© 2011 Triveni Enterprises
Vikas Nagar, Lucknow, INDIA
editor@jeb.co.in
Full paper available on: www.jeb.co.in

J. Environ. Biol.
32, 271-276 (2011)
ISSN: 0254-8704
CODEN: JEBIDP

Effects of varying dilutions, pH, temperature and cations on spermatozoa motility in fish *Larimichthys polyactis*

Author Details

Minh Hoang Le	Department of Aquaculture, Pukyong National University, Busan 608-737, Korea
Han Kyu Lim (*Corresponding author*)	Aquaculture Management Division, National Fisheries Research and Development Institute, Gijang, Busan 619-902, Korea e-mail: limhk@nfrdi.go.kr
Byung Hwa Min	Aquaculture Management Division, National Fisheries Research and Development Institute, Gijang, Busan 619-902, Korea
Mi Seon Park	Aquaculture Management Division, National Fisheries Research and Development Institute, Gijang, Busan 619-902, Korea
Maeng-Hyun Son	Aquaculture Management Division, National Fisheries Research and Development Institute, Gijang, Busan 619-902, Korea
Jung Uie Lee	Aquaculture Management Division, National Fisheries Research and Development Institute, Gijang, Busan 619-902, Korea
Young Jin Chang	Department of Aquaculture, Pukyong National University, Busan 608-737, Korea

Publication Data

Paper received:
28 April 2009

Revised received:
21 May 2010

Accepted:
01 June 2010

Abstract

The objectives of this present study were to assess the effects of varying dilutions, pH, temperature and cations on spermatozoa motile parameters (SMPs) in fish *Larimichthys polyactis*. Optimal SMPs were observed when semen was diluted in artificial seawater (ASW) at a ratio of 1 to 100, with temperature of 10°C and pH 8.0. The spermatozoa of *L. polyactis* were immotile in distilled water and motile in solution containing different cations. Maximum SMPs were obtained in each solution containing 0.4 mol NaCl, 0.4 mol KCl, 0.2 mol $CaCl_2$ and 0.2 mol $MgCl_2$. This study provides baseline knowledge of *L. polyactis* spermatozoa sensitivity of pH, temperature and cationic effects.

Key words

Larimichthys polyactis, Spermatozoa motility, Dilution, Temperature, pH, Cations

Introduction

Spermatozoa of both freshwater and marine fish species are immotile in the testis and seminal plasma. Motility of spermatozoa occurs after they are released into surrounding aqueous environment during natural reproduction or into a diluent during artificial reproduction (Darszon *et al*., 1999; Alavi and Cosson, 2006; Cosson *et al*., 2008a,b). Spermatozoa motility is a prerequisite parameter in determining fish semen quality and fertilizing capacity (Alavi *et al*., 2004; Alavi and Cosson, 2005a,b; Abascal *et al*., 2007). Spermatozoa motility is also influenced by several factors, such as temperature (Willot *et al*., 2000; Alavi and Cosson, 2005b), pH (Ingermann *et al*., 2002; Alavi and Cosson, 2005 a,b; Zuccarelli *et al*., 2007), cations (Darszon *et al*., 1999; Linhart *et al*., 2003; Cosson, 2004; Alavi and Cosson, 2006; Alavi *et al*., 2007), osmolality (Linhart *et al*., 2003; Cosson, 2004; Alavi and Cosson, 2006; Alavi *et al*., 2007; Zuccarelli *et al*., 2007) and dilution ratio (Alavi *et al*., 2004; 2005a,b; 2007) in either aqueous environment or diluent. Understanding the effects of these factors is helpful to the aquaculture industry as it allows for the development of optimal artificial reproduction methods and contributes towards the knowledge-base of better short- and long-term fish semen preservation conditions (Alavi *et al*., 2007; Cosson *et al*., 2008a,b).

Larimichthys polyactis is traditionally an important commercial fish species in Korea. It migrates out to the East China

Sea in winter and returns to the Yellow Sea to spawn in spring (Kim *et al*., 1997). Its spawning season extends from April to June, peaking in May in the Eastern part of the Yellow Sea (Kim *et al*., 1997). Although basic reproductive and life-history information on this species is available (Trewavas, 1977), data on the effect of pH, temperature, cations and semen/diluent ratio on spermatozoa motility remains unknown. Given the paucity of knowledge on the above stated parameters, this study aimed to examine the effects of varying dilution, pH, temperature and cations on spermatozoa motility in *L. polyactis*. The effects of these factors on spermatozoa motility were assessed in terms of spermatozoa motile parameters (SMPs); velocity, movable ratio and duration of motility.

Materials and Methods

All experiments were carried out at the National Fisheries Research and Development Institute, Korea, during spawning season of June 2008. Male fish (23.31±0.17 cm total length and 128.76±0.57 g body weight) were maintained in a 2 m^3 tank supplied with seawater at a temperature of 17~21°C, salinity of 32~33 psu and 5~6 mgO_2l^{-1} at a flow rate of 0.2 ls^{-1}. Fresh semen samples were obtained by serial waves of abdominal pressure and stored in 1.5 ml Eppendorf tubes on ice until analysis.

SMPs were determined immediately after initiation of sperm activation until 100% of spermatozoa were immotile. The experiment was carried out on three males. All experiments were performed in triplicate at room temperature (20~22°C). Motility of spermatozoa was measured after mixing 1 µl of semen with 99 µl of diluent. The successive positions of the recorded spermatozoa heads were observed at 200× magnification under microscope (Axioskop 2 plus Zeiss, Gottingen, Germany).

Artificial seawater (ASW containing 27g NaCl, 0.5 g KCl, 1.2 g $CaCl_2$, 4.6 g $MgCl_2$, 0.5 g $NaHCO_3$ in a litter of distilled water and pH 7.8) was used for effect of varying dilution, pH and temperature.

Semen of three males was used to determine the effect of varying dilutions on spermatozoa motility. The effect of varying dilutions on spermatozoa motility was evaluated with ASW at the ratios of 1:25, 1:100 and 1:400 (semen:ASW).

Semen of three males was used to determine the effect of pH on the motility of *L. polyactis* spermatozoa. The influence of pH on spermatozoa motility was assessed using ASW titrated over a range of pH values (6.0, 7.0, 8.0, 9.0 and 10.0) at the ratio of 1:100 (1 µl of semen with 99 µl of ASW). The pH of ASW was measured by pH meter (Istek, Korea).

Semen of three males was used to test the effect of temperature on spermatozoa motility. To assess the influence of temperature on spermatozoa motility, semen samples were diluted with ASW adjusted to different temperature values of 0, 10, 20, 30 and 40°C at ratio of 1:100.

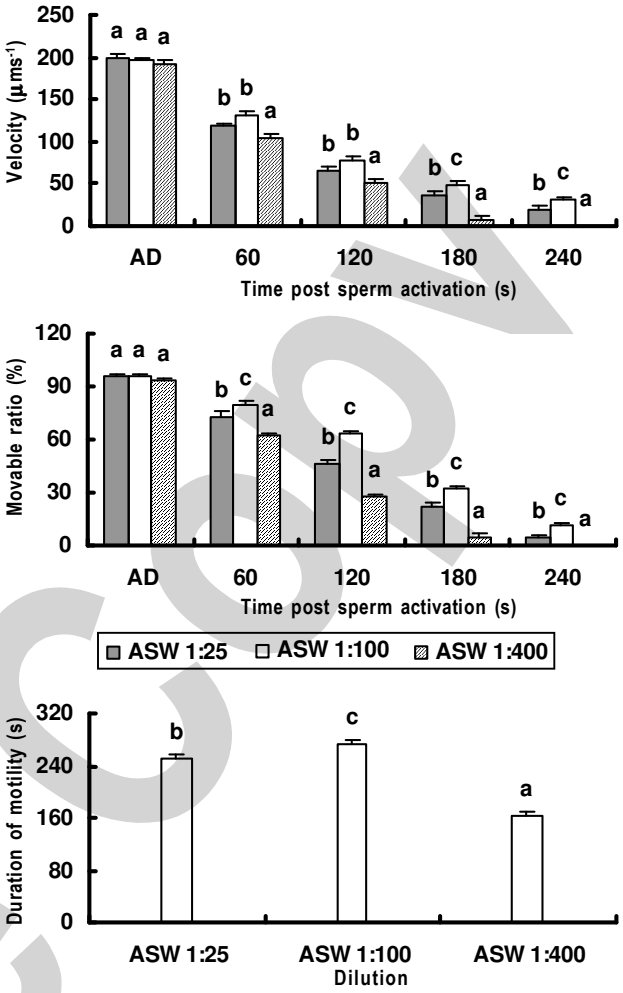

Fig. 1: Effect of varying dilutions on velocity, movable ratio and duration of motility in fish *L. polyactis* spermatozoa after activation in artificial seawater (ASW). Values with the different alphabetic letters on each bar indicate significant differences between dilution ratios ($p<0.05$). AD= After dilution

The effect of cations (Na^+, K^+, Ca^{2+} and Mg^{2+}) on *L. polyactis* spermatozoa motility also was carried out on three male. Semen samples were activated with solutions containing 0, 0.2, 0.4, 0.6, 0.8, 1.0 M NaCl, KCl, $CaCl_2$ and $MgCl_2$ at the ratio of 1:100 (1 µl of semen with 99 µl of solution containing NaCl, KCl, $CaCl_2$ and $MgCl_2$).

Data representing influence of dilution ratio, pH, temperature and cations on spermatozoa motility were analyzed by one-way ANOVA using SPSS version 16.0. The Ducan test was used for *post hoc* comparisons. Results are presented as mean ± SE. Differences with a probability value (P) of 0.05 ($P<0.05$) were considered significant.

Results and Discussion

Sperm dilution is a key to the stimulation of spermatozoa motility and the maintenance of its fertilizing ability of diluted fish sperm. High dilution produces a homogeneous sperm suspension which is suitable for synchronous motility observation

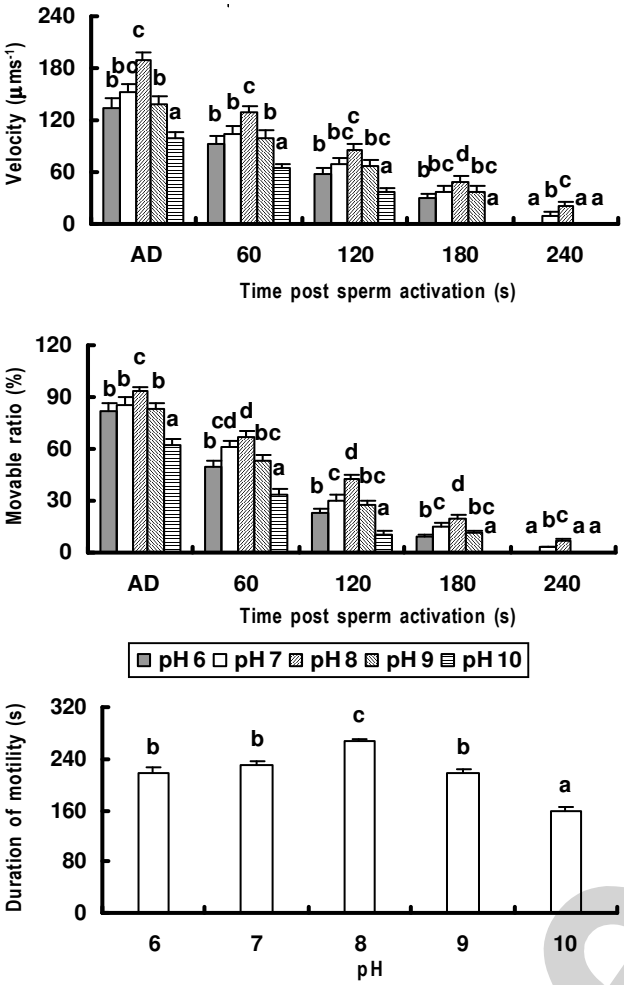

Fig. 2: Effect of different pH values on velocity, movable ratio and duration of motility in fish *L. polyactis* spermatozoa after activation. Values with the different alphabetic letters on each bar indicate significant differences between pH values (p<0.05). AD= After dilution

