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EFFECT OF OSMOTIC PRESSURE ON SPERM MOTILITY OF MEKONG GIANT CATFISH AND CHAO PHRAYA CATFISH

By

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ABSTRACT:- Motility of sperm cells of the catfishes *Pangasius gigas* and *Pangasius hypophthalmus* was estimated over a range of osmotic pressures. The osmotic pressure of threshold activation (25% motile sperm cells in a sample) was 240 ± 10 mOsm/kg for the sperm of *P. gigas*, and 221 ± 12 mOsm/kg for *P. hypophthalmus*. The osmotic pressure of complete activation (highest percentage of motile cells observed in a sample) was 186 ± 9 mOsm/kg for the sperm of *P. gigas*, and 98 ± 21 mOsm/kg for *P. hypophthalmus*. These experiments were performed using dilutions of calcium-free Hanks' balanced salt solution (C-F HBSS) which is highly ionic. Similar experiments were performed using diluted glucose solutions to evaluate the activation of sperm in ion-deficient solutions. The osmotic pressure in glucose solutions of threshold activation was 248 ± 27 mOsm/kg for sperm of *P. gigas*, and 209 ± 16 mOsm/kg for *P. hypophthalmus* ; the osmotic pressure of complete activation was 187 ± 12 mOsm/kg for sperm of *P. gigas*, and 98 ± 24 mOsm/kg for *P. hypophthalmus*. These values were not significantly different from those obtained using diluted C-F HBSS. The role of particular ions in the activation of sperm of these species is unknown. Reduction in osmotic pressure

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appears to play a major role in activation, with the sperm of *P. gigas* being more sensitive to reduction of osmotic pressure than the sperm of *P. hypophthalmus*.

Key words : sperm, activation, motility, osmolality, fertilization

INTRODUCTION

Asian catfishes of the family Pangasiidae are important to commercial fisheries and aquaculture. The taxonomy of these fishes has been problematic, and a systematic revision has been published recently recognizing 2 genera within the family, comprising 21 species (Roberts and Vidthayanon 1991). Perhaps the most notable of these fishes is the Mekong giant catfish, or pla buk (*Pangasius gigas* ; formerly *Pangasianodon gigas*, Chevey), which can attain sizes of 300 kg or larger and apparently can reach 200 kg in 3 years of growth (Roberts and Vidthayanon 1991). This fish is of considerable cultural importance in Thailand and Laos, and has probably been the prize of traditional fisheries for centuries. Declining harvests of pla buk suggested that the species might become endangered and the Thai Fisheries Department had initiated an artificial breeding program since 1967. Likewise, there has been a declining harvest of the Chao Phraya catfish, or sawai (*Pangasius hypophthalmus* ; formerly *Pangasius sutchi* Fowler). This species and its interspecific hybrid with pla buk (*P. hypophthalmus* female x *P. gigas* male) called "buk sawai" is currently used for aquaculture in Thailand.

Since artificial breeding of fishes requires collection, storage, and use of gametes (Stoss 1983), in order to enhance these efforts sperm can be stored by cryopreservation for later use. Sperm of *P. gigas* was successfully cryopreserved and used to produce hybrids with *P. hypophthalmus* (Mongkonpunya et al. 1992). Further refinement of these techniques is required, however. Fish sperm are activated upon contact with water, and are motile for about a minute, after which they rapidly become inviable. The purpose of this study was to examine the relationship between osmotic pressure and sperm motility of *P. gigas* and *P. hypophthalmus*. Our objectives were to: 1) estimate the motility of sperm diluted in solutions ranging in osmotic pressure from 10 mOsm/kg to 300 mOsm/kg; 2) identify the osmotic pressures that yield threshold activation (25% motility) and complete activation in ionic solutions, and 3) identify the osmotic pressures that yield threshold and complete activation in ion-deficient (glucose) solutions.

