

A SPECIALIZED ROLE FOR THE PACIFIC HERRING EGG

CHORION IN SPERM MOTILITY INITIATION

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INTRODUCTION

The Pacific herring, *Clupea pallasii*, is a commercially and ecologically important marine teleost that spawns in bays and estuaries having freshwater input (Alderdice and Velsen, 1971). Like many other teleost fish, herring reproduce by broadcast spawning of sperm and eggs. Typically, males spawn first, and released sperm serves to attract females as well as other males to the spawning site (Stacey and Hourston, 1982; Carolsfeld and Sherwood, 1995). Herring eggs are extremely adhesive, and attach to a variety of solid substrates upon which the fertilized eggs undergo development through hatching. Herring spawning biomass in San Francisco Bay, which is the basis of a large commercial fishery, experienced a decline during the late 1980's and early 1990's (CDFG, 1992). During this period, salinities in the Bay increased dramatically due to drought conditions and diversion of freshwater runoff. This has led to the hypothesis that altered salinities may adversely affect herring reproduction.

It has long been recognized that the eggs of many invertebrates and vertebrates possess properties that enhance or activate sperm (reviewed by Morisawa 1994). For example, the jelly coat of sea urchin eggs induces the sperm acrosome reaction, a prerequisite for successful fertilization. Similarly, mammalian eggs possess factors that capacitate sperm and induce the acrosome reaction, as well as facilitate binding of the sperm to the egg plasma membrane. The plasma membrane of teleost eggs is surrounded by a thick chorion that is impenetrable to sperm except in the region of the micropyle, located at the animal pole. Fertilization occurs only when a motile sperm finds and traverses the micropylar canal to fuse with the egg plasma membrane. With the exception of the Japanese bitterling, in which the micropyle region has chemoattractant properties, the micropyle of most teleosts is considered to play a passive role, serving primarily as a barrier to the passage of supernumerary sperm and as a pathway for the fertilizing sperm.

The micropyle region of the eggs of Pacific herring (*Clupea pallasii*) has been shown to play an active role in the activation of herring sperm (Yanagimachi and Kanoh, 1953; Yanagimachi, 1957; Yanagimachi *et al.*, 1992; Pillai, *et al.*, 1993; Griffin *et al.*, 1996). Unlike the sperm of most

organisms, herring sperm are virtually immotile at the time of spawning, however, contact with the micropyle region of the herring egg initiates sperm motility. Our laboratory has focused on the basic mechanisms of the above phenomenon during herring gamete interaction, as well as the role of environmental factors in modulating fertilization.

SPERM MOTILITY INITIATING FACTOR

Our laboratory has isolated and partially characterized a component of the herring egg shown to induce motility, termed sperm motility initiating factor, or SMIF (Pillai *et al.*, 1993; Griffin *et al.*, 1996). SMIF is a 105 kDa basic glycoprotein that is localized to the region surrounding the vestibule, which contains the micropylar opening. Evidence for sperm motility initiation by the micropyle region of the *C. pallasii* chorion was first observed by Yanagimachi and Kanoh (1953). Immotile sperm in contact with the micropyle region were observed to become motile, while sperm in contact with other regions of the chorion remained immotile. We have recently observed that the number of motile sperm in the micropyle region of eggs is significantly reduced in the presence of a polyclonal antibody generated against SMIF (Griffin, *et al.*, 1996). Similarly, fertilization rates of eggs incubated in antibody are also reduced. Immunostaining of anti-SMIF treated eggs confirmed a specialized role for SMIF at the site of sperm-chorion interaction; specifically, the region surrounding the vestibule which contains the micropylar opening (Fig. 1).

