, steps 1 to 3 are mandatory hatchery procedures; steps 4 and 5, can be conducted in coastal waters, using a procedure known as "remote setting" (Roland and Broadly 1990).

The general procedure for remote setting is straightforward and uses simple techniques. Hatchery-raised "eyed" larvae (pediveliger), which are ready to set, are transported in a moist container to the grower. On arrival, these larvae are introduced into large, insulated tanks containing filtered (50-100 μm), aerated, and heated (20-27°C) seawater, and various cultch material, such as a hard fixation substrate, like shell chips, French pipes, Vexar, or Chinaman hats. The larvae metamorphoses and sets on the cultch over a relatively short period of time (1-7 days). Cultch samples are examined after spat settlement to determine the success of the settling method. Thereafter, cultch with the newly settled spat is transferred from holding tanks to a nursery area in open coastal waters. Remote setting minimizes the capital outlay at the hatchery, allowing operations to focus on large-scale production of ready-to-set larvae. It also gives individual farmers the ability to produce oyster seed independently of natural conditions and/or transfer restrictions.

Remote setting of the Pacific oyster (*Crassostrea gigas*) on the west coast of Canada and the United States has been extensively researched since the 1970s, and is currently conducted on a commercial scale. *C. virginica*, on the other hand, has received considerably less attention (Supan and Wilson, 1994). To the best of our knowledge, a single reported investigation has been conducted on the possibility of applying the remote setting technique to *C. virginica* in eastern Canada (Méthé, 1996). Results were encouraging and clearly demonstrated that the oyster industry in the southern Gulf of St. Lawrence could benefit from the use of that technique. The reason is that natural spatfall at this northernmost range of the species occurs in mid-summer, leaving little time for spat growth before the onset of winter. By remotely setting the larvae in early spring, Méthé (1996) considerably extended the spat grow-out period and obtained large seed oysters (96-428% larger, compared with naturally set seed) by the fall of the same year.

The oyster company, La Maison BeauSoleil, was interested in pursuing research on the topic, using an innovative technology, known as "bouncing buckets." These buckets were developed in British Columbia, and have been used in other countries, namely Brazil. To our knowledge, the buckets had only been used as a nursery system to rear *C. gigas* spat. Here, the intent was to determine whether the buckets were suitable for both the remote setting (fixation of larvae) and nursing of *C. virginica*.

However, for remote setting to be feasible on a commercial scale, it is necessary to make it advantageous financially. One option to achieve this consists of excluding the costs associated with the setting tanks, building, water heating, phytoplankton production, maintenance labour, etc. For this study we proposed that the eyed larvae could be transferred from the hatchery directly into buckets previously set at sea. Although the approach of a direct transfer from the hatchery into the field appears feasible, from a commercial perspective, it required formal testing of its technical applicability.

Thus, as a first scientific objective, the setting capacity of *C. virginica* pediveligers in field bouncing buckets was quantified. A second objective was to demonstrate that the newly settled spat contained in bouncing buckets could grow into large seeds by the fall of the same year. Wild seeds collected on Chinese hats are typically small (< 25mm). In the autumn, they are first transferred into *small* mesh Vexar

bags for continued growth, and the following year are placed into *large* mesh Vexar bags. A remote setting approach could allow the proponent to extend the summer growth period, by advancing the larvae setting date from mid to late July (typical in N.B.) to early June. Méthé (1996) showed that such an extended summer growth period could produce large (> 25 mm) oyster seeds by the fall of the same year. The proponent of the present project, La Maison BeauSoleil, was motivated by the idea that remotely settled seeds could be transferred from bouncing buckets directly into *large* mesh bags, skipping the intermediate step of placing the seed into *small* mesh bags. Thus, as a final objective, we sought to assess the daily growth of different densities of early juvenile oysters in bouncing buckets with different screen sizes.

METHODOLOGY

Larvae source

C. virginica eyed larvae (pediveligers) were obtained from hatchery facilities of the Aquarium and Marine Center in Shippagan (N.B.) where they are regularly produced in large numbers as part of ongoing research projects. The Aquarium regularly produces pediveliger larvae of 300 µm in length, precisely the size that successfully settled remotely in Méthé`s (1996) trials. During the winter of 2006, the pediveliger larvae produced at the Shippagan Aquarium showed poor metamorphosis and settlement rates within the Aquarium; this was resolved in subsequent larvae production. Broodstock at is collected from the Caraquet (N.B.) area and was conditioned to spawn.

