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Effect of cations on sperm motility of mangrove red snapper *Lutjanus argentimaculatus*

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Abstract
The objective of this study was to determine the effect of cations on sperm motility of mangrove red snapper *Lutjanus argentimaculatus*. The research was conducted in order to determine the optimal concentration of cations as Na^+, Ca^{2+}, K^+ or Mg^{2+}. The concentrations of these cations were 0.2, 0.4, 0.6 or 0.8 M, respectively. The percentage of motile cells and duration of sperm motility were estimated after dilution sperm into cation media. The results showed that the concentration of 0.4 M (NaCl, KCl, $CaCl_2$) or 0.6 M $MgCl_2$ reached the best duration and percentage of sperm motility. We used these media as artificial insemination media for fertilizing matured eggs. The results showed that the fertilized and hatching rates in 0.4M NaCl were higher than that in other treatments. In conclusion, our findings suggest that a medium such as 0.4 M NaCl can be used as an artificial insemination medium for artificial propagation of mangrove red snapper.

Keywords: Mangrove red snapper, *Lutjanus argentimaculatus*, cation, motility, sperm

1. Introduction

Spermatozoa of all marine fish species are immovable in the testis or seminal plasma. However, motility of spermatozoa happens after they are liberated into surrounding aqueous environment during natural reproduction or into a diluent during artificial reproduction [2, 3, 5, 6]. Sperm motility parameters such as the percentage of motile cells (MOT) and duration of sperm motility (DSM) are key parameters in estimating fish spermatozoa quality and fertilizing capacity [1, 4, 6]. Sperm motility parameters are influenced by several factors, such as temperature, pH, cations, osmolality and dilution ratio [2, 3, 10, 12]. Understanding the effects of these factors is a good method for artificial insemination and a good techniques for sperm chilled storage (short-term storage) or cryopreservation (long-term storage) [5, 6].

Mangrove red snapper *Lutjanus argentimaculatus* is a tropical marine fish species of high demand and economic value. However, little research on reproductive physiological information has been performed with this species [7]. Vuthiphandchai *et al.* [14] have described the sperm cryopreservation of this species. In addition, a study on the effect of pH, temperature, osmolality and semen/diluent ratio on sperm motility parameters of mangrove red snapper was also conducted by Le and Nguyen [11]. However, research on the effect of cations on sperm motility parameters of this species remains unknown. Given the paucity of knowledge on the above-stated parameters, this study aimed to assess the effects of cations on sperm motility parameters (MOT and DSM) in mangrove red snapper *Lutjanus argentimaculatus*. The effects of these factors on sperm motility were assessed in terms of sperm motility parameters: motile cells, duration and also in fertilizing capacity**.**

2. Materials and Methods

2.1 Fish and gamete collection

All experiments were conducted at the laboratory of aquatic animal reproductive physiology, Institute of Aquaculture, Nha Trang University, Viet Nam. Adult mangrove red snapper (BW=4.3±0.6 kg, TL= 57.7±2.5 cm) were collected from the wild population. Milt was collected during the spawning season by abdominal massage after anesthetizing with ethylene glycol monophenyl ether (Merk, Germany; 100 ppm v:v). The urogenital areas were blotted dry with a paper towel in order to avoid fecal, urine, mucus and seawater contamination. Milt was stripped and put into 15 ml Eppendorf tube on crushed ice until analysis (no more than 3 hours).

2.2 Spermatozoa motility assessment
Spermatozoa motile parameters (percentage of motile cells (MOT) and duration of sperm motility (DSM) were determined immediately after initiation of sperm activation and continued until 100% of spermatozoa were immotile. Motility of sperm was evaluated after mixing 1 µl of sperm with 99 µl of diluent. The consecutive positions of the recorded sperm heads were observed at 400× magnification under a light microscope (Olympus BX41, Japan). Sperm movement was then recorded (Olympus camera, 60 frames/s, 10s film duration). MOT was determined using a CASA (computer aided for sperm analysis) plug-in developed for Image J (see website: http://rsb.info.nih.gov/ij/download.html). The DSM (duration of sperm motility) was calculated as the time taken for estimated proportion of sperm after activation to sperm motility fell below 10% [9, 12]. Each experiment was performed on three males. All experiments were implemented in triplicate at room temperature (25-27ºC).