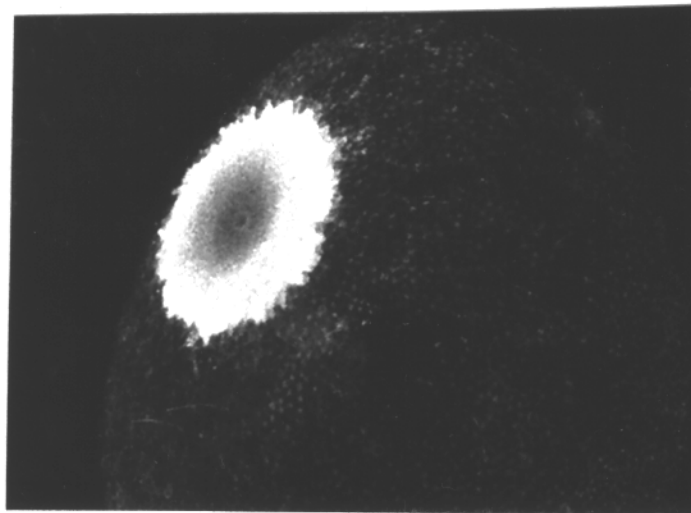


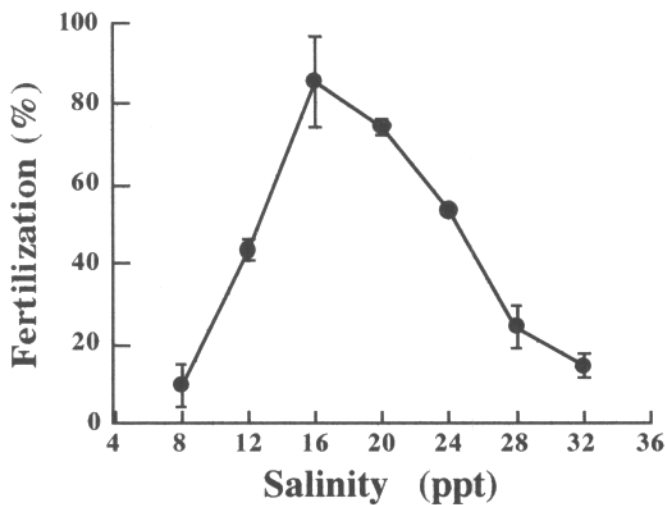
Figure 1. Anti-SMIF labeling of the herring chorion demonstrating staining in the micropylar region only. Intensity of staining increases from the micropylar opening towards the periphery of the micropyle.

SMIF appears to be a non-diffusible component of the micropyle, in that sperm motility eventually ceases if a spermatozoon swims away from and does not re-contact the micropyle. Furthermore, extensive washing in 1/2 sea water does not significantly reduce the motility initiating ability of chorions (Yanagimachi, 1957; Yanagimachi *et al.*, 1992). SMIF can, however, be removed from egg chorions using 1/2 sea water which is acidified to pH 3.5, and this “soluble” SMIF is a potent inducer of sperm motility *in vitro*. SMIF clearly differs from a family of small molecular weight peptides, termed herring sperm activating peptides (HSAP’s), which have also been reported to enhance sperm motility (Morisawa *et al.*, 1992; Morisawa, 1994; Oda *et al.* 1995).

EFFECTS OF SALINITY ON FERTILIZATION AND SMIF-INDUCED SPERM MOTILITY

Our laboratory has been investigating the effects of salinity and specific ions on herring fertilization and early development in the San Francisco Bay population. It has previously been shown that the optimal salinity for both fertilization and development is approximately half-strength seawater (Yanagimachi, 1953; Yanagimachi and Kanoh, 1953; reviewed by Alderdice and Hourston, 1985). Our results for the San Francisco Bay population during the 1992-93 season confirm this (Fig 2 A, B). A role for specific ions is suggested by preliminary results indicating that increasing concentrations of sodium in 1/2 sea water decrease fertilization rates in a dose-dependent manner. The roles of other ions remain to be clarified.

A. Effect of Salinity on Fertilization



B. Effect of Salinity on Hatching

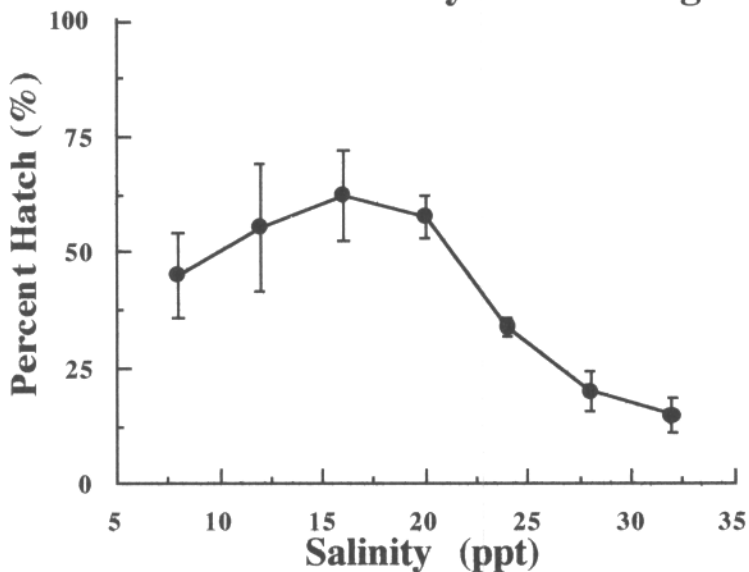


Figure 2 A,B. Graphs illustrating the effects of salinity (in ppt) on percent fertilization (A) and hatching success (B) in Pacific herring.

Alterations in salinity affect SMIF induced sperm motility *in vitro* as well, with very low or very high salinities reducing motility (Fig. 3). This led us to extend our initial investigations to include both the salinity and ionic requirements for initiation of herring sperm motility, as well as to further characterize the role of SMIF in inducing sperm motility (Yanagimachi *et al.*, 1992; Pillai *et al.*, 1993). Initiation of herring sperm motility is highly dependent on ionic conditions in the surrounding medium. Motility initiation in the micropyle region requires extracellular calcium and potassium, as evidenced by lack of SMIF induced motility in calcium or potassium free 1/2 strength artificial seawater (1/2 Ca⁺⁺ or K⁺ FASW) (Yanagimachi *et al.*, 1992). However, manipulation of intracellular levels of these cations alone is not sufficient to trigger motility. Interestingly, vigorous motility can be initiated in 1/2 low sodium (2 mM) artificial seawater (1/2 Na⁺FSW), in the absence of SMIF. The likelihood of herring sperm experiencing seawater lacking sodium is remote (the Na⁺ concentration in 1/2 sea water is approximately 220 mM), thus the physiological relