Effects of substrate size and cleaning regime on growth and survival of captive-bred juvenile freshwater pearl mussels, *Margaritifera margaritifera* (Linnaeus, 1758)

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Abstract

The freshwater pearl mussel is critically endangered and most English populations are at risk of extinction unless conservation measures are implemented immediately. The study objectives were to test a culture system for rearing *Margaritifera margaritifera* in captivity, and to investigate the effects of substrate size (0.25-1 mm and 1-2 mm) and cleaning regime (weekly and monthly) on survival and growth. In total, 1207 and 518 juveniles were reared to 362 (12 months) and 758 days (25 months) respectively. After 362 days, survival was significantly higher in 1-2 mm substrate treatments cleaned monthly (55 ± 6%) and lowest in 0.25-1 mm substrate cleaned weekly (14 ± 3%). Growth was significantly higher in 1-2 mm substrates cleaned weekly (length = 1.15 ± 0.21 mm) and lowest in 0.25-1 mm substrates cleaned monthly (length = 0.83 ± 0.23 mm). Juveniles from most treatments did not display size-dependent over-winter survival, but a significant correlation was found between shell length and survival in the 0.25-1 mm weekly treatment. This low-maintenance system utilised features of previously described systems and growth and survival rates were comparable to, if not better than, other studies culturing *M. margaritifera.* The system could be scaled-up to rear significant numbers of juveniles in captivity.

Keywords: Margaritiferidae, captive rearing, low-maintenance system, mussel conservation

Introduction

Freshwater molluscs are among the most endangered invertebrates in the world (Machordom et al., 2003; Primack, 2006) and are disproportionately imperilled compared with other groups (Williams et al., 1993). Understandably the number of studies on freshwater bivalves has increased substantially over the past 30-50 years with the quickest growing subject area being ecology and conservation (Haag, 2012; Lopes-Lima et al., 2014). Unsustainable pressures such as habitat modification, unnatural silt and nutrient input, pressures on host fishes and deteriorating water quality are responsible for the decline of the freshwater pearl mussel, *Margaritifera margaritifera* (Linnaeus, 1758). This species declined by over 90% in the 20th Century (Bauer, 1988) prompting urgent conservation action from both Government agencies and NGO's to improve pearl mussel habitat and, in some countries, to begin captive rearing activities for populations particularly at risk. The type of conservation strategy employed by different counties (i.e. emphasis on catchment improvements compared with captive breeding) depends upon a variety of factors including size of remaining populations, catchment size, and types of pressure. In England there are approximately 12 populations of *M. margaritifera*, all of which are in decline (Chesney & Oliver, 1998). In 2007 the decision was taken to remove a subset of individuals from the most imperilled English populations for captive rearing whilst catchment improvements, including pearl mussel habitat restoration, took place. The Freshwater Pearl Mussel Ark is a captive rearing programme funded by Freshwater Biological Association (FBA), Natural England and the Environment Agency, with the FBA managing captive rearing activities. The overall objectives of the project are to hold sub-populations from target rivers to protect against local population extinction, and to rear juvenile mussels for release into natal rivers.

A major benefit of ex-situ mussel culture is that environmental parameters can be controlled to optimise habitat conditions and ultimately increase juvenile survival. Percentage survival of larval and juvenile stages in the wild is low (Young & Williams, 1984) even in sustainable populations.

Captive rearing can offer a short-term solution to boost population size until natural recruitment levels can be re-established in the wild. Captive rearing is not a long-term solution for declining populations and must be coupled with catchment-wide habitat improvements to reduce pressures on pearl mussel habitat. Substantial improvements in habitat quality are required to allow more sustainable levels of juvenile survival and enable demographic recovery of wild populations.

Research in the early 2000's into optimising captive rearing conditions for freshwater mussels (mainly by North American and some European practitioners) has led to the near perfection of these techniques for certain species (Lopes-Lima et al., 2014). Captive rearing of particularly sensitive species such as *M. margaritifera* however has proven slightly more problematic and has required significant investigation. Several different methods of propagating *M. margaritifera* have been trialled in Europe (Gum et al., 2011) including bankside encystment and immediate release of encysted salmonids (Altmüller & Dettmer, 2006), rearing juveniles in trays or baskets (Hastie & Young, 2003; Taylor, 2007; Lange & Selheim, 2011; Scriven et al., 2011; Eybe et al., 2013; Lavictoire et al., 2014), suspending cages containing juveniles in raceways or rivers (Buddensiek, 1995; Schmidt & Vandré, 2010), allowing juveniles to excyst directly into raceways (Preston et al., 2007; Moorkens, 2011) and holding juveniles in boxes containing water and placing them in incubators (Lange & Selheim, 2011; Eybe et al., 2013). Whilst previous studies have investigated growth and survival of *M. margaritifera* juveniles under various culture conditions (Buddensiek, 1995; Hruška, 1999; Schmidt & Vandré, 2010; Lange & Selheim, 2011; Eybe et al., 2013), they have lacked specific information on periodic growth and survival rates over extended periods of time, mainly due to low numbers of surviving individuals. Hruška (1999) detailed the rearing of 30,000 individuals to over 3 years old but did not include information on original numbers or survival rates. Further studies with periodic monitoring and a standardised reporting system are required to better understand the factors affecting growth and survival of very young (< 1 year) juveniles in captivity.

Previous studies focussing on captive rearing of mussel species (mainly North American) have found that certain environmental conditions in culture are important for optimal growth and survival. These include substrate size (Beaty & Neves, 2004; Liberty et al., 2007) and depth (Yeager et al., 1994; Beaty & Neves, 2004; Jones et al., 2005), maintenance (cleaning) regime (O'Beirn et al., 1998; Liberty et al., 2007), diet (Gatenby, 1997; Lange, 2005; Kovitvadhi et al., 2006; Schmidt & Vandré, 2010; Eybe et al., 2013) and mussel stocking density (Eybe et al., 2013).

This investigation sought to test an experimental culture system for *M. margaritifera* using a flow-through system with water sourced from a mesotrophic lake. Substrate size and cleaning regime were tested to identify optimal conditions for the species within this system and to assess which method provided the highest level of survival.

Materials and methods

Experimental work was undertaken at the Freshwater Biological Association headquarters in Cumbria, UK. Water was sourced from Windermere, a large mesotrophic lake, within a catchment with historic records for *M. margaritifera*. Water used in experiments had particles larger than 20 µm removed using a Hydrotech 501 filter. Water temperature followed the lake's natural temperature regime.

A pilot study, carried out between June 2011 and April 2012, informed experimental design for the work described here. Data collection took place over a 12 month period from June 2012. Two different substrate mixes (0.25-1 mm and 1-2 mm) and two cleaning frequencies (weekly and monthly) were tested, giving a total of four treatment types. Substrate was sourced from around the FBA facility on the western shore of Windermere. Before use, substrate was air-dried before it was sieved to the required clast sizes (either 0.25-1 mm or 1-2 mm).

A down-welling, flow-through system was designed which supplied filtered lake water to juveniles at a rate of approximately 67 $ml.s^{-1}$. Thirty six square holes were cut out of a styrene sheet

fixed to the sides of a glass aquarium (995 mm x 357 mm x 510 mm). *Artemia* sieves (Hobby, Germany) with a mesh size of 0.9 mm were adhered to the styrene sheet to create a fixed support structure for the experimental sieves. Removable experimental sieves (mesh size 0.18 mm) were inserted into the fixed sieves, providing the sole pathway for water flow. Water entered the top of the system via a spray bar, passed through the sieves containing juveniles and substrate, and exited from the bottom of the system through the down-pipe (Fig. 1).

Nine replicates of each of four treatments (36 experimental sieves in total) were set up. Each sieve contained one of the experimental substrate mixes to a depth of approximately 1 cm (50 g dry weight). Treatment sieves containing substrate were exposed to flowing lake water for a minimum of 21 days prior to the start of the experiment to allow biofilm development on the substrate, as per Gum et al. (2011). Juveniles propagated from a single population provided by the FBA's Freshwater Pearl Mussel Ark Project (Sweeting & Lavictoire, 2013) were used. One hundred newly excysted active juveniles were added to each experimental sieve (total 3,600 individuals). For each sieve, 30 individuals were selected at random and measured (length and height to nearest 50 µm) before being added. The position of experimental sieves within the aquarium was assigned on a random basis so that each column within the aquarium had one of each of the four treatments. Sixteen days after the experiment commenced, the surface of the styrene sheet was siphoned and 11 juveniles were found indicating escapement. *Artemia* sieves with a 0.3 mm mesh were placed over the experimental sieves so juveniles could not escape.

Every week, sieves in the weekly cleaning treatment (18 sieves in total) were removed from the aquarium and substrate was gently emptied into a glass container. Substrate was elutriated to suspend organic particles and the elutriate poured through a 0.18 mm mesh sieve to retain any suspended juveniles. Sieves were inspected under a low power microscope (x 20) and any juveniles replaced into the experimental sieve along with the substrate. The 0.3 mm sieve (cover) and 0.9 mm

sieve (fixed within aquarium) were also cleaned before the experimental sieve was replaced. The same process was repeated on a monthly basis for treatments requiring monthly cleaning.

Approximately every two months (51, 112, 167, 247, 308 and 362 days post excystment) the numbers of surviving juveniles and dead shells were recorded. In addition, thirty live individuals from each sieve were chosen at random and measured. Where fewer than 30 individuals remained, all juveniles were measured. Dead juveniles were measured and removed from sieves. Sampling in this manner constituted a cleaning event as organic matter was removed during sampling.

Sampling for growth and survival ceased after 12 months but the system and cleaning regimes were retained for a further 13 months. Sieves were sampled for the final time at 758 days post-excystment. Survival and size were recorded as before but instead of being returned to sieves, all juveniles were removed to a modified salmon egg tray containing substrate measuring 1-2 mm as described in Sweeting & Miles (2010). Statistical analysis on juvenile size and survival are reported for the first 12 months only, unless otherwise stated.

Data analysis

Central Limit Theorem (Elliott, 1993) was applied to assume normality where appropriate. Standard deviation values are provided after mean values. One-way Analysis of Variance (ANOVA) with post hoc Tukey's HSD tests were used to assess the significance of survival, survival rates and size between treatments on the same sampling occasion when data were normal. Where data were not normal Kruskal-Wallis tests were employed. Two-way ANOVA's were used to investigate the interaction of substrate size and cleaning regime and their effects on both survival and size in treatments on day 362. Repeated Measures ANOVA's with pairwise comparisons were used to test survival between 0 – 362 days to see if survival changed at specific times. For Repeated Measures ANOVA's a Greenhouse-Geisser correction was applied if the assumption of sphericity was not met. Student's *t*-tests were used to compare length pre- and post-winter to help establish if juveniles

displayed size-dependent over-winter survival within treatments. Spearman's Rank Correlation Coefficient tests were used to test the significance of correlations between shell length and height, shell growth and temperature, and mean survival and shell length.

Juvenile length and survival across rows and columns in the aquarium were tested in June 2013 to rule out any bias due to sieve positioning (lateral and top-to-bottom positioning within the aquarium). There was no significant difference in survival between different columns ($F_{(8,27)} = 0.196$, $P = 0.989$) or rows ($F_{(3,32)} = 0.025$, $P = 0.994$) indicating that any significant results between treatments were not due to sieve positioning. The same was true for mean juvenile length in June 2013; there were no significant differences between different columns ($F_{(8,27)} = 0.097$, $P = 0.999$) or rows ($F_{(3,32)} = 0.163$, $P = 0.920$).

Initial size in June 2012 was also tested to ensure juveniles in each sieve had the same starting length. Starting lengths of individuals in all 36 sieves were not significantly different ($F_{(35,1044)} = 1.35$, $P = 0.083$) at the beginning of the experiment (mean length = 0.40 ± 0.02 mm).

Results

Size

Spearman's Rank Correlation Coefficient tests found juvenile length and height were significantly correlated ($P < 0.001$); 0.25-1 mm weekly treatment ($r_{s(1216)} = 0.980$), 0.25-1 mm monthly treatment ($r_{s(1837)} = 0.965$), 1-2 mm weekly treatment ($r_{s(1876)} = 0.974$) and 1-2 mm monthly treatment ($r_{s(1888)} = 0.968$). As such only the length parameter was used for analysis in this study.

Length of juveniles on each sampling occasion was considered in turn to establish any effects of the different treatments over time (Fig. 2). Juvenile sizes were all significantly different ($P < 0.001$) among treatments on days 51 ($F_{(3,1076)} = 77.295$), 112 ($F_{(3,964)} = 195.723$), 167 ($F_{(3,951)} = 158.522$), 247 ($F_{(3,941)} = 175.247$), 308 ($F_{(3,906)} = 162.465$) and 362 ($F_{(3,883)} = 167.377$). The same pattern was observed

throughout the experiment; 1-2 mm weekly > 0.25-1 mm weekly > 1-2 mm monthly > 0.25-1 mm monthly. Table 1 summarises these results giving mean length (mm) on each sampling occasion. A 2-way ANOVA was carried out for the June 2013 sample (362 days) and a significant interaction was found between substrate size and cleaning regime ($F_{(1,883)} = 7.414$, $P = 0.007$). Analysis of simple main effects found significant interactions at all levels.

Shell growth in *M. margaritifera* is positively correlated with temperature ($r_{s(214)} = 0.76$, $P < 0.001$). Apparent cessation of growth was observed below approximately 10 °C (Fig. 3). Mean daily growth rates during the warmest period (June-August 2012) were almost 11 times higher than during the coolest period (February-April 2013). Average growth per day highlighted that growth slowed over winter but did not halt completely (Table 2).

Survival

We report survival rates comparable to, and in most cases greater than, previous studies of a similar duration on other species of freshwater mussel (Fig. 4a), and specifically *M. margaritifera* (Fig. 4b). In this investigation, higher survival was observed in the 1-2 mm monthly treatment with an average of over 55 % survival after 12 months and 23 % after 25 months. The lowest survival was in the 0.25-1 mm weekly treatment with the other two treatments displaying intermediate survival (Tables 3 and 4; Fig. 5).

Juvenile survival between treatments was significantly different on all sampling occasions ($P < 0.001$); 51 days ($F_{(3,32)} = 128.30$), 112 days ($F_{(3,32)} = 148.285$), 167 days ($F_{(3,32)} = 145.296$), 247 days ($F_{(3,32)} = 140.117$), 308 days ($F_{(3,32)} = 145.350$) and 362 days ($F_{(3,32)} = 64.670$). The same pattern in survival was observed on all sampling occasions; 1-2 mm monthly > 1-2 mm weekly > 0.25-1 mm monthly > 0.25-1 mm weekly. A 2-way ANOVA showed no significant interaction between the effects of substrate size and cleaning regime on survival in June 2013 after 362 days ($F_{(1,32)} = 0.805$, $P = 0.376$).

Survival over time

Survival differences within treatments over time were tested with Repeated Measures ANOVAs to see if there were specific times when survival changed. Mean survival was different over the course of the experiment for all treatments ($P < 0.001$); 0.25-1 mm weekly ($F_{(2.021, 16.171)} = 1147.196$), 0.25-1 mm monthly ($F_{(6, 48)} = 315.484$), 1-2 mm weekly ($F_{(2.126, 17.008)} = 324.543$) and 1-2 mm monthly ($F_{(2.030, 16.243)} = 167.912$). Post-hoc tests revealed that survival was the same only in the 1-2 mm weekly treatment between October & December 2012 ($P = 0.086$) and in the 0.25-1 mm weekly treatment between December 2012 & February 2013 ($P = 0.122$), February & June 2013 ($P = 0.128$) and April & June 2013 ($P = 0.288$).

Survival rate

Survival rates were considered to further examine data taking into account the high initial mortality observed in the 0.25-1 mm weekly treatment. Survival rates between the treatments were significantly different ($P < 0.002$) on days 51 ($F_{(3,32)} = 128.303$), 112 ($F_{(3,32)} = 41.388$), 167 ($F_{(3,32)} = 9.743$), 308 ($F_{(3,32)} = 6.553$) and 362 ($H_{(3,32)} = 15.179$) but were not statistically different on day 247 ($F_{(3,32)} = 0.619$, $P = 0.608$).

When mean survival rate was plotted against mean shell length, no relationship was found for either of the 1-2 mm treatments (Fig. 6). In the 0.25-1 mm treatments however, there was a positive relationship, which is stronger in the treatment which is cleaned weekly. Spearman's rank correlation coefficient tests found no significant correlation in the 0.25-1 mm monthly treatment ($r_{s(4)} = 0.515$, $P = 0.296$) but there was in the 0.25-1 mm weekly treatment ($r_{s(4)} = 0.947$, $P < 0.01$). Larger individuals displayed significantly higher survival compared with smaller individuals in this treatment.

Over-winter survival

To establish whether juveniles displayed size-dependent over-winter survival, *t*-tests were carried out comparing juvenile size pre- and post-winter (October 2012 and April 2013). Across all treatments, juveniles were significantly larger post-winter ($P < 0.001$); 0.25-1 mm weekly ($t_{(272)} = -4.377$), 0.25-1 monthly ($t_{(522)} = -5.239$), 1-2 mm weekly ($t_{(538)} = -3.717$), 1-2 mm monthly ($t_{(530)} = -5.027$). However, there were more juveniles in larger size classes in April 2013 compared to October 2012. This implies that, rather than there being high mortality of smaller juveniles over winter, individuals have grown, thereby pushing them into larger size classes.

