Indian J. Fish., 61(4) : 131-133, 2014

Note

Effect of selected chemicals on inducing relaxation in the dog conch *Laevistrombus canarium* (Linnaeus, 1758)

V. VENKATESAN AND C. KALIDAS*

Central Marine Fisheries Research Institute, Cochin - 682 018, Kerala, India
**Tuticorin Research Centre of Central Marine Fisheries Research Institute, Tuticorin - 628 001, Tamil Nadu, India*
e-mail: venkatcmfri@yahoo.co.in

ABSTRACT

Adult dog conchs *Laevistrombus canarium* (60.6±1.23 mm shell length) were subjected to selected potential relaxant chemicals *viz.,* magnesium chloride ($MgCl_2$), magnesium sulphate ($MgSO_4$), EDTA (ethylenediaminetetraacetic acid disodium), 2-phenoxyethanol (PE), menthol (ME) and the time taken to induce relaxation and recovery were studied. All the treatments were observed continuously for 30 min post-exposure. Among the selected relaxants, $MgCl_2$ (30 g l^{-1}) successfully induced adequate relaxation in 24.5 min. Relaxed conch presented an extended mantle and exhibited slow reaction to physical manipulation. Conchs exposed to all other treatments, retracted into their shells and did not show any sign of relaxation during the exposure period.

Keywords: Dog conch, *Laevistrombus canarium*, Magnesium chloride, Relaxant chemicals, Relaxation

Relaxant chemicals are extensively used on marine molluscs for a variety of purposes including reduction of stress associated with handling and transportation of farmed molluscs (Ross and Ross, 1999); physical examination, sizing, and pearl-seeding (White *et al.,*1996); suppression of spawning during handling (Heasman *et al.,*1995); safe non-mechanical removal of animals from culture tanks, prevention of mechanical injury during routine husbandry (White *et al.,*1996); facilitation of pearl culture operations (Aquilina and Roberts, 2000); sex identification and bioactive compound recovery (Noble *et al.,*2009).

Till date, natural pearl production in the dog conch, *Laevistrombus canarium* has not been reported. However, it has been attempted in another species *i.e.,* queen conch *Strombus gigas* (Creswell and Davis, 1991). Pearl oysters do not need relaxation prior to surgery (Acosta-Salmon *et al.,* 2004) whereas gastropods require some degree of relaxation (Aquilina and Roberts, 2000). For production of cultured pearl, nucleus and mantle tissue are implanted in the gonad of a recipient pearl oyster (Acosta-Salmon *et al.,* 2004) and a similar process is necessary for producing cultured pearl in conch. Any attempt of pearl seeding in conch is difficult because it can completely retract its body into the shell. Moreover, the gonad is located in the upper spire of the shell, and hence it is hard to reach through the conch shell aperture. Relaxation of the conch is necessary prior to pearl seeding. Relaxants used should evoke rapid response, be 100% effective, allow for complete recovery of the exposed animal and be non-toxic for both cultured animals and the operator (Acousta-Salmon and Davis, 2007). Several studies have been undertaken on the effect of a variety of relaxants on induction of relaxation in marine molluscs *viz., Pecten fumatus* (Heasman *et al.,*1995); *Haliotis midae* (White *et al.,*1996); *Pinctada albino* and *Pinctada margaritifera* (Norton *et al.,*1996); *Strombus gigas* (Acosta-Salmon and Davis, 2007) and *Saccostrea glomerata* (Butt *et al.,* 2008). In the present study, an attempt was made to test the effect of selected relaxant chemicals in the dog conch *Laevistrombus canarium* for facilitating pearl seeding.

Live dog conchs (60.6±1.23 mm in shell length) were collected from the Mandapam trawl landing centre (lat. 09º17′11.3″ N and long 79º 09′17.1″ E) along the Palk Bay. The collected specimens were immediately placed in plastic containers having aerated seawater and were shifted to the laboratory. In the laboratory, shell length of the conchs was measured with vernier calipers to the nearest 0.1 mm, and the body weight was taken to the nearest 0.01 g with an electronic balance. In the wet

laboratory, they were maintained in 75 l FRP tanks with sand substratum having flow-through water exchange system. Conchs were fed with seagrass and brown seaweed. After 4-5 h of acclimatisation, these animals were tested for relaxation with six potential relaxant chemicals.

Water soluble anaesthetic chemicals reported for different molluscs *viz.,* 2-phenoxyethanol (PE) at 3 ml l^{-1} (Norton *et al.,*1996; White *et al.,*1996); menthol (ME) at 0.25 g l^{-1} (Norton *et al.,*1996); magnesium chloride ($MgCl_2$) at 30 g l^{-1} (Heasman *et al.,*1995); Magnesium sulphate ($MgSO_4$) at 22 g 100 ml^{-1} and EDTA (ethylenediaminetetraacetic acid disodium) at 5 g 100 ml^{-1} (White *et al*.,1996) were selected for the experiments. The control animals were exposed to fresh seawater with no chemical treatment. $MgCl_2$, $MgSO_4$ and EDTA readily dissolved in seawater. ME crystals were weighed and placed in a small amount of warm seawater (30°C) to melt. After melting the crystals, the solution was poured into 2 l bucket and mixed by manual stirring. PE was dissolved in seawater in a small bucket and stirred to disperse the chemical in small droplets. After preparation, temperature, salinity and pH of the treatment solutions were measured.