2.3 Effect of cations on sperm motility
The effect of cations (Na^+, K^+, Ca^{2+} and Mg^{2+}) on mangrove red snapper spermatozoa motility parameters was also evaluated with sperm from three males. Sperm samples were diluted with each solution containing 0.2, 0.4, 0.6 or 0.8 M NaCl, KCl, $CaCl_2$ or $MgCl_2$ at the ratio of 1:100 (1 µl of semen with 99 µl of each solution). Each observation was repeated three times.

2.4 Fertility trials
The fertilization rate (%) and the hatching rate (%) of eggs fertilized with the experimental sperm samples were evaluated using a ratio of 1:1,000,000 (egg:sperm) in each medium such as 0.4 M NaCl, 0.4 M KCl, 0.4 M $CaCl_2$, 0.6 M $MgCl_2$, ASW (artificial seawater) or seawater. The fertilized eggs were incubated in these media at 29 - 30ºC and the dead eggs which became muddy or opaque were discarded from each medium after fertilization. The fertilization rate was calculated by the number of gastrula stage embryos per the number of eggs. The hatching rate was assessed as the percentage of hatched fry from their fertilized eggs.

2.5 Data analysis
Data were expressed as mean ± standard deviation (SD) or standard error (SE). The data on the effect of cations on sperm motility parameters were analyzed by one-way ANOVA using SPSS version 22.0. The Duncan test was used for *post hoc* comparisons. A probability value *p* of 0.05 was considered significantly different.

3. Results and Discussion
The percentage of motile cells (MOT) at 10s (85.0±2.9%) in NaCl at the concentration of 0.4 M was higher than that in 0.2, 0.6 or 0.8 M ($p<0.05$). This parameter at 180s was 17.7±1.5% higher than the other treatments (p<0.05). In addition, the duration of sperm motility (DSM: 251.0±12.4 s) in NaCl at the concentration of 0.4 M was higher than that in 0.2, 0.6 or 0.8 M (p<0.05). So, the medium 0.4M NaCl was considered as a good medium for sperm activation (Figure 1).

The concentration of 0.4 M KCl resulted in the best sperm motility parameters (MOT at 10s: 85.0±2.9%, MOT at 120s: 21.7±1.7%, DSM: 163.0±9.1 s) compared to other treatments ($p<0.05$). As a result, the KCl at the concentration of 0.4 M was used for fertility trials in this study (Figure 2).

The MOT at 10s, MOT at 120s and DSM (88.3±1.7%, 15.0±2.9% and 120.67±1.32 s, respectively) were maximal in medium containing 0.4 M $CaCl_2$ after sperm activation. Sperm motility parameters (MOT and DSM) rapidly decreased in media containing 0.6 M or more (Figure 3).

The DSM (156.0±6.1s), MOT at 10s (71.7±2.9%) and MOT at 120s (18.3±4.4%) in $MgCl_2$ at the concentration of 0.6 M was higher than to compare to other treatments ($p<0.05$) (Figure 4). These results showed that mangrove red snapper sperm is sensitive to $CaCl_2$ or $MgCl_2$. Therefore, the sperm motility parameters in these media were also used for fertility trials.

At the ratio of 1,000,000:1 (sperm:egg), the fertilization rate and hatching rate (67.1±2.5%, 52.5±2.4%) in 0.4 M NaCl medium were higher than that in the other treatments ($p<0.05$). There were no significant differences in fertilization rate and hatching rate in 0.4 M KCl (57.2±4.4%, 42.3±2.0%), ASW (57.8±1.3%, 41.3±2.0%) or seawater (52.1±1.8%, 39.2±3.9%) media ($p>0.05$) (Figure 5).

The sperm motility parameters (MOT or DSM) in marine fish were affected and controlled through their sensitivity to cations concentrations. Moreover, the mechanisms of initiation of sperm motility in several marine fish species were reviewed by several authors [3, 6]. According these authors, they suggested that (1) sperm motility in tilapia was controlled by extracellular Ca^{2+} ions combined with Na^+ solution as well as osmotic pressure; (2) the presence of Ca^{2+} allowed motility to occur at high osmotic pressure; and that extracellular Ca^{2+} was not required for initiation of motility in puffer fish sperm but it was a prerequisite for the initiation of sperm motility in herring. In contrast to these species, sperm motility parameters were immediately activated by Na^+, K^+, Ca^{2+}, Mg^{2+} ions. Nevertheless, our findings suggest that the optimal sperm motility parameters in mangrove red snapper were stimulated in 0.4 M NaCl, 0.4 M KCl, 0.4 M $CaCl_2$ or 0.6 M $MgCl_2$. These results were similar to the other marine fishes species such as in rabbit fish *Siganus guttatus* [13] at 0.4 M NaCl, 0.4 M KCl, 0.4 M $CaCl_2$, 0.6 M $MgCl_2$. However, the optimal cation concentrations in mangrove red snapper were different to another marine fish species such as in yellow croaker *Larimichthys polyactis* [10] at 0.4 M NaCl, 0.4 M KCl, 0.2 M $CaCl_2$, 0.2 M $MgCl_2$; tiger grouper *Epinephelus fuscoguttatus* [8] at 0.6 M NaCl, 0.4 M KCl, 0.4 M $CaCl_2$, 0.4 M $MgCl_2$; and waigieu seaperch *Psammoperca waigiensis* [12] at 0.6 M NaCl, 0.6 M KCl, 0.2 M $CaCl_2$, 0.2 M $MgCl_2$. Therefore, the optimal concentration of each cation in marine fish species is species-specific. Further research needs to carry out the surrounding concentrations of these media to get more information about the mechanisms of effects of ions on sperm motility parameters in this species.

It is clear that sperm motility parameters play an important role in evaluating the fertilizing ability of fish sperm. In addition, the fertilizing ability not only clearly depends on the activated sperm ratio per egg, but also on time duration of sperm motility [4, 6]. It is possible to increase the fertilizing capacity of the fish sperm by using suitably activated solutions that can keep sperm motility for a long time. In the present study, the highest sperm motility parameters were attained in the medium of 0.4 M NaCl. Therefore, the highest fertilizing rate and hatching rate were observed after stimulation of sperm in above medium. Thus, the medium of 0.4 M NaCl can be used for the artificial propagation of mangrove red snapper in the future.

In conclusion, our findings suggest that a medium such as 0.4 M NaCl can be used for artificial propagation of mangrove red snapper.

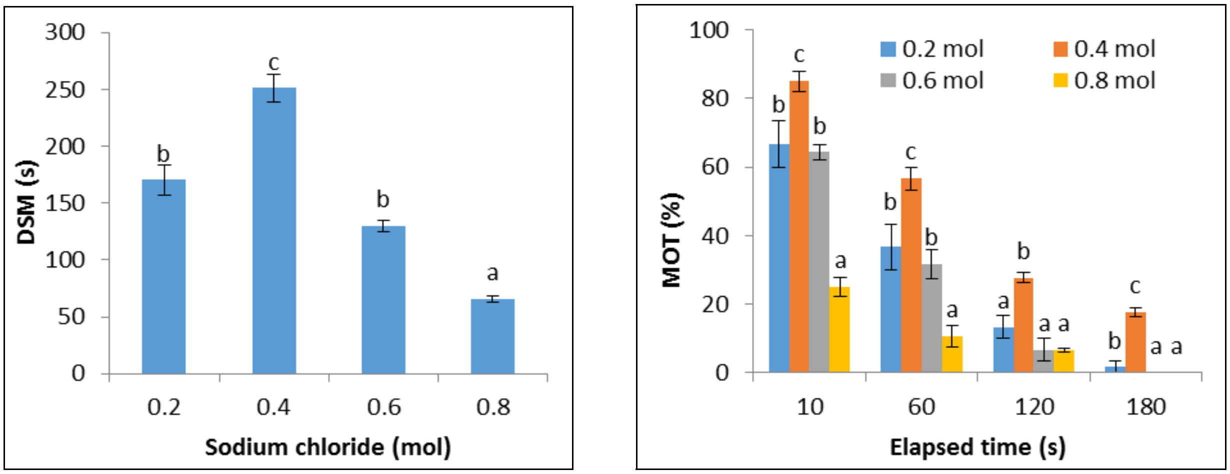

Fig 1: Effect of sodium chloride concentrations on the percentage of motile cells (MOT) and duration of sperm motility (DSM) in mangrove red snapper *Lutjanus argentimaculatus* sperm after activation. Values with the different alphabetic letters indicate significant differences between sodium ion concentrations (P<*0.05*).

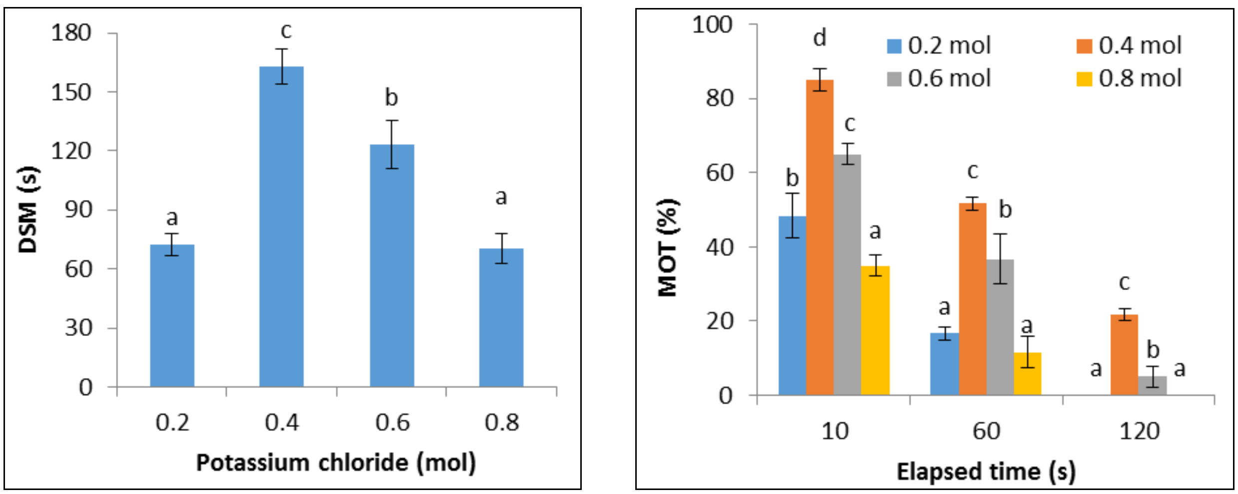

Fig 2: Effect of potassium chloride concentrations on the percentage of motile cells (MOT) and duration of sperm motility (DSM) in mangrove red snapper *Lutjanus argentimaculatus* sperm after activation. Values with the different alphabetic letters indicate significant differences between sodium ion concentrations (*P<0.05*).

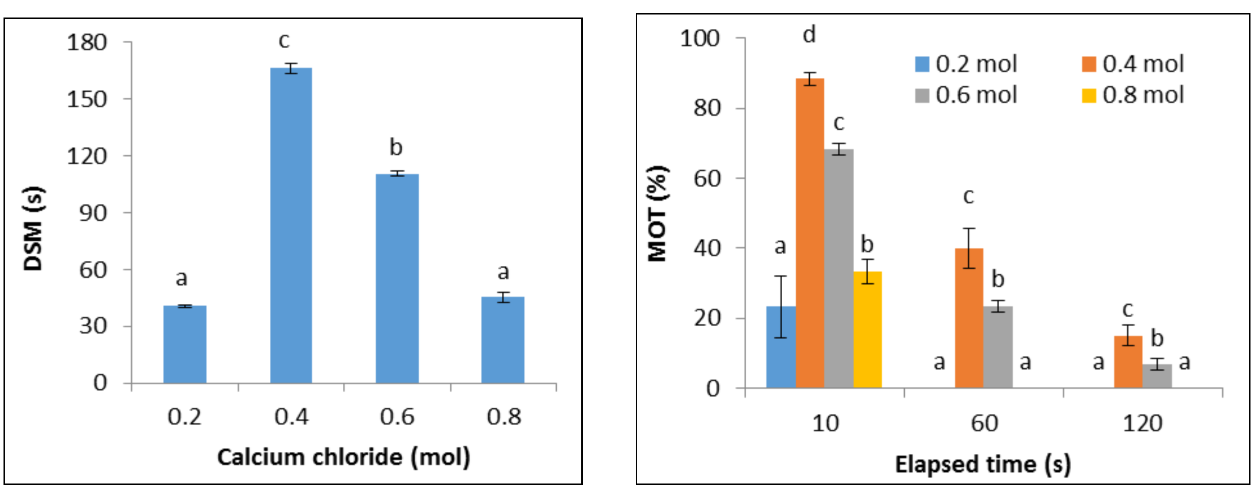

Fig 3: Effect of calcium chloride concentrations on the percentage of motile cells (MOT) and duration of sperm motility (DSM) in mangrove red snapper *Lutjanus argentimaculatus* sperm after activation. Values with the different alphabetic letters indicate significant differences between sodium ion concentrations (*P<0.05*).

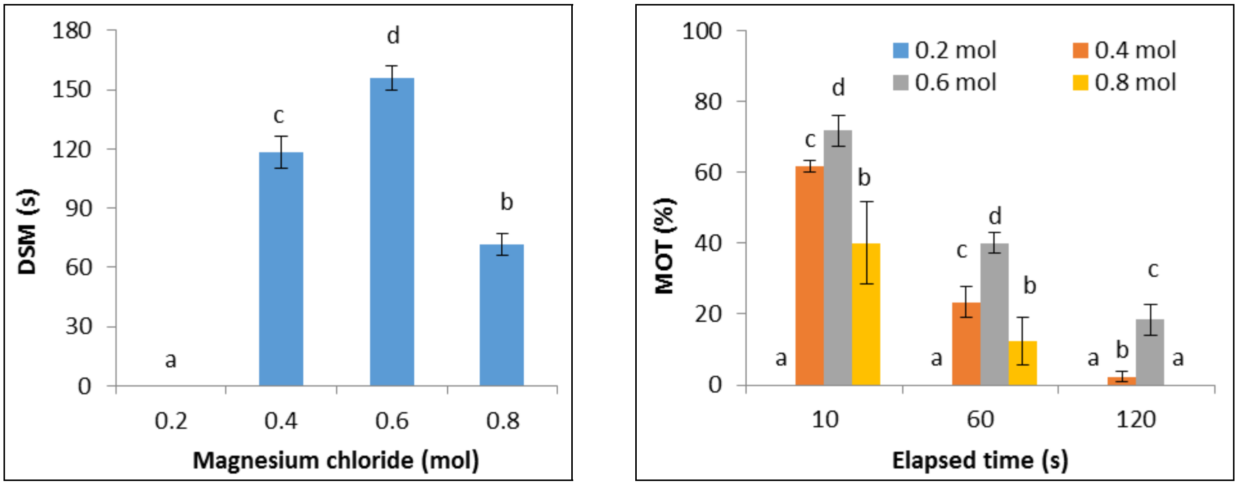

Fig 4: Effect of magnesium chloride concentrations on the percentage of motile cells (MOT) and duration of sperm motility (DSM) in mangrove red snapper *Lutjanus argentimaculatus* sperm after activation. Values with the different alphabetic letters indicate significant differences between sodium ion concentrations (*P<0.05*).