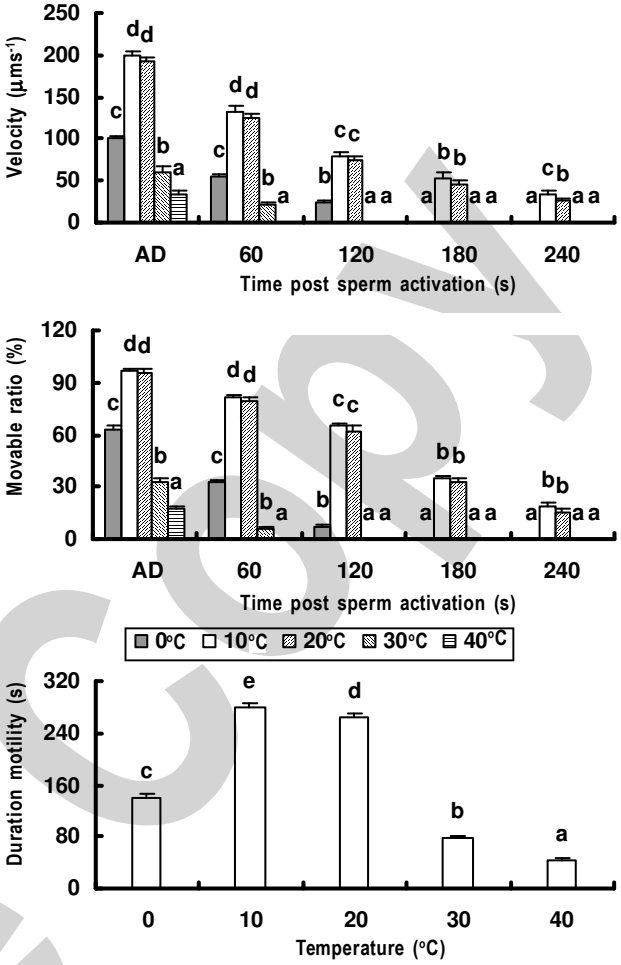

Fig. 3: Effect of different temperature values on velocity, movable ratio and duration of motility in fish *L. polyactis* spermatozoa after activation. Values with the different alphabetic letters on each bar indicate significant differences between temperature values (p<0.05). AD= After dilution

and for studying of the biochemical changes that occur during and after activation (Alavi *et al*., 2004; 2007). The effect of varying dilutions on SMPs is shown in Fig. 1. After activation, movable ratio values were 95.98±0.92%, 96.03±1.06% and 93.75±1.41% at dilution ratios of 1:25, 1:100 and 1:400, respectively. In addition, velocity at dilution ratios of 1:25, 1:100 and 1:400 were 199.26±5.15 µms^{-1}, 195.56±3.51 µms^{-1} and 191.85±4.81 µms^{-1}, respectively. No significant differences were observed in movable ratio and velocity between 1:25, 1:100 and 1:400 (p>0.05) after activation. However, differences for movable ratio and velocity were statistically significant among varying dilution ratios from 60 to 240 s. Further, duration of motility was reached 251.22±7.28, 273.56±5.58 and 164.56±6.31 s at the ratios of 1:25, 1:100 and 1:400, respectively. At this time, dilution ratio 1:100 showed the best result for spermatozoa motility activation. Therefore, dilution 1:100 (semen:diluent) was used to examine the effects of pH, temperature and cations on spermatozoa motility in *L. polyactis*.

The SMPs observed after dilution depends on pH of ASW, showed for pH 6.0 to 10.0 in Fig. 2. Maximum velocity, movable ratio and duration of motility occurred at pH 8.0 after dilution. The values of duration were 218.78±8.70, 229.44±6.72, 266.00±4.91, 216.11±7.63 and 157.67±7.38 s at pH 6.0, 7.0, 8.0, 9.0 and 10.0, respectively. There were no significant differences in duration of motility at pH 6.0, 7.0 and9.0 (p>0.05). The result of this experiment showed that pH 8.0 was optimal for SMPs. The indirect and direct influence of pH on spermatozoa motility has been shown to be significant (Cosson *et al*., 2008a). Diluent pH also effects on fertilizing ability of spermatozoa (Alavi and Cosson, 2005 a,b). Our experiment showed that optimum pH for spermatozoa motility was pH 8.0. This result confirmed that alkaline diluent condition increase SMPs of *L. polyactis* spermatozoa, also reported for other species such as Persian sturgeon *Acipenser persicus*, Mississippi paddlefish *Polyodon spathula* and Shovelnose sturgeon *Scaphirhynchus platorynchus* (Alavi *et al*., 2004) and the rainbow trout *Oncorhynchus mykiss* (Cosson, 2004). It has been reported that a change in the