Discussion

The objective of this study was to test the effectiveness of the proposed culture system for rearing juvenile mussels (*M. margaritifera*) and to examine the effects of different substrate sizes and cleaning regimes on growth and survival. Here we describe a culture method employing some features from previous studies (e.g. Hastie & Young, 2003; Lange, 2005; Barnhart, 2006; Preston et al., 2007; Gum et al., 2011) to create a low-maintenance system which utilises a natural temperature regime. The proportion of mussels escaping experimental containers was minimal (2.5%) and can be prevented in future by placing covers over containers from the beginning. Juveniles were reared to an age of 758 days (25 months) with optimum treatments achieving 55% mean survival after 12 months and 23% after 25 months. No other captive rearing facility has reported this level of survival over similar timescales for *M. margaritifera* (Fig. 4b). Growth during the first 362 days (12 months) was comparable with other studies (e.g. Hruška, 1999; Scheder et al., 2011; Scriven et al., 2011; Eybe et al., 2013). Juveniles in this study achieved growth rates of between 170 - 220% (0.68 – 0.89 mm) during the first growth season, which compares favourably with Hruška (1999) reporting 250%, and Eybe et al. (2013) with rates between 150-200 %. While it is not possible to directly compare growth from different captive rearing programmes due to differences in culture systems, temperature regimes, handling, and population-specific growth rates, the desired outcome of growth and survival from the techniques used in this study are positive compared with other reported methods.

This investigation concurred with other studies which have found that juveniles display a large size range after a relatively short period (Beaty & Neves, 2004; Barnhart, 2006; Schmidt & Vandré, 2010). By the end of the first year, some individuals were 2.5 times larger than others. This pattern has been shown to continue as juveniles get older with some individuals reaching over 9 times the length of conspecifics by 5 years old (Sweeting & Lavictoire, 2013).

Significantly higher survival was observed in larger substrates (1-2 mm) which concurs with previous studies on unionids (e.g. Liberty et al., 2007). Substrate size preference appears species-specific but may also be affected by the culture system. For example, different substrate size preferences were found for *Villosa iris* (Lea, 1829) in different culture systems in Virginia. Superior growth and survival were found in substrates < 0.2 mm in a recirculating system (Hua et al., 2013) but higher survival was recorded in substrates between 0.5-0.85 mm in a flow-through system (Liberty et al., 2007). *M. margaritifera* is slower growing and has a longer juvenile stage compared to most other freshwater mussels, so rearing techniques from other species may have limited relevance. There remains a lack of understanding of the specific habitat requirements (physical, chemical and biological) for *M. margaritifera*, both in the wild and in captivity. For captive rearing programmes to become more successful a greater understanding of the factors limiting survival is needed.

Individuals in the 0.25-1 mm weekly treatment showed size-dependent survival, something which was not apparent in the other treatments. This may be because this treatment appeared to be the least suitable for *M. margaritifera* (displaying the highest mortality), so only the largest individuals were able to survive. If smaller substrate sizes impeded water flow to an extent where food or oxygen supply was not sufficient then size-dependent survival would also have been observed in the 0.25-1 mm monthly treatment. Likewise, if a weekly cleaning regime was too stressful for juveniles, size-dependent survival would have been observed in the 1-2 mm weekly treatment, but it was not. It appears that the combination of small substrate size and frequent

(weekly) cleaning does not provide suitable conditions for *M. margaritifera* juveniles to thrive. This finding is important when considering catchment management and habitat improvements to ensure that enough coarse substrate is available for juveniles.

Cleaning of substrate on a weekly basis has a detrimental effect on survival compared to monthly cleaning (Table 4). Differences in survival rates between treatments were significant which infers that observed differences in survival were not artefacts of high initial mortality in the 0.25-1 mm weekly treatment after approximately 112 days. Poorer survival and growth in treatments cleaned more frequently has been documented in studies on different mussel species (O'Beirn et al., 1998; Liberty et al., 2007) due to stress or accidental damage/loss during sampling.

Whilst survival was compromised in treatments cleaned more regularly, growth was found to be significantly higher, contradicting findings of studies on some unionid species (e.g. Liberty et al., 2007). This higher growth rate may be because cleaner substrate conditions allow pedal-feeding juveniles to forage for more or better quality food. Whilst this finding is interesting, higher growth rates for *M. margaritifera* should not be sought at the expense of survival in captive rearing programmes. It appears that most captive-bred species of mussel require enough cleaning to remove fine particles in order that normal foraging behaviour is not affected, but too much cleaning may cause stress and damage/loss, leading to higher mortality (O'Beirn et al., 1998). As reported in other studies, (e.g. Buddensiek 1995), juvenile growth was found to be negligible at low temperatures and near-cessation (approximately 0.3 μm per day) of growth occurred below 10 °C, corroborating the findings of Hruška (1999).

Across all treatments, mortality was highest during the first growth season (June-October 2012), after which mortality was relatively low over winter. This implies that survival is relatively stable when temperature (and therefore metabolic rate) is low. This was unexpected as it was assumed that mortality would increase over winter for those juveniles lacking sufficient nutritional reserves. Buddensiek (1995) found high mortality during the first few months post-excystment but

also found complete mortality of smaller mussels (< 0.7 mm) over-winter; a result which has not been replicated in this study. Size-dependent over-winter survival was not observed in this investigation, contrasting with the findings of Buddensiek (1995).

It is unclear if there is any intraspecific competition within sieves at a density of 100 juveniles in 34 cm^3 of substrate, and testing different juvenile densities is an aspect which requires further investigation. Eybe et al. (2013) found significantly higher growth in containers with 200 mussels suggesting that density-dependent competition was occurring in treatments with higher numbers. Barnhart (2006) achieved good survival for several North American freshwater mussel species at densities of 2000 individuals in small cups. Similarly Beaty (1999) found no density dependent effects for *V. iris*. Higher densities however may lead to higher instances of fungal infection which can spread rapidly and kill large numbers of juveniles (L. Lavictoire, pers. observation). This system may safeguard against the spread of fungal infection because juveniles are contained in separate, removable sieves. Eybe et al. (2013) found fungal infections could be problematic and could spread rapidly, especially in containers with a density of 500 individuals per 500 ml water.