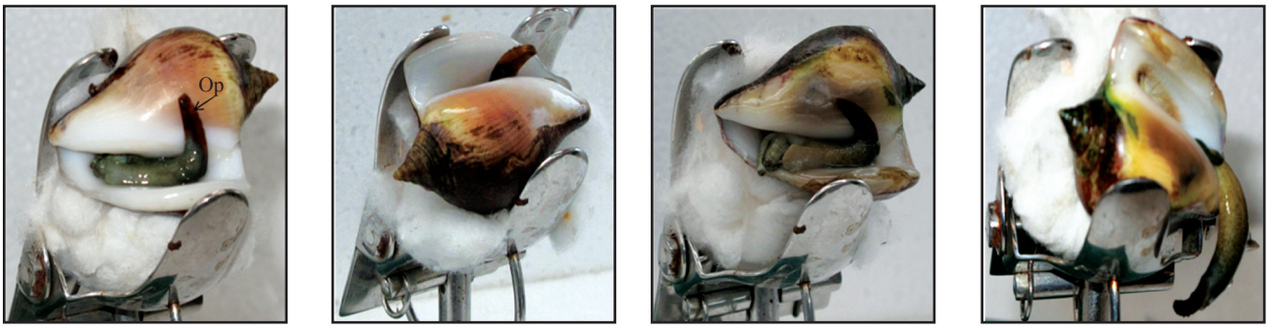
The methodology followed was as per Norton *et al*., (1996) and Acosta-Salmon and Davis (2007). Animals were individually stocked in 2 l buckets for each treatment in five replicates. Buckets were provided with UV filtered (5 µm) seawater without aeration, and water flow. Animals were initially placed upside down in the bucket to check for righting response *i.e.,* the ability to regain normal position. When the animal righted themselves or moved from their original position, they were inverted again. This process was repeated until animals were unable to right themselves. Righting response was also used as the measure to check recovery after treatment. Animals were tested for relaxation by removing them from the treatment, placing them on a pearl oyster shell stand, and gently but firmly pulling the foot using operculum. If the animal reacted to pulling they were returned to the relaxant. This step was repeated every 5 min until the animal relaxed completely. All animals were tested for relaxation by this method for 30 min post-exposure. Subsequently, the animals were returned to the buckets containing aerated fresh seawater in an upside down position and were again monitored for recovery. Recovery was measured as the time taken for righting response to occur.

The physical characteristics such as temperature, pH and salinity in all treatments were within optimal levels for *L. canarium,* except in $MgCl_2$ which showed an increase in salinity to 49 ppt (Table 1). However, this abnormal rise of salinity for a short duration exposure did not cause any adverse effect in *L. canarium*. Acosta-Salmon and Davis (2007) also reported similar observation with *S. gigas.*

Conchs in the control showed normal behaviour and did not retract into their shell and remained at the edge of the shell aperture (Fig. 1a). Of the five anesthetic treatments used to induce relaxation in *L. canarium*, only $MgCl_2$ induced adequate relaxation (with in 24.5 min post-exposure) and indicated suitable for pearl seeding trials. In all other relaxant treatments, conchs retracted into their shells without any visible sign of relaxation during the treatment period (Fig.1b). Survival was 100%

Table 1. Physico-chemical characteristics of the treatment media

Treatment	Temperature (°C)	pH	Salinity (‰)
PE	28.5	8.1	35.4
ME	28.2	8.2	36.1
$MgCl_2$	29.3	7.8	49.1
$MgSO_4$	29.1	8.1	35.7
EDTA	28.9	8.0	35.3
Control	28.0	8.2	36.5



(a) (b) (c) (d)

Fig.1. (a) Control conch, (b) Conch exposed to relaxants other than $MgCl_2$ with operculum retracted into the shell, Conch exposed to $MgCl_2$ before (c) and after (d) pulling operculum

in all the treatments, except in EDTA where two conchs in the treatment died within 48 h of exposure.