MATERIALS AND METHODS

Catfish used in this study were *Pangasius gigas* (pla buk, or Mekong giant catfish) and *Pangasius hypophthalmus* (Chao Phraya catfish). The *P. gigas* (n = 2)

were netted from the Mekong river by commercial fishermen during May of 1994 (weights: 133 and 188 kg). The *P. hypophthalmus* (n = 12) were of domesticated pond-raised stock (10-15 kg) from a commercial farm in Nakorn Sawan, and the Thai Department of Fisheries, Ayuttaya Fisheries Center. Undiluted semen samples of *P. gigas* and *P. hypophthalmus* were collected by abdominal stripping. In all cases, only high quality sperm samples with fast swirling movement and more than 75% motile sperm were used.

In preliminary studies with full-strength Hanks' balanced salt solution (HBSS; Tiersch et al. 1994) prepared at 270 mOsm/kg, low levels of activation (~ 10% motility) were found for the sperm of *P. gigas*. To avoid activation of sperm during storage, calcium-free Hanks' balanced salt solution (C-F HBSS) was prepared at ~ 300 mOsm/kg. From this stock solution, eleven test solutions were prepared by stepwise dilution in decrements of 10% (~ 30 mOsm/kg). Eleven glucose dilutions were also prepared from a 50% stock solution (A. N. B. Laboratory Co., Bangkok). All chemicals were of reagent grade and the water used for the dilutions was triple-distilled (10 mOsm/kg).

Sperm motility was scored by placing 1 µl of semen on a slide, activating by the addition of 20 µl of test solution, and viewing with a light microscope at 100-x magnification. The activation of sperm of *P. gigas* and *P. hypophthalmus* was characterized by a pronounced mass swirling action in addition to vigorous forward swimming of the individual sperm cells. Because motility was maintained for <1 min, all estimates were recorded within 10 sec of addition of test solutions. Motility was scored in four categories (Table 1) for each sperm sample exposed to

Table 1. System used to score the motility of catfish sperm. Motility of <25% was considered to be sub-threshold activation. Motility of >25% with a mass swirling motion was considered to be above threshold. Scores of >2 were generally characterized by a mass swirling motion in addition to vigorous forward motion of the individual sperm cells.

%MotileSperm	Score	Remarks
0 to <25	1	Sub-threshold, no swirling
25 to <50	2	No swirling or slow swirling
50 to <75	3	Moderate swirling
75 to <100	4	Fast swirling

each of the eleven test solutions. Osmotic pressure was measured by osmometer (HR 33T Dew Point Microvolt Meter, Wescor, USA) for the test solutions in the absence of sperm. The motility scores obtained from samples of each species were averaged for each test solution.

The osmotic pressure of threshold and complete activation was determined in C-F HBSS for 2 semen samples of *P. gigas* and 12 samples of *P. hypophthalmus*. Threshold activation was defined as the initiation motility (activated by the test solutions) of 25% motile sperm cells in the sample. The highest percentage of motility observed for each sample when activated with distilled water was defined as complete activation. The lowest osmotic pressure that yielded threshold activation, and the highest osmotic pressure that yielded complete activation were recorded for each sample.

To test the effect of osmotic pressure on motility in ion-deficient solutions, the preceeding experiment was repeated for the same samples using diluted glucose solutions. The paired comparison t-test was used to test the differences in the osmotic pressures of threshold and complete activation, and between the C-F HBSS and glucose solutions.

RESULTS AND DISCUSSION

From our general observation, sperm of *P. gigas* were activated at higher osmotic pressures than were sperm of *P. hypophthalmus*. However, at osmotic pressures above 300 mOsm /kg (in C-F HBSS), minimal activation of sperm (<5% motility, without swirling) of either species was observed. Between 280 and 310 mOsm/kg, sub-threshold activation (<25%, motility score <1) without mass swirling movement was observed in *P. gigas* sperm (Figure 1). This level of sub-threshold activation occurred between 260 mOsm/kg and 300 mOsm/kg for sperm of *P. hypophthalmus*. Threshold activation, as indicated by slow or no swirling motion and 25% motility, was observed at 240 ± 10 mOsm/kg for *P. gigas* sperm (Figure 1), and at 221 ± 12 mOsm/kg for *P. hypophthalmus* sperm (Figure 2). These values were not significantly different between the two species ($P > 0.05$). However, the highest osmotic pressure (186 ± 9 mOsm/kg) yielding complete activation of *P. gigas* sperm was significantly higher ($P < 0.01$) than the highest osmotic pressure (98 ± 21 mOsm/kg) yielding complete activation of *P. hypophthalmus* sperm.

