POWDER BLASTED MICROSYSTEM FOR HATCHING, OBSERVATION AND STUDY OF *CARIDINA MULTIDENTATA* LARVAE

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Abstract

Several glass microsystems for shrimp eggs cultivation were fabricated by powder blasting technology from cover glasses and microscopy glass slides. Due to modular assembly of the microsystems, different designs of the microchip can be used. In our study, successful cultivation of the shrimp *Caridina multidentata* eggs was achieved.

Key words: *Powder blasting, Microsystems, Shrimp larvae, Caridina multidentata*

Introduction

Shrimps of the species *Caridina multidentata* (Stimpson, 1857)[1] Johanne Rogers duce a Republica Federata missa“,“publisher“:“[Philadelphia: Academy of Natural Sciences]“,“number-of-pages“:“158“,“source“:“Internet Archive“,“abstract“:“Offprints: Proceedings of the Academy of Natural Sciences of Philadelphia, Feb. 1857-Jan. 1860; Includes bibliographical references; pars 1. Turbellaria dendrocoela -- pars 2. Turbellarieorum nemertineorum -- pars 3. Crustacea maioidea -- pars 4. Crustacea cancroidea et corystoidea -- pars 5. Crustacea ocypodoidea -- pars 6. Crustacea oxystomata -- pars 7. Crustacea anomoura -- pars 8. Crustacea macrura; extracted picklist; MSC copy bound with: Report on the Crustacea (Brachyura and Anomura are highly prized among the aquarists as the so-called „algae eaters“. However, their rearing in aquarium is very demanding and therefore they are often caught in the wild as the cheaper solution. This mostly unregulated catch can lead to the decimation of wild shrimp populations which can further lead to the loss of biodiversity of aquatic ecosystems [2–4]often taking action only after a critical stock suffers overfishing or collapse. The invertebrate ornamental fishery in the State of Florida, with increasing catches over a more diverse array of species, is poised for collapse. Current management is static and the lack of an adaptive strategy will not allow for adequate responses associated with managing this multi-species fishery. The last decade has seen aquarium hobbyists shift their display preference from fish-only tanks to miniature reef ecosystems that include many invertebrate species, creating increased demand without proper oversight. The once small ornamental fishery has become an invertebrate-dominated major industry supplying five continents. Methodology/Principal Findings: Here, we analyzed the Florida Marine Life Fishery (FLML. Their transport is difficult and there is often a die-off of the shrimps soon after their introduction into the aquarium due to the effects of exhaustion, long-lasting stress, or the inability of adults to get accustomed to aquarium conditions.

The difficulty of rearing these shrimps is due to its complex development. In contrary to widespread species of aquarium shrimps like genus *Neocaridina* and *Halocaridina* or species *Caridina cantonensis* (Yü,1938) [5], *Caridina multidentata* hatch in freshwater condition but then undergoes several additional larval stadiums after hatching [6]. These larval stages are tied to salt water with a full salinity of 32–35 ppt. Salinity below or above this range results in severe mass loses ranging from 98 % to 100 % in the mysid-stage.

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Since the *Caridina multidentata* follow the r-selected species strategy, number of unhatched eggs, which are dropped off by the shrimp when the larvae starts to hatch, is large. Only about half of the eggs hatch from the eggshell from which only few larvae survive to maturity in aquarium conditions. Dropped eggs will not hatch on its own because they require a constant supply of fresh water and dissolved oxygen, which is normally supplied by the shrimp carrying eggs [7].

In our study, we present powder blasted glass microsystem as an alternative method for hatching of the dropped eggs to further increase the number of larvae that can be raised to maturity.

Enabling the rearing of this shrimp among commercial aquarium breeders by using a simple device to significantly increase the number of surviving larvae could help limit the hunting of this shrimp in the wild.

Experimental

The several microsystem designs were fabricated using powder microblasting technology. All parts of microsystems were created with powder blasting lathe (Comco, Inc.) with 50 μm Al_2O_3 abrasive particles and the nozzles with 0.46 mm and 0.76 mm inner diameter. Common microscopy glass slides (Menzel Gläser cut glass slides (76 mm × 26 mm × 1 mm) (Fisher Scientific, spol. s. r. o.)) were used as a substrate materials. The microsystem is modular, as shown in Fig. 1, panel B. All microfabricated parts were bonded with UV glue (Conloc 665, TGK GmbH, Germany) and exposed to the UV light dose of 100 000 mJ/cm^2 by photolithographic system with digital control of exposition (NUV Illumination System – Inverted, Newport Corporation, USA).