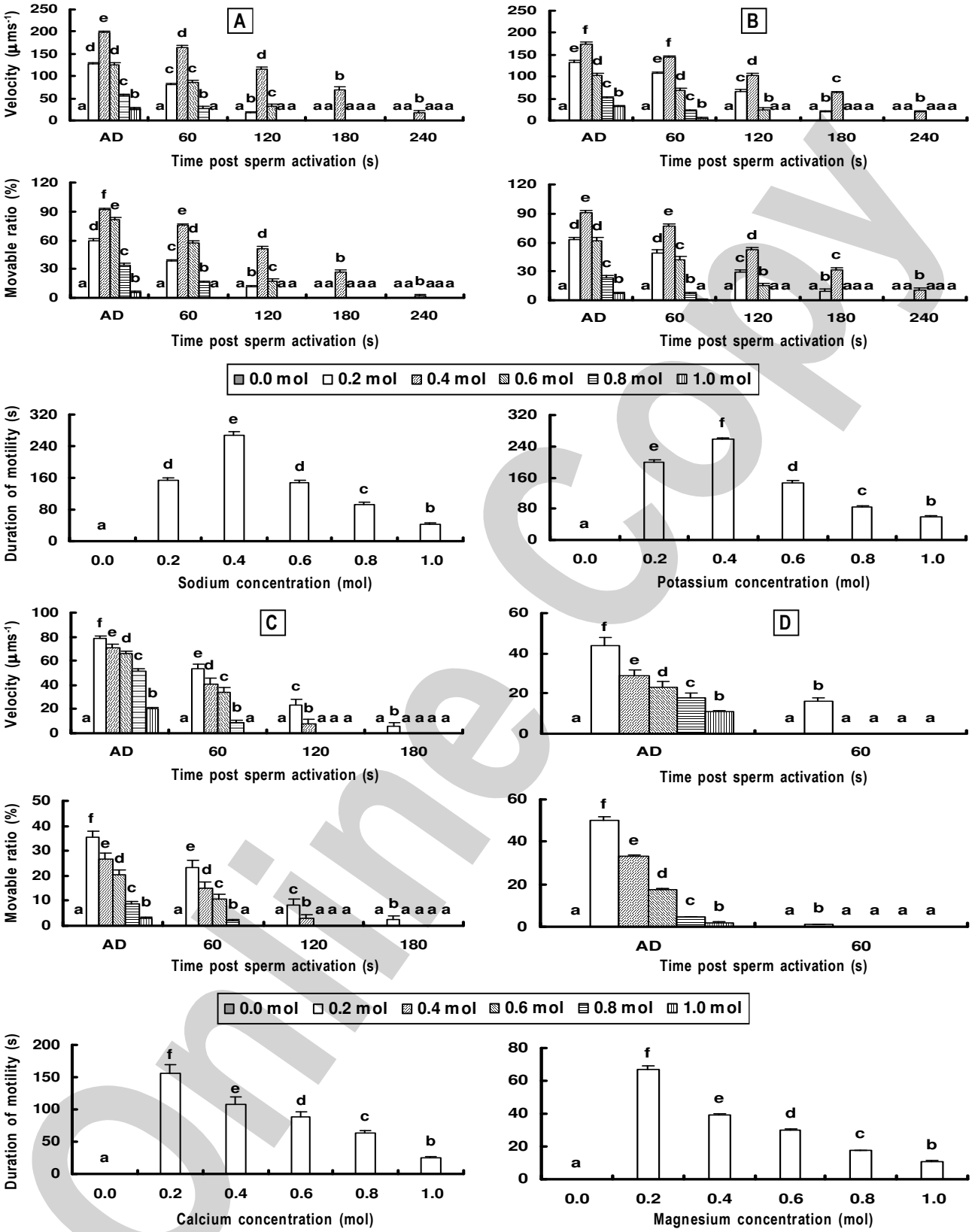

Fig. 4: Effect of different cationic concentrations on velocity, movable ratio and duration of motility in fish *L. polyactis* spermatozoa after activation. (a) sodium; (b) potassium; (c) calcium; (d) magnesium. Values with the different alphabetic letters on each bar indicate significant differences between sodium ion concentrations ($p<0.05$). AD= After dilution

external pH value induces a change in the internal pH (Alavi and Cosson, 2005b). Sea bass *Dicentrarchus labrax* spermatozoa have been shown to be motile in diluted seawater buffered between pH 5 and 10, with the optimum activity achieved around pH 9, also the same for halibut *Hippoglossus hippoglossus* and for turbot *Scophthalmus maximus* (Cosson, 2004).