The osmolality values for threshold and complete activation were significantly different ($P < 0.001$) within each species. The zone of activation (between the values for threshold and complete activation) was ~ 50 units of mOsm /kg for *P. gigas*, and ~ 120 units mOsm/kg for *P. hypophthalmus*. Within this

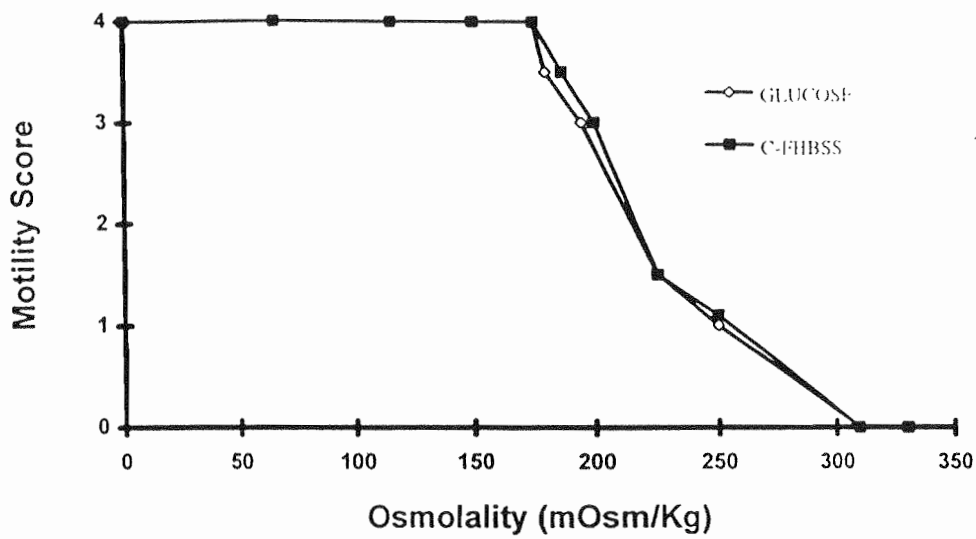

Figure 1. Motility of *Pangasius gigas* sperm activated in diluted C-F HBSS (black squares) or glucose (white squares). Each point represents the mean value of 2 fish. The arrows indicate the osmotic pressure of threshold (25% motile sperm with slow swirling motion) and complete activation (>75% motile sperm with fast swirling).

zone, a change of 10 mOsm/kg in osmotic pressure resulted in a change in motility of ~ 10% in sperm of *P. gigas* and ~ 4% in sperm of *P. hypophthalmus*.

The activation of sperm of each species with glucose solutions showed patterns identical to those observed for activation with C-F HBSS. For *P. gigas* sperm, the osmotic pressures that yielded threshold (248 ± 27 mOsm/kg) and complete (187 ± 12 mOsm/kg) activation with diluted glucose solutions (Figure 1) were not different ($P > 0.05$) from those observed for activation with C-F HBSS. Similarly for *P. hypophthalmus* sperm, the osmotic pressures that yielded threshold (209 ± 16 mOsm /kg) and complete (98 ± 24 mOsm/kg) activation (Figure 2) were not different ($P > 0.05$) from those observed for activation with C-F HBSS.

Mechanisms of sperm activation involving dilution of a specific ion would have predicted activation at higher osmotic pressures in the ion-deficient solutions. For example, motility of salmonid sperm could be induced at the osmolality of seminal fluid (300 mOsm/kg) if the concentration of potassium ions was below that of the seminal fluid (Morisawa et al. 1983a). Sperm of freshwater cyprinids were activated, however, by a decrease in osmotic pressure independent of potassium concentration (Morisawa et al. 1983b). The result was similar in the case for