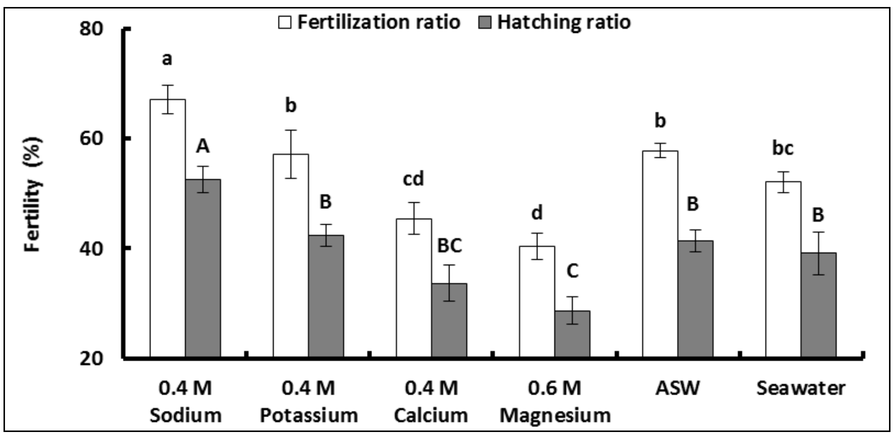

Fig 5: Fertilization ratio and hatching ratio of mangrove red snapper *Lutjanus argentimaculatus* in different fertility environments. Values with the different alphabetic letters (capital for hatching ratio, small for fertilization ratio) indicate significant differences between fertility environments (*P<0.05*).

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5. References

1. Abascal FJ, Cosson J, Fauvel C. Characterization of sperm motility in sea bass: the effect of heavy metals and physicochemical variables on sperm motility. Journal of Fish Biology. 2007; 70:509-522.
2. Alavi SMH, Cosson J. Sperm motility in fishes: (I) effects of temperature and pH. Cell Biology International. 2005; 29:101-110.
3. Alavi SMH, Cosson J. Sperm motility in fishes: (II) Effects of ions and osmolality. Cell Biology International. 2006; 30:1-14.
4. Browne RK, Kaurova SA, Uteshev VK, Shishova NV, McGinnity D, Figiel CR *et al.* Sperm motility of externally fertilizing fish and amphibians. Theriogenology. 2015; 83:1-13.
5. Cosson J, Groison AL, Suquet M, Fauvel C, Dreanno C, Billard R. Marine fish spermatozoa: racing ephemeral swimmers. Reproduction. 2008; 136:277-294.
6. Cosson J, Groison AL, Suquet M, Fauvel C, Dreanno C, Billard R. Studying sperm motility in marine fish: an overview on the state of art. Journal Applied Ichthyology. 2008; 24:460-486.
7. FAO. http://www.fao.org/fishery/species/3134/en. Food and Agriculture Organization of the United Nations Accessed. 2016.
8. Hoang TH, Le MH. Several properties of milt and effects of cations on sperm motility in tiger grouper (*Epinephelus fuscoguttatus* Forsskal, 1775). Journal of Mekong Fisheries. 2014; 3:23-32 (in Vietnamese).
9. Le MH, Brown PB. Effects of time after hormonal stimulation on milt properties in waigieu seaperch *Psammoperca waigiensis*. The Israeli Journal of Aquaculture - Bamidgeh. 2016; 68:9.
10. Le MH, Lim HK, Min BH, Park MS, Son MH, Lee JU *et al.* Effects of varying dilutions, pH, temperature and cations on sperm motility in fish *Larimichthys polyactis*. Journal of Environmental Biology. 2011; 32:271-276.
11. Le MH, Nguyen DT. Effects of dilution ratio, pH and osmolality on sepermatozoa motility in silver red snapper *Lutjanus argentimaculatus*. Journal of Fisheries Science and Technology. 2015; 3:28-32 (in Vietnamese).
12. Le MH, Pham QH. Sperm motilities in wigieu seaperch, *Psammoperca waigiensis*: Effects of various dilutions,

pH, temperature, osmolality, and cations. Journal of the World Aquaculture Society. 2016. Accepted.
13. Vo TNG, Le MH, Phan VU, Pham QH. Effects of dilution ratio, pH and osmolality on sperm motility in rabbit fish *Siganus guttatus*. Journal of Fisheries Science and Technology. 2014; 3:26-30. (in Vietnamese).
14. Vuthiphandchai V, Chomphuthawach S, Nimrat S. Cryopreservation of red snapper (*Lutjanus argentimaculatus*) sperm: Effect of cryoprotectants and cooling rates on sperm motility, sperm viability, and fertilization capacity. Theriogenology. 2009; 72:129-138.