Mature eggs were fertilized at a water temperature of approximately 22°C. The larvae were then grown at the Aquarium until most (> 70 %) reached a diameter of 15-17 µm and developed an eyespot. Other larval quality characteristics, such as time elapsed to swim up and the color of the gut, were recorded before shipment of the larvae to the remote site. For this project, we acquired Aquarium pediveliger larvae and quantified their settlement rates at remote locations. In May 2006, a total of over 3M larvae were transferred according to the schedule in Table 1.

Remote setting

Bouncing buckets (Fig. 1) are essentially low-cost upwellers that can be attached to longlines at culture sites. A screened opening on the bottom of the bucket allows the vertical circulation of water. The top portion is also open; a Styrofoam ring keeps this portion above sea level to allow air to circulate freely. Aeration is important to equalize the water temperature, distribute the larvae uniformly around the tank, and to produce an even set. The wave action bounces the bucket, in a manner that prevents sedimentation and compacting of oysters and cultch material on the bottom mesh screen.

Two variables were manipulated in an attempt to optimize the larvae settling rates. The first was the setting material, which presumably has a major influence on setting rates. We investigated two micro-cultch sizes of 200 and 400 µm which consisted of oyster shells, crushed to microscopic size and aged (6 months). Cultch was conditioned by pre-exposing it to seawater; as a result it becomes covered with a thin film of bacteria that attracts larvae at the time of settlement.

The second variable relates to the downwelling treatment in tanks set up on a floating platform, and more specifically to identifying the minimum treatment required for successful settlement of larvae in the bouncing buckets. Rather than placing oysters on longlines, they were held on a floating platform, 3 tanks were used to contain 9 bouncing buckets. Each of the 3 tanks held buckets with 150-µm screen mesh bottoms and 400-µm micro-cultch previously soaked for 24 h in the receiving tanks. Each bucket was submitted to a typical hatchery procedure, such as the downwelling current (Fig. 2a, 2b). Approximately 35,000 pediveligers were introduced into each bucket. At the end of the 5-day setting period, half of the experimental buckets were deployed at the culture site, while the other half were brought to the laboratory for spat count and measurements. While the bulk of the experiment was conducted at the Chiasson Office site, two additional sites, Néguac and Aldouane, were also included to determine whether there was any site-effect on setting rates. This portion of the experiment required the deployment of 50 buckets (10 per setting material category) at each of the two sites. We investigated three scenarios: a downwelling current over 5 consecutive days, a downwelling current for 2 h every day for 5 consecutive days (with buckets transferred to

longlines during non-treatment periods), and no downwelling treatment (direct transfer of eyed-larvae from the hatchery to buckets attached to longlines).

We also took the experiment a step further with the aim of performing remote setting in bouncing buckets using eyed larvae. Based on the assumption that they may benefit from natural food sources and the environment, pediveliger larvae were poured into buckets (Fig. 3a, 3b) that had already been soaked at the experimental sites. Groups of bouncing buckets each contained micro-cultch of varying quantity and size. Additionally, eyed larvae densities were varied for each treatment (larvae/bucket). This in-situ experiment consisted of 30 bouncing buckets that were not submitted to the above controlled environment; instead, they were attached to a longline at the culture site one week prior to the arrival of the hatchery larvae; 35,000 swimming larvae were added to each of the 30 buckets at the beginning of the 5-day trial described above.

One third of the buckets was examined for spat count after 5 days, and another third after 10 days; the remaining 10 buckets were left on the longline for long-term monitoring of survival and growth. Following the successful completion of the initial trial, we proceeded with a full experiment to assess the value of bouncing buckets as a setting environment for *C. virginica*. The experiment was conducted at the Chiasson Office site.

The total number of bouncing buckets that were manipulated during the course of this project provides an appreciation of the scope of the experiment. A total of 382 buckets appeared to be a reasonable number for statistically assessing the effect of two materials (× 3 sites) and two downwelling regimes (× 1 site) on the setting success of *C. virginica* within bouncing buckets. The total count also includes an assessment of the nursing capability of the buckets.