The highest and lowest SMPs were observed when semen was diluted in ASW at temperature of 10 and 40°C, respectively (Fig. 3). After dilution, no significant differences were observed in terms of movable ratio and velocity at 10 and 20°C ($p>0.05$) except velocity at 240 s. However, the duration of motility showed significant differences at 10 and 20°C ($p<0.05$). Therefore, the best temperature for spermatozoa motility was concluded to be 10°C. Our result was similar to Siberian sturgeon *Acipenser baeri*, as reported by Williot *et al*. (2000). Spermatozoa motility parameters and fertilizing ability also depend on temperature of diluents (Alavi and Cosson, 2005b). The results of this study showed that SMPs were affected by diluent temperature. This is in accordance with previous studies on Salmonids and Acipenserids (Alavi and Cosson, 2005b). Consequently, our observations indicate that SMPs were best at 10°C temperature.

The motility of fish spermatozoa is controlled through their sensitivity of cationic concentrations. This phenomenon is related to cationic channel activities in the membrane and governs the motility mechanisms of anxonemes (Alavi and Cosson, 2006).

Spermatozoa of *L. polyactis* were immotile in distilled water and motile in solution containing different cations. Maximal and minimal velocity and movable ratio, as well as duration of motility, were observed in solutions containing 0.4 mol NaCl and 1.0 mol KCl, respectively (Fig. 4 a, b). The values of duration of motility, movable ratio and velocity after dilution were 267.56±8.18 s, 91.67±1.43% and 197.67±3.80 μms^{-1} in 0.4 mol NaCl and 42.00±3.54 s, 6.22±0.62% and 27.67±2.11 μms^{-1} in 1.0 mol NaCl (Fig. 4a). And, the results of duration of motility, movable ratio and velocity in KCl solution after dilution were 257.78±3.32 s, 91.44±1.76% and 175.00±3.78 μms^{-1} in 0.4 mol and 58.11±2.94 s 7.44±0.50% and 32.89±2.28 μms^{-1} in 1.0 mol (Fig. 4b). Concentration of NaCl and KCl more than 0.4 mol showed significant difference in SMPs ($p<0.05$). Ciereszko *et al*. (2002) reported activation inhibition of spermatozoa motility in sea lamprey at the NaCl and KCl concentration of higher than 40 mmol just after initiation of movement and 2 min after activation. NaCl and KCl concentrations over 20 mmol were inhibitory. In this study, duration of motility after activation at 0.4 mol of NaCl and KCl was 267.56±8.18 and 257.78±3.32 s, respectively. The velocity (43.70±4.02 μms^{-1}), Movable ratio (49.84±2.02%) and duration of motility (66.78±2.41 s) were maximal in solution containing 0.2 mol $CaCl_2$ after dilution (Fig. 4c). A rapid decrease in the movable ratio and velocity was observed in solutions containing 0.4 mol $CaCl_2$ or more. The results of this study showed the high sensitivity of *L. polyactis* spermatozoa to concentrations of $CaCl_2$. Although these data confirm a key role for Ca^{2+} in the activation of spermatozoa in *L. polyactis*, there are many questions that are still to be unanswered on the mechanisms and function of inter-cellular and extra-cellular calcium signaling in sperm motility of *L. polyactis*. It was shown that Ca^{2+} ions influx occurring during the short motility period is responsible for circling observed in trout *Salmo gairdneri* spermatozoa (Cosson, 2004). The effect of Ca^{2+} on flagella circling is especially easy to control when using demembranated/reactivated sperm models (Cosson, 2004). Maximum SMPs including velocity (78.33±2.57 μms^{-1}), movable ratio (35.22±2.69%) and duration of motility (156.56±12.78 s) were obtained in solution containing 0.2 mol $MgCl_2$ after activation (Fig. 4 d). $MgCl_2$ concentration of more than 0.2 mol significantly decreased SMPs. There is limited information about the effects of Mg^{2+} ions on spermatozoa motility in teleosts (Alavi *et al*. 2004), including the *L. polyactis*. Studies on the intracellular mechanisms of sperm motility in teleost fish confirm a key role for Mg^{2+} in the initiation of sperm motility activation, especially in demembranated sperm (Alavi *et al*. 2004; Cosson, 2004). This report is the first to show positive effects of Mg^{2+} on motility characteristics of *L. polyactis* spermatozoa when the concentration of Mg^{2+} are increased beyond 0.2 mol in solution.

In conclusion, the mechanisms of initiation of motility in *L. polyactis* spermatozoa are not completely elucidated, especially the events occurring in the intracellular environment. In addition, Na^+ is major inhibitory factor of sperm motility in *L. polyactis*. Cationic factors can stimulate the initiation of activation of sperm, but the biological sensitivity of sperm to cationic concentrations must be concern during determination of diluent composition in fish farms.