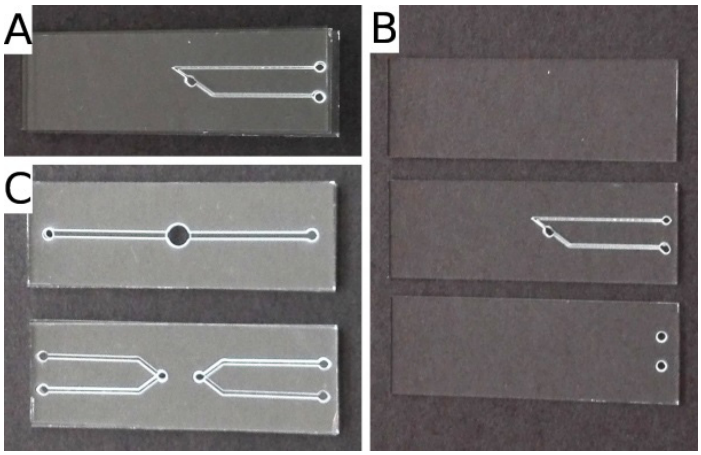

Figure 1: Microsystem for shrimp egg cultivation

Panel A - assembled microsystem (basic version). Panel B - parts of the microsystem from top to bottom: substrate, inlet and outlet channel with cultivation chamber, inlet and outlet ports. Panel C - different designs of the microsystem.

The cultivations of shrimp eggs were tested within the fabricated systems under binocular magnifier with camera (Canon EOS 600D, fixed focal length of lens 50 mm) and appropriate software (Canon EOS Utility).

The adult shrimps were obtained from local pet store (PetProfi s.r.o., ZOO shop Benji, Děčín 1, Czech Republic). The nomenclature was adopted and determination was done according to the publication [6]. The eggs were collected from the shrimp carrying eggs at time of visibly developed eyes of the larvae in the egg and were introduced into the microsystem. The eggs cultivation tests were performed in perfusion conditions. Water in perfusion conditions were actuated by a microfluidic pressure system OB1 (Pepiniere Paris Sante Cochin – ELVESYS, France)). To mimic the motion of shrimp carrying the eggs, the perfusion condition was set to the sets of pulses with 30 seconds between each set of pulses. Each set of pulses consisted of 5 pulses of duration 1 second each pulse with 0.5 second time between individual pulses. The flow of the water in the microsystem was set to 200 μl/min in pulsing state and to 10 μl/min in the rest state of the microsystem. The tests were performed in duplicates with two eggs as the tests served only as a proof of concept.

Results and discussion

Designed microsystem was powder blasted and tested with shrimp eggs. Due to powder blasting technology, finalization of the cultivation system was very fast and tests could be performed within one day or several hours, depending on the system complexity.

The shrimp eggs were successfully cultivated within the fabricated microsystems in perfusion conditions. We found out that if the design of the cultivation chamber was optimized for the water flow, the shrimp eggs cultivations could be performed. The cultivation of the shrimp egg is presented in Fig. 2, panels A–F. Hatched larvae can move freely in the cultivation chamber and can be pulled out of the microsystem simply by switching the water flow in the microsystem to the opposite direction.

In our test all eggs hatched confirming our proof of concept that microsystems can be adapted for rearing the shrimp eggs. To authors knowledge rearing of shrimp eggs was never done before in microsystems. Our results are in good terms with results in [8–15]"container-title":"Scientific Reports", "page":"36385","volume":"6","source":"www.nature.com","abstract":"The zebrafish is a powerful genetic model organism especially in the biomedical chapter for new drug discovery and development. The genetic toolbox which this vertebrate possesses opens a new window to investigate the etiology of human diseases with a high degree genetic similarity. Still, the requirements of laborious and time-consuming of contemporary zebrafish processing assays limit the procedure in carrying out such genetic screen at high throughput. Here, a zebrafish control scheme was initiated which includes the design and validation of a microfluidic platform to significantly increase the throughput and performance of zebrafish larvae manipulation using the concept of artificial cilia actuation. A moving wall design was integrated into this microfluidic platform first time in literature to accommodate zebrafish inside the microchannel from 1 day post-fertilization (dpf where other organisms e.g. fishes, fish embryos, nematodes, fruit flies, bacteria can be successfully cultivated inside microsystems.