Nursery trials

The bouncing buckets technique has proven to be effective in the Pacific region for the nursery stage of oysters. We tested feasibility of using this technique as nursery in Canada's most northern populations of *C. virginica*. For this experiment, oyster juveniles of 800 µm, 1,000 µm, 1,200 µm, and 2,200µm were used for these trials, using

different densities (13,450, 4,000, 7,600 and 4,400, respectively) and screen sizes (700 or 350 μm).

RESULTS

Remote setting

Mortality was nearly 100% in each bucket for all treatments. Survivability of eyed larvae and recruitment numbers in bouncing buckets could have been jeopardised by predation, failure to settle, and lack of suitable substrates for settlement. Certain combinations of micro-cultch quantity and size and larvae density seemed to be more effective than others, based on qualitative observations. Additional fine-tuning of the bouncing buckets technique is required before further trials of remote setting are conducted. Of the 3,035,333 larvae introduced into buckets, only 86 larvae successfully settled onto the cultch material. Results were similar for buckets attached to longlines (in open water) and buckets contained in the downwelling system at a remote site (Table 2). At the Shippagan aquarium, however, larvae recruitment was successfully conducted using the same generation of larvae. This suggests that the failure of recruitment in the field was linked to the buckets (micro-currents, physical barrier) and/or the environment (physico-chemical parameters, predation), rather than larval quality (health).

Following the field outcome, we attempted the procedure in a more controlled environment. In January 2007, larvae were introduced into smaller-scale buckets at the Shippagan Aquarium. Unfortunately, the outcome was also negative because of massive larvae mortality at the hatchery during the winter of 2007.

Nursery trials

Conducting the nursery stage in bouncing buckets has proven effective on the Pacific coast of Canada, and our results suggest interesting growth data for juvenile oysters at different densities. Buckets containing 7,600 oysters of 1.2 mm in a standard bouncing bucket with a 700 μm mesh had the most promising daily growth (M = 0.1216 mm; SD = 0.0213 mm). Buckets containing 13,450 oysters of 0.8 mm in a standard bouncing bucket with a 350 μm mesh had the most promising daily growth (M = 0.1028 mm; SD = 0.0185 mm). For a total of 100 days, the treatment with the best daily

growth was the one containing 4,000 oysters of 1 mm in a standard bouncing bucket with a 700 μm mesh (M = 0.1329 mm; SD = 0.0299 mm). The overall mean growth for all treatments averaged ~0.12 $mm*d^{-1}$ and independent of oyster density in the buckets (Table 3).

DISCUSSION

Méthé (1996) showed a settling success rate between 6% and 60%, based on a controlled setting environment (tanks); an average settlement above 20% would have been considered a success for bouncing buckets in open water. Unfortunately, our remote setting trials in the field were not as effective, mostly due to factors such as micro-cultch conditioning and biotic factors, such as predation (e.g., by *Crangon sp*.) and natural mortality. The top opening of the bucket is subject to the action of small breaking waves, which gives predators an entry point to access oyster larvae. Epifauna fouling, including mussel spat (*M. edulis*) both on the mesh screen and within the bucket, was observed. This type of fouling occurred in a very short period of time, suggesting that there was reduced water exchange throughout the bucket during the experiment (Fig. 4a, 4b). The site of the remote setting is an important decision, with regard to parameters such as salinity. Oyster larvae also do not set well in water that is too fresh (e.g., under 10 ppt) (Supan, 1994). Proximity to wharfs and docks should be a consideration since bilge water that often contains oil and fuel that can contaminate the area.

We conducted trials with the conditioning time of the micro-cultch on a smaller scale before transferring the larvae for successful recruitment in the lab. The size of the micro-cultch size (200 and 400 μm) was another parameter examined. Unfortunately, massive larvae mortality occurred in the hatchery, due to fungal contamination of the water and cultch. Therefore no conclusion can be reached on that element. The settlement of marine larvae is influenced by a wide range of physical and biological factors. It is still poorly understood how the nature of the substrate and the biofilm interact in regulating settlement patterns of invertebrate larvae (Faimali *et al.* 2004). Micro-cultch acclimation time in the natural environment before remote-setting trials could have an effect on recruitment success. Bio-films on cultch material could have an important impact on oyster larval recruitment on the substrate.