Culture conditions which allow water to flow through interstitial spaces but do not allow too much fine organic or particulate matter to infiltrate can supply juveniles with suitable habitat conditions with good levels of oxygen and food (Liberty et al., 2007). The benefits of rearing juveniles in substrate > 1 mm diameter could be improved oxygen and food supply and the more efficient removal of potentially toxic ions such as ammonia or nitrite, found to be a limiting factor for juvenile survival (Eybe et al*.,* 2013). Further investigations considering interstitial dissolved oxygen concentration, nitrite and ammonia concentration, and flow characteristics through different substrates and types of culture system (e.g. down-welling versus laminar flow) are required to better understand the habitat requirements of young juveniles. Investigation is also required to establish the natural diet of *M. margaritigera* and the importance of different algae and bacteria species as

food items for juveniles. Whilst diet was not studied during this investigation, a comprehensive list of recorded phytoplankton in Windermere can be found in Reynolds & Irish (2000).

The exact age (or size) at which juvenile *M. margaritifera* metamorphose and switch from pedal- to filter-feeding is unknown but observations made during this experiment suggest that juveniles were still pedal-feeding at 12 months old but had switched to filter-feeding by 25 months old. This down-welling system is therefore suitable for juveniles which are pedal- as well as filter-feeding. Further investigation is required to establish if substrate requirements are different for pedal-feeding versus filter feeding juveniles.

Although mussel culture systems have been somewhat perfected (Lopes-Lima et al., 2014) culture of *M. margaritifera* remains challenging. This culture system was successful in rearing 1207 juveniles to 12 months old and 518 juveniles to 25 months old. Juveniles were easy to find when sampling due to the small size of containers. This is important when designing culture systems to maximise efficiency. If scaled up, this system could potentially rear up to 2000 juveniles to 12 months old to a size of > 1 mm using 1-2 mm substrate cleaned monthly, requiring minimal maintenance time (approx. 3hrs/month). This work has informed breeding practices for the Freshwater Pearl Mussel Ark Project at the FBA. A rearing protocol using 1-2 mm substrate cleaned approximately every two to three weeks in modified fish-egg trays (described in Sweeting & Miles, 2010) was introduced for all new juvenile cohorts collected from 2013. Initial results show good numbers of juveniles surviving after 24 months. Early success in this modified system allows tentative optimism that aspects of this investigation can be used to scale-up propagation of threatened populations at this facility and could be replicated elsewhere.

Captive rearing programmes are an important activity to safeguard the most vulnerable populations and provide more time for catchment restoration to improve pearl mussel habitat in the wild (Gum et al., 2011), especially where problems are diverse and difficult to solve. It is important

to understand the limiting factors of juvenile culture and to maximise survival in captivity but these initiatives should not replace restoration activities in the wild.

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Figure captions

Fig. 1. Experimental set-up. Water enters at the top of the system via a spray bar (SB) and flows through sieves (S) containing substrate and juveniles (arrows show direction of flow). Water exits via the downpipe (DP).

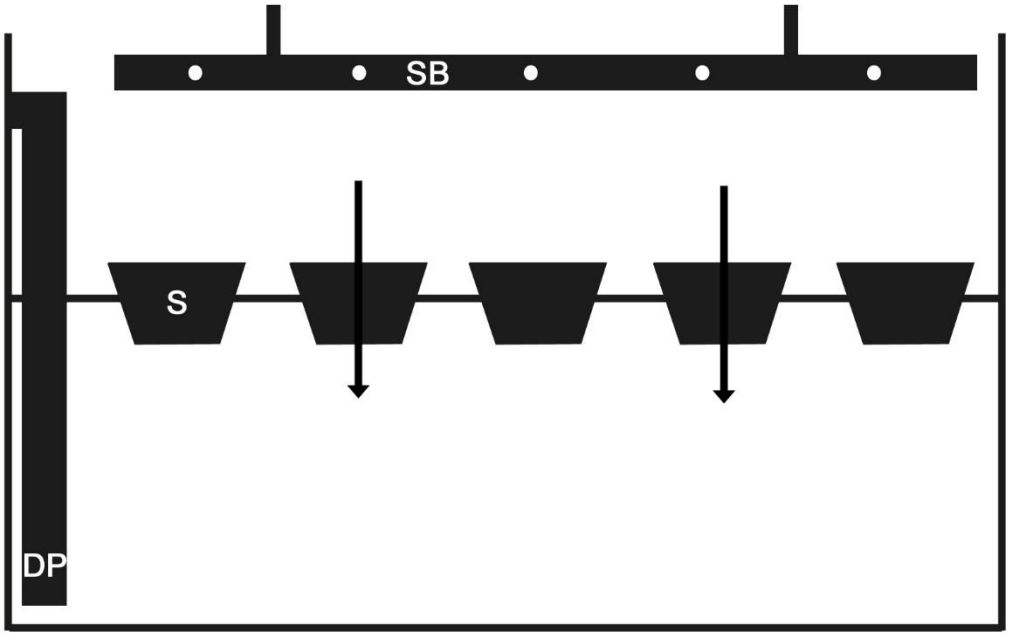

Fig. 2. Juvenile length (mm) with SD bars, and daily mean temperature (°C) during the course of the experiment. The x-axis is provided both in days since experiment commenced and per month to show how growth relates to time of year.