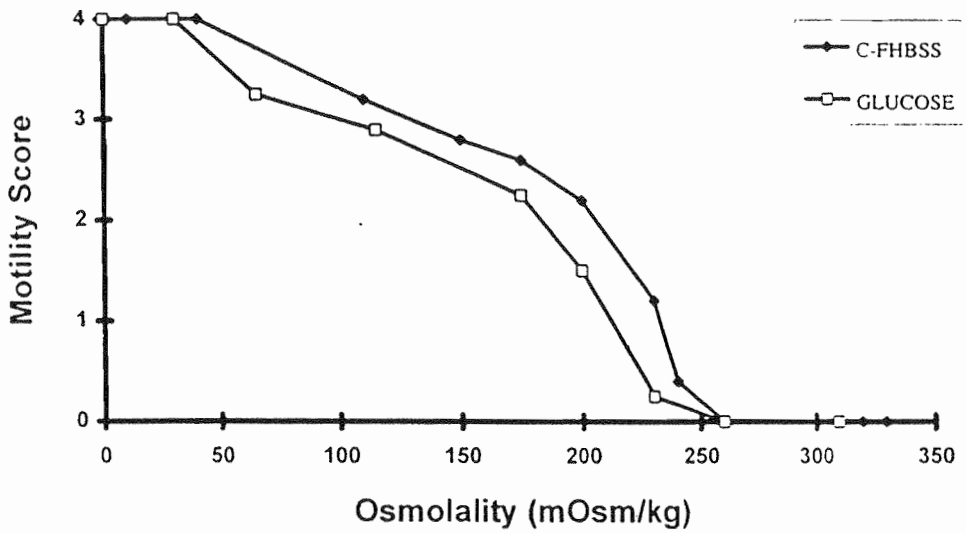

Figure 2. Motility of *Pangasius hypophthalmus* sperm activated in diluted C-F HBSS (black squares) or glucose (white squares). Each point represents the mean value of 12 fish.

channel catfish *Ictalurus punctatus*, (Bates et al.1996) and pangasiid catfishes as demonstrated in this study. Thus while the role of specific ions in sperm activation requires further study, it would seem that osmotic pressure plays a major role in the activation of sperm of *P. gigas* and *P. hypophthalmus* in the presence or absence of extracellular ions.

This study showed the importance of osmotic pressure in the activation of sperm of *P. gigas* and *P. hypophthalmus*. By manipulating osmotic pressure, we would be able to activate sperm motility over the range of 10-300 mOsm/kg. This finding is useful in selecting storage buffers, and in diluting sperm suspensions to assure complete motility for fertilization or determination of sperm quality. Our study suggests that the storage of sperm of these species in C-F HBSS at osmotic pressures above 320 mOsm, or about 20-25% above the osmolality of the seminal fluids (our unpublished observations: 237 ± 2 mOsm/kg for *P. gigas* and 252 ± 4 mOsm/kg for *P. hypophthalmus*) would provide sufficient protection from activation by osmotic mechanisms.

Motility is often used as an indicator for quality of fish sperm. This estimation, however, is subjective and can be error-prone. The accurate assessment of sperm quality by motility estimation is dependent on testing at osmotic pressures below 180 mOsm/kg for sperm of *P. gigas*, and below 90 mOsm/kg for sperm of *P. hypophthalmus*. Thus, for greatest accuracy, distilled water (in at least a 10-fold

excess) should be used to dilute semen samples of these species for determination of motility.

Male pangasiid catfish may possess adaptations suited to reproduction in swiftly flowing water. Sperm of *P. gigas* and *P. hypophthalmus* were remarkably motile, exhibing mass swirling as well as forwards swimming of individual sperm cells. In *P. gigas*, the weight of the testes can be equal to 10% of the body weight of males collected during April and May at Chieng Khong in the Chieng Rai province of Thailand (our unpublished data). It follows that a testes weighing as much as 30 kg could contain trillions of sperm. Large volumes of highly motile sperm could thus serve to ensure fertilization of tremendous numbers of eggs in strong water currents.

Even so, the harvests of *P. gigas* and *P. hypophthalmus* are declining. Culture of these species offers potential for supplementation of wild stocks, aids in the development of local economies and provides sufficient fish for the market. Development of improved methods of artificial spawning of these fishes will become increasingly important as the aquaculture industry grows. Indeed, buk sawai, the hybrid of *P. hypophthalmus* and *P. gigas*, offers great potential in aquaculture and can only be produced by artificial spawning. Further studies should address refrigerated storage of gametes, and improved cryopreservation procedures for these species.

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