Due to the modularity the numbers of cultivation chambers can be adjusted thus enabling mass parallelization. Because there is exactly one egg per cultivation chamber and each cultivation chamber can be directly addressed, it is possible to change the stream of freshwater to saltwater for each cultivation chamber individually to continue with the cultivation of larvae in the microsystem e.g. for research purposes, retrieve only already hatched larvae from the microsystem and continue the cultivation only with the rest of the eggs or retrieve only dead, defective or fungal diseased eggs from the microsystem to prevent spoiling of the rest of the eggs in contrary to commercially used Zugske or Chase bottles [16] if the cultivation is already running.

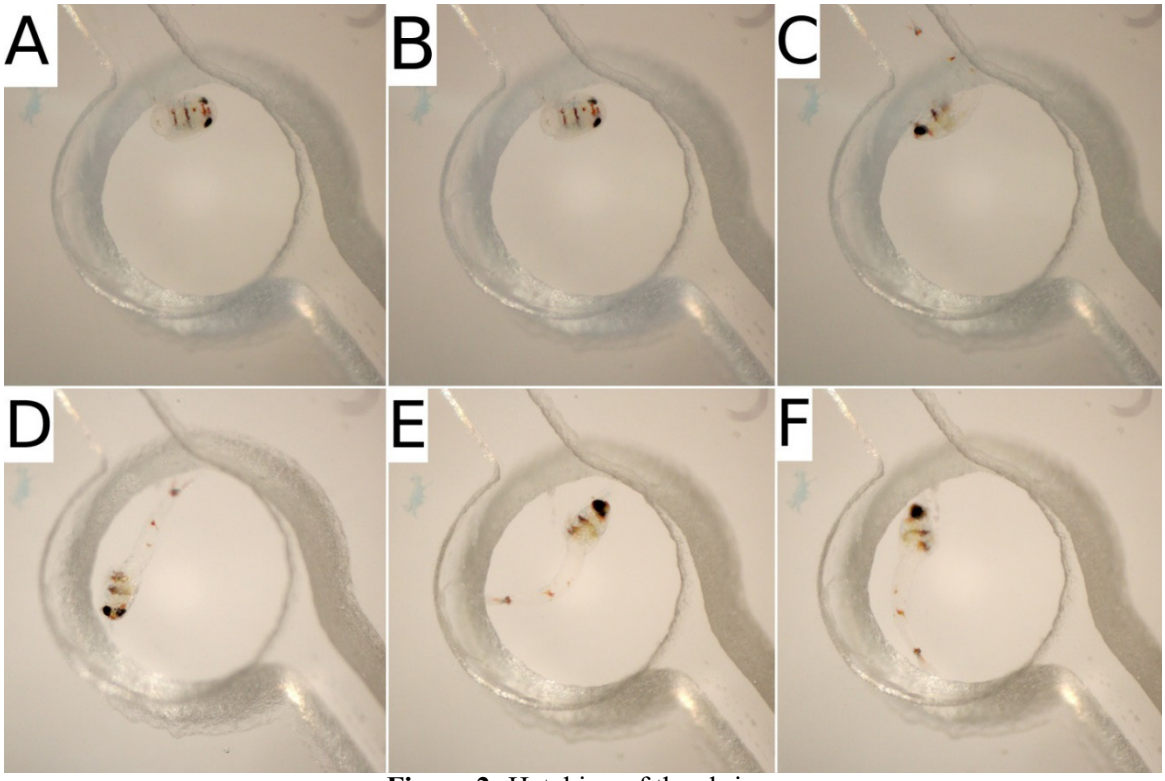

Figure 2: Hatching of the shrimp

Panel A - 1 day before hatching, panel B - 10 minutes before hatching, panel C - 1 minute after hatching. Panels D, E, F - 1 day after hatching, the larvae can swim and move freely in the cultivation chamber

Conclusion

The presented powder blasted microsystem is reusable, quick and easy to assemble and operate. Due to the modular approach of the systems assembly, the design and the size (width, length, height) and number of the shrimp egg cultivation chambers can be easily adjusted thus enabling mass parallelization and can be adopted for eggs of any other shrimp.

The microsystem design is simple enough to be implemented in plastic injection molding mass production technology which will reduce it cost.

The performed tests have shown that the fabricated system can be adjusted for further study of shrimp larval development.

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