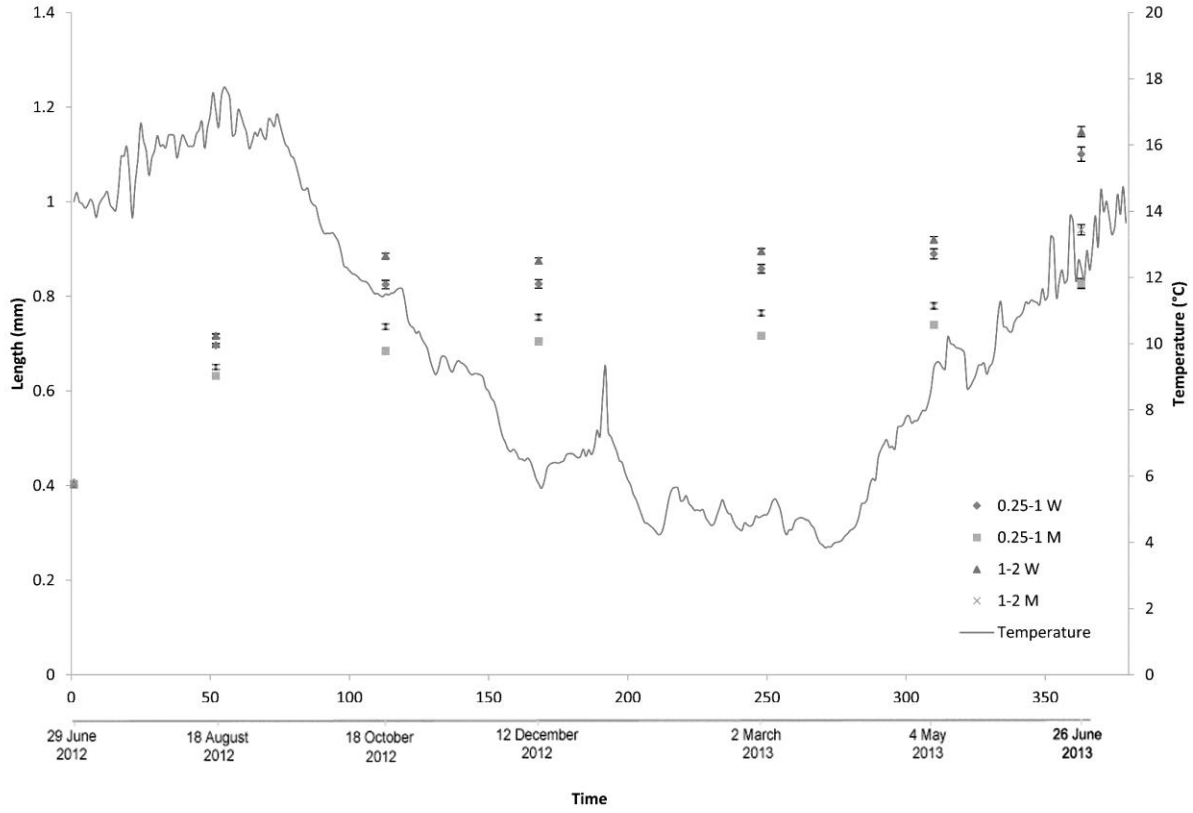

Fig. 3. Mean shell growth against daily mean temperature for the six sampling occasions.

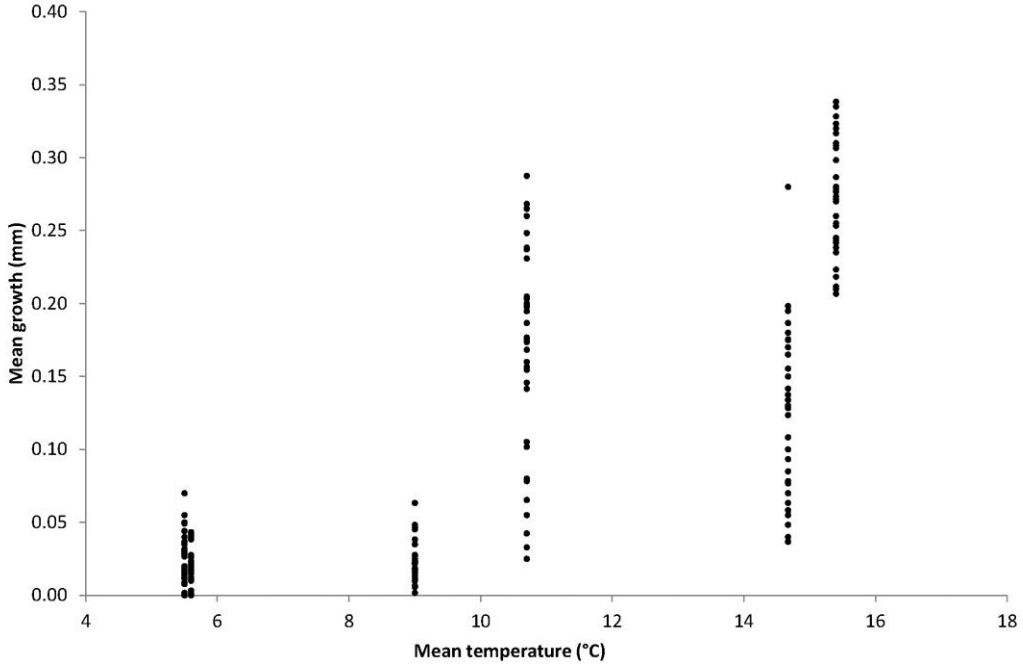

Fig. 4. Mean survival in (a) other published bivalve captive rearing studies and (b) more specifically in *M. margaritifera* studies. Mean survival up until July 2014 has been provided for this study. * Indicates figures based upon estimates; Buddensiek (1995) figures taken from survival graphs; Hastie & Young (2003) Baskets estimated a sample of known volume and multiplied up to indicate likely survival in total volume. ^ Indicates only one treatment selected for illustrative purposes; this study investigated survival of 8 different species over different timescales. For all studies where several treatments are reported, only the best survival results are reported here for comparison.