Movements of animals exposed to $MgCl_2$ after recovery were found similar to that of control animals. Animals exposed to treatments other than $MgCl_2$, closed their operculum in the initial 3 sec, then they became calmer after 13 sec and were unable to retract operculum back into their shells (Fig.1b). During relaxation, the conchs exposed to $MgCl_2$ showed an extended mantle and a relaxed foot (Fig.1c and d). Similar behaviour was also reported by Acosta-Salmon and Davis (2007) in *S. gigas* except for the time taken by the animals to relax, which was slightly higher in case of *S. gigas* (*i.e.,* 20 min). *S. gigas* is larger in size compared to *S. canarium* and it has been reported that the time needed for relaxation is generally longer for larger molluscs (Mc Craw, 1958; Meier-Brook, 1976). There are several reports on use of $MgCl_2$ for relaxation in other marine molluscs *viz., Ostrea edulis* (Culloty and Mulcahy, 1992); *Pecten fumatus* (Heasman *et al.,* 1995); *P. albino* and *P. margaritifera* (Norton *et al.,* 1996); *Strombus gigas* (Acosta-Salmon and Davis, 2007); *Saccostrea glomerata* (Butt *et al.,* 2008) and *Dicathais orbita* (Noble *et al.,* 2009). In the present study, no other chemicals tested induced relaxation in *L. canarium* with respect to their respective concentration and time. However, the chemicals such as 2-phenoxyethanol (White *et al.,*1996 in *H. midae*; Mamangkey *et al.* 2009 in *P. maxima*), menthol (Mamangkey *et al.,* 2009 in *P. maxima*), magnesium chloride (Heasman *et al.,* 1995 in *Pecten fumatus*; Butt *et al.,* 2008 in *Saccostrea glomerata*; Acosta-Salmon and Davis, 2007 in *Strombus gigas*), magnesium sulphate (White *et al.,* 1996 in *H. midae*; Heasman *et al.,* 1995 in *P. fumatus*), and EDTA (White *et al.,* 1996 in *H. midae*) have been successfully used to relax other invertebrate species. Strong muscular contraction was noted in *S. gigas*, with all these chemicals other than magnesium chloride. Aquilina and Roberts (2000) reported that pearl seeding requires rapid relaxation and extension as well as softening of the foot which was observed in the present study with respect to magnesium chloride.

Acknowledgements

The authors express their sincere gratitude to the Director and also to the Head, Molluscan Fisheries Division, CMFRI, Kochi for their support and encouragements.

Date of Receipt : 22.05.2013
Date of Acceptance : 21.06.2014

References

Acosta-Salmón, H., Martínez-Fernández, E. and Southgate, P. C. 2004. A new approach to pearl oyster broodstock selection: can saibo donors be used as future broodstock. *Aquaculture*, 231: 205-214.

Acosta-Salmón, H. and Davis, M. 2007. Inducing relaxation in the queen conch *Strombus gigas* (L.) for cultured pearl production. *Aquaculture*, 262: 73-77.

Aquilina, B. and Roberts, R. 2000. A method for inducing muscle relaxation in the abalone, *Haliotis iris*. *Aquaculture*, 190: 403-408.

Butt, D., O'Connor, S. J., Kuchel, R., O'Connor, W. A. and Raftos, D. A. 2008. Effects of the muscle relaxant, magnesium chloride, on the Sydney rock oyster (*Saccostrea glomerata*). *Aquaculture*, 275: 342-346.

Creswell, L. and Davis, M. 1991. Queen conch, the well-bred queen of the Caribbean. *World Aquacult.,* 22 (1): 28 - 40.

Culloty, S. C. and Mulcahy, M. F. 1992. An evaluation of anaesthetics for *Ostrea edulis* (L.). *Aquaculture*, 107: 249 - 252.

Heasman, M. P., O'Connor, W. A. and Fraser, A. W. J. 1995. Induction of anaesthesia in the commercial scallop, *Pecten fumatus* Reeve. *Aquaculture*, 131: 231 - 238.

Mamangkey, N. G. F, Acosta-Salmón, H. and Southgate, P. C. 2009. Use of anaesthetics with the silver-lip pearl oyster, *Pinctada maxima* (Jameson). *Aquaculture*, 288: 280-284.

Mc Craw, B. 1958. Relaxation of snails before fixation. *Nature,* 4608: 575.

Meier-Brook, C. 1976. The influence of varied relaxing and fixing conditions on anatomical characters in a *Planorbis* species. *Basteria,* 40: 101 – 106.

Noble, W. J., Cocks, R. R., Harris, J. O. and Benkendorff, K. 2009. Application of anaesthetics for sex identification and bioactive compound recovery from wild *Dicathais orbita*. *J. Exp. Mar. Biol. Ecol.,* 380: 53-60.

Norton, J. H., Dashorst, M., Lansky, T. M. and Mayer, R. J. 1996. An evaluation of some relaxants for use with pearl oysters. *Aquaculture,* 144: 39-52.

Ross, L. G. and Ross, B. 1999. *Anaesthetic and sedative techniques for aquatic animals*. Blackwell Science, Oxford, UK., 176 pp.

White, H. I., Hecht, T. and Potgieter, B. 1996. The effect of four anaesthetics on *Haliotis midae* and their suitability for application in commercial abalone culture. *Aquaculture*, 140: 145-151.