Some authors have accomplished remote setting using aged oyster cultches similar to ours (Gilcrist *et al.* 2005). Zhao *et al.* (2003) and Taylor *et al.* (1998) demonstrated an important inductive aspect of the biofilm on goldlip pearl oyster (*Pinctada maxima*) larval settlement. The best recruitment occurred on rough surfaces with a biofilm, compared with a smooth surface with no biofilm The same conclusion was reached recently with a different species of pearl oyster (*Pinctada martensii*) (Su *et al.* 2007). The inductive effect appeared to be closely associated with the bacteria in the biofilm (Hamer *et al.* 2001). Further, based on recent experiments by Avendano-Herrera and Riquelme (2006), a biofilm composed of bacteria and diatoms, such as *Navicula veneta,* improved the settling of marine larval shellfish. Bourne *et al.* (2004) confirmed the presence of bacteria in the biofilm, by electron microscopy. They observed that the clear surface was initially occupied by a biofilm and by species not using that biofilm as a positive cue. This film then became a cue for other invertebrate species, and changes in the biofilm modify further settlement patterns (Keough, 1998). For the tropical oyster, *Crassostrea iredalei*, larvae recruited in the hatchery seemed to be attracted to substrates pre-soaked in tissue extracted from several bivalve species (Devakie and Ali, 2002).

The optimum quantity of micro-cultch per bucket is an important question in term of the relationship between a suitable substrate to settle on and food availability. As anticipated, the buckets with the lowest density of oysters used in the nursery trials had the best daily growth. A lower density gives a better water flow in the bucket, minimising obstruction of the underlying screen with silt and epifauna. Additionally, competition for food is reduced.

CONCLUSION

Our results suggest that there are several issues which must still be addressed for remote setting to be considered successful for oyster recruitment. Predation of larvae, micro-cultch conditioning, larvae densities, screen size, and micro-cultch size and quantity need to be studied further. Promising preliminary results of bouncing buckets as potential oyster nurseries were demonstrated. Lessons learned in the course of conducting these experiments are valuable to gain a better understanding of the challenge that remain to ensure practical and economic feasibility of this technique.

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Table 1: Larvae transfer schedule.

Transfer date (2006)	Larval stage	Approximate number of larvae
June 9th	Eyed	600 000
June 12th	Eyed	80 000
June 14th	Eyed	400 000
June 17th	Eyed	500 000
June 19th	Eyed	600 000
July 5th	14 days	500 000
July 14th	Eyed	355 333
July 17th	Eyed	84 000
Total		**3 119 333**

Table 2: Mean number of fixed larvae per bucket.

Date (2006)	Mean No. of settled oysters per bucket placed in field downwelling system	in open water
June 9th	0	0
June 12th	12	0
June 14th	0	0
June 17th	0	19
June 19th	0	35
July 5th	0	0
July 14th	10	0
July 17th	0	0

Table 3: Mean growth rates for the nursery component.

Oyster initial size (µm)	Bucket mesh size (µm)	Density (number of oysters per bucket)	Mean growth rate ($\mu m * d^{-1} \pm SD$)
1,000	700	4,000	132.9 ± 29.9
1,200	700	7,600	121.6 ± 21.3
800	350	13,450	102.8 ± 18.5

Figure 1: Bouncing buckets.

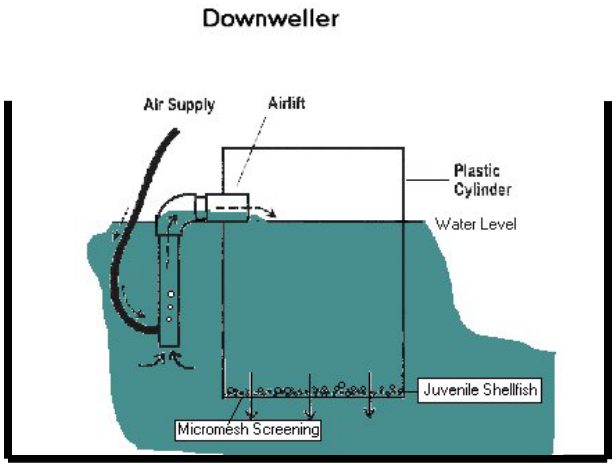

Figure 2a: Sketch of downwelling treatment to be applied to bouncing buckets.

Figure 2b: On-site downwelling system constructed on a floating platform, containing nine bouncing buckets.

Figure 3a: Longline of bouncing buckets.

Figure 3b: Eyed larvae being poured into buckets.

Figure 4a: Epifauna, mussels set and silt accumulation in a bucket.

Figure 4b: Clean new mesh (left) compared to epifauna and silt covered mesh (right).