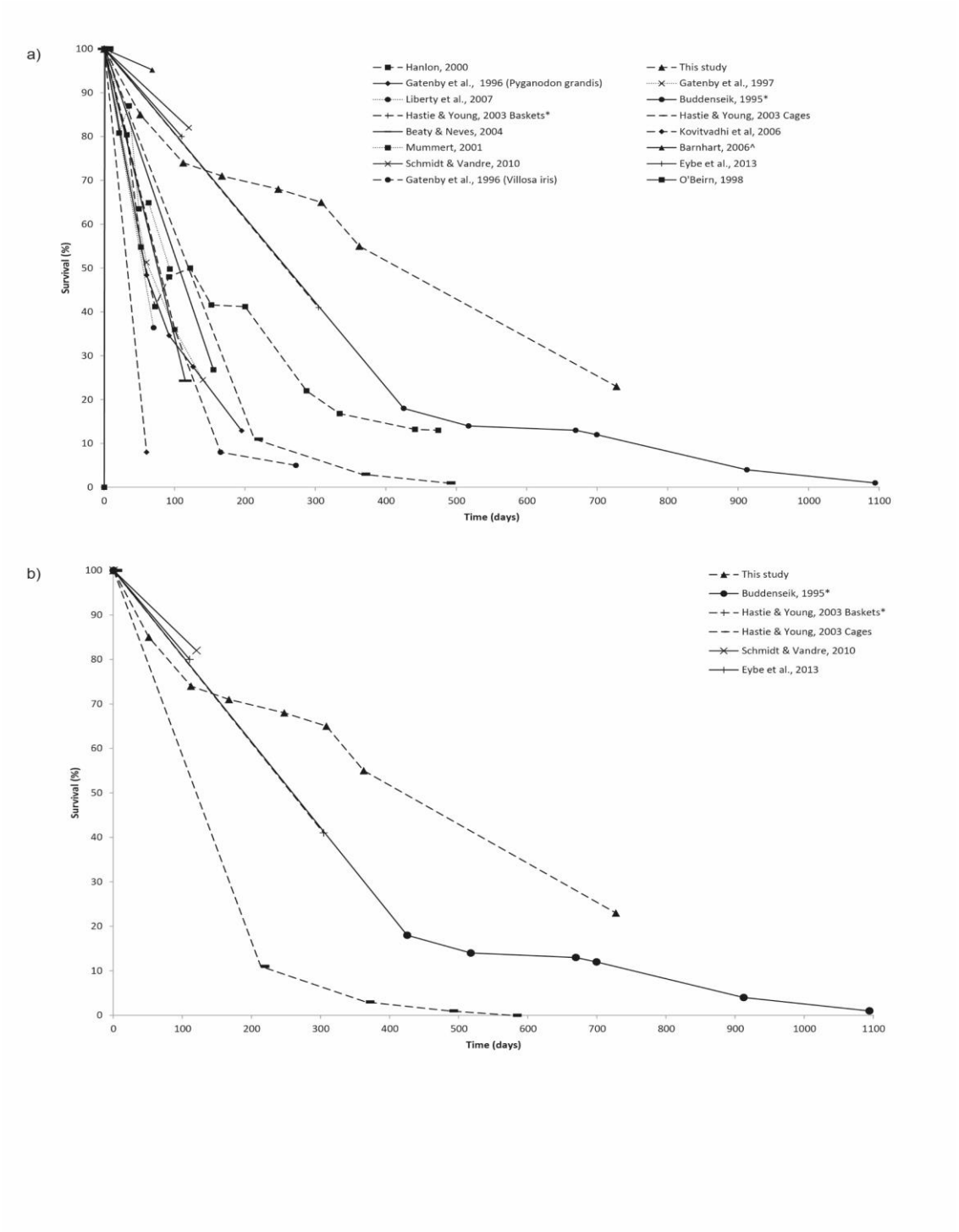

Fig. 5. Mean juvenile survival for each treatment with SD bars. The x-axis is provided both in days since experiment commenced and per month to show how survival relates to time of year.

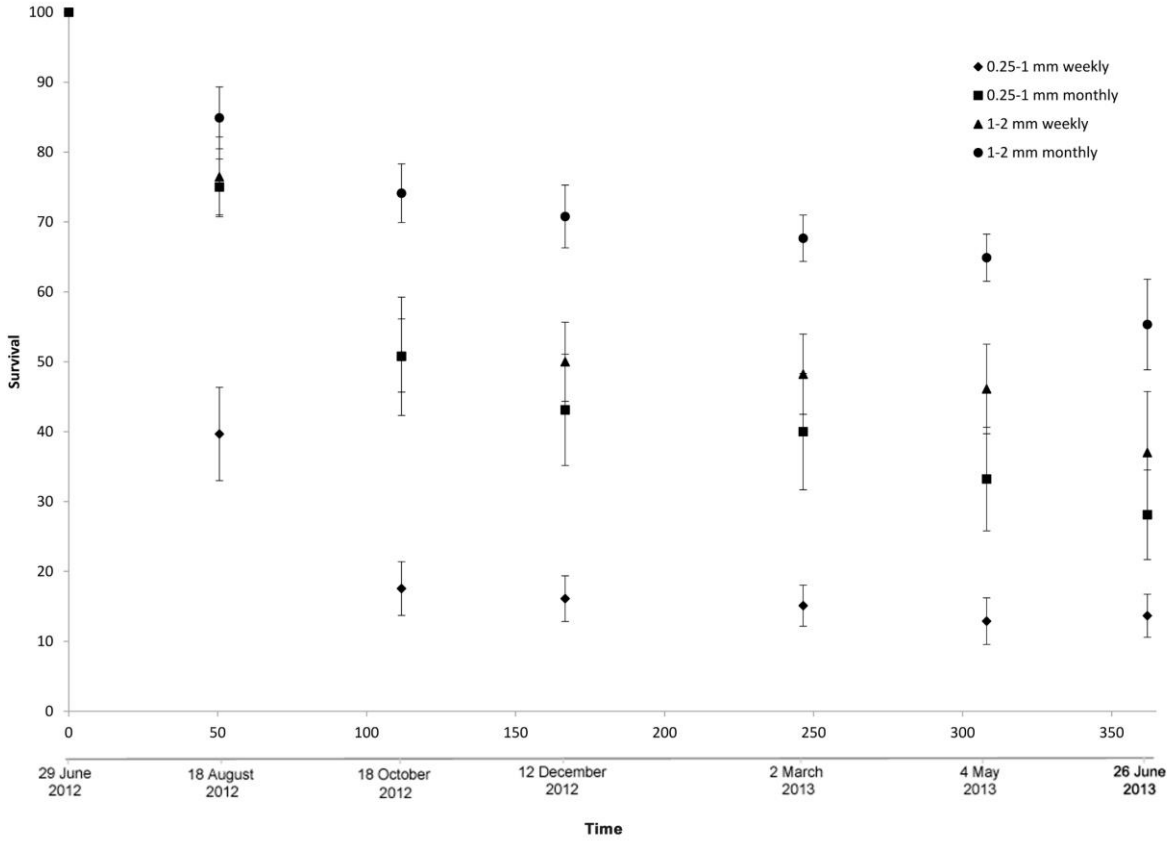

Fig. 6. Mean survival rate (%) against mean shell length (mm) for each treatment. Survival rate was > 100 % on one occasion in the 0.25-1 mm weekly treatment due to sampling error.