Acknowledgments

This study was supported by project at the National Fisheries Research and Development Institute (RP-2009-AQ-022).

References

Abascal, F.J., J. Cosson and C. Fauvel: Characterization of sperm motility in sea bass: The effect of heavy metals and physicochemical variables on sperm motility. *J. Fish Biol.*, **70**, 509-522 (2007).

Alavi, S.M.H. and J. Cosson: Sperm motility and fertilizing ability in the Persian sturgeon *Acipenser persicus*. *Aquacult. Res.*, **36**, 841-850 (2005a).

Alavi, S.M.H. and J. Cosson: Sperm motility in fishes: I. Effects of temperature and pH. *Cell Biol. Int.*, **29**, 101-110 (2005b).

Alavi, S.M.H. and J. Cosson: Sperm motility in fishes: II. Effects of ions and osmolality. *Cell Biol. Int.*, **30**, 1-14 (2006).

Alavi, S.M.H., J. Cosson, M. Karami, B.M. Amiri and M.A. Akhoundzadeh: Spermatozoa motility in the Persian sturgeon, *Acipenser persicus*: Effects of pH, dilution ratio, ions and osmolality. *Reproduction*, **128**, 819-828 (2004).

Alavi, S.M.H., M. Rodina, T. Policar, P. Kozak, M. Psenicka and O. Linhart: Semen of *Perca fluviatilis* L.: Sperm volume and density, seminal plasma indices and effects of dilution ratio, ions and osmolality on sperm motility. *Theriogenology*, **68**, 276-283 (2007).

Ciereszko, A., K. Dabrowski, G.P. Toth, S.A. Christ and J. Glogowski: Factors affecting motility characteristics and fertilizing ability of sea Lamprey spermatozoa. *Trans. Am. Fish. Soc.*, **131**, 193-202 (2002).

Cosson, J.: The ionic and osmotic factors controlling motility of fish spermatozoa. *Aquacult. Int.*, **12**, 69-85 (2004).

Cosson, J., A.L. Groison, M. Suquet, C. Fauvel, C. Dreanno and R. Billard: Marine fish spermatozoa: Racing ephemeral swimmers. *Reproduction*, **136**, 277-294 (2008a).

Cosson, J., A.L. Groison, M. Suquet, C. Fauvel, C. Dreanno and R. Billard: Studying sperm motility in marine fish: An overview on the state of art. *J. Appl. Ichthyol.*, **24**, 460-486 (2008b).

Darszon, A., P. Labarca, T. Nishigaki and F. Espinosa: Ion channels in sperm physiology. *Physiol. Reviews*, **2**, 481-510 (1999).

Ingermann, R.L., M. Holcomb, M.L. Robinson and J.G. Cloud: Carbon dioxide and pH affect sperm motility of white sturgeon *Acipenser transmontanus*. *J. Exp. Biol.*, **205**, 2885-2890 (2002).

Kim, S.A., S.G. Jung and C.I. Zhang: The effect of seasonal anomalies of seawater temperature and salinity on the fluctuation in yields of small yellow croaker, *Larimichthys polyactis*, in the Yellow Sea. *Fish. Oceanogr.*, **6**, 1-9 (1997).

Linhart, O., J. Cosson, S.D. Mims, M. Rodina, D. Gela and W.L. Shelton: Effects of ions on the motility of fresh and demembranate spermatozoa of common carp *Cyprinus carpio* and paddlefish *Polyodon spathula*. *Fish Physiol. Biochem.*, **28**, 203-205 (2003).

Trewavas, E.: The sciaenid fishes (croakers or drums) of the Indo-west Pacific. *Trans. Zool. Soc. Lond.*, **33**, 253-541 (1977).

Williot, P., E.F. Kopeika and B.F. Goncharov: Influence of testis state, temperature and delay in semen collection on spermatozoa motility in the cultured Siberian sturgeon *Acipenser baeri* Brandt. *Aquaculture*, **189**, 53-61 (2000).

Zuccarelli, M.D., N. Jensen and R.L. Ingermann: Inhibitory effect of osmotic concentration, potassium and pH on motility of the sperm of the North American burbot *Lota lota maculosa*. *J. Fish Biol.*, **70**, 178-189 (2007).