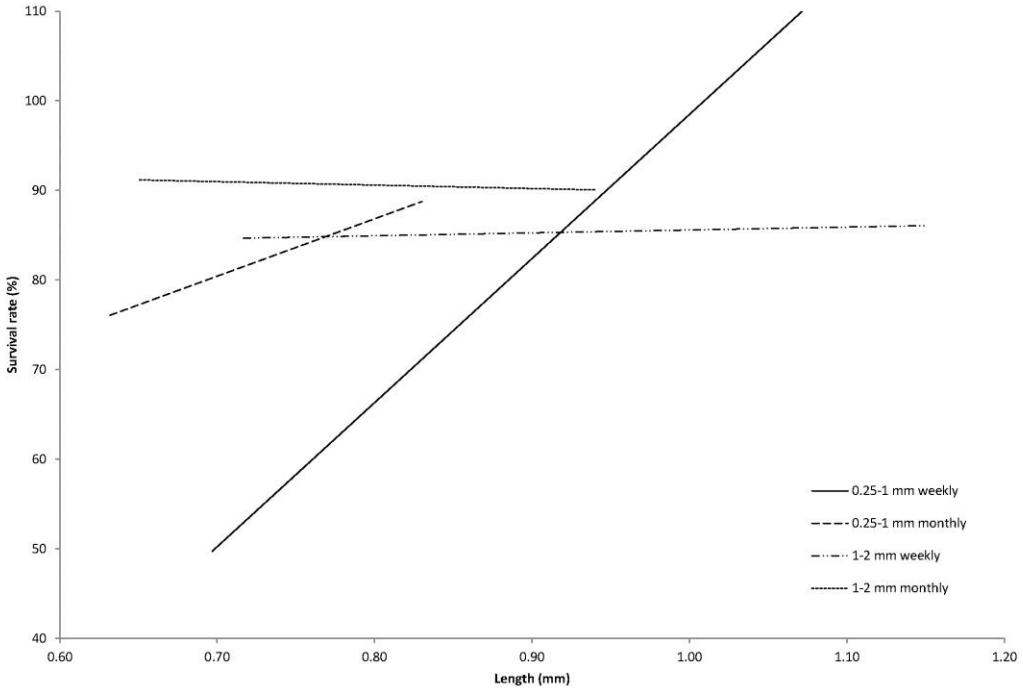

Table 1. Mean length ± SD (mm) of juveniles on different sampling occasions in order of largest to smallest. Size was significantly different between all treatments ($P < 0.001$). Tukey's HSD tests showed significant differences within each sampling point ($P < 0.05$) except where indicated 167 days (*$P = 0.31$) and 308 days post excystment (^ $P = 0.17$).

	51 days	112 days	167 days	247 days	308 days	362 days
1-2 mm weekly	0.72 ± 0.07	0.89 ± 0.09	0.88 ± 0.10*	0.90 ± 0.09	0.92 ± 0.10^	1.15 ± 0.17
0.25-1 mm weekly	0.70 ± 0.07	0.84 ± 0.11	0.86 ± 0.11*	0.87 ± 0.10	0.90 ± 0.12^	1.10 ± 0.17
1-2 mm monthly	0.65 ± 0.08	0.74 ± 0.10	0.76 ± 0.10	0.77 ± 0.10	0.78 ± 0.11	0.94 ± 0.18
0.25-1 mm monthly	0.63 ± 0.07	0.69 ± 0.10	0.71 ± 0.10	0.72 ± 0.10	0.74 ± 0.11	0.83 ± 0.16

Table 2. Average growth per day (µm) for individuals in each treatment and mean daily temperature (± SD) between sampling points. Negative values are due to sampling error and do not indicate shrinkage.

Treatment	0-51 days	51-112 days	112-167 days	167-247 days	247-308 days	308-362 days
Mean daily temp. (°C) over period	15.4 (1.0)	14.7 (2.0)	9.0 (1.7)	5.6 (1.1)	5.5 (1.5)	10.7 (1.3)
0.25-1 mm weekly	6.15	2.10	0.42	-0.01	0.66	3.67
0.25-1 mm monthly	4.18	1.01	0.31	0.15	0.27	1.76
1-2 mm weekly	6.80	2.52	-0.24	0.22	0.46	4.00
1-2 mm monthly	4.58	1.58	0.35	0.11	0.21	3.20

Table 3. Summary survival statistics. Total number of individuals surviving, percentage survival and the range of percentage survival for all treatments in June 2013.

Treatment	Total no. survived	Survival (%)	Survival range (%)
0.25-1 mm weekly	123	14	8-19
0.25-1 mm monthly	253	28	19-40
1-2 mm weekly	333	37	18-47
1-2 mm monthly	498	55	45-63

Table 4. Mean number of juveniles surviving (± SD) at each sampling point in order of highest to lowest survival. Survival was significantly different between all treatments. Tukey's HSD tests showed significant differences within each sampling point ($P < 0.05$) except where indicated on days 51 (*P = 0.93) and 112 (^ P = 0.97). Results have been rounded to whole juveniles.

	51 days	112 days	167 days	247 days	308 days	362 days
1-2 mm monthly	85 ± 4	74 ± 4	71 ± 4	68 ± 3	65 ± 3	55 ± 6
1-2 mm weekly	76 ± 6*	51 ± 5^	50 ± 6	48 ± 6	46 ± 6	37 ± 9
0.25-1 mm monthly	75 ± 4*	51 ± 8^	43 ± 8	40 ± 8	33 ± 7	28 ± 6
0.25-1 mm weekly	40 ± 7	18 ± 4	16 ± 3	15 ± 3	13 ± 3	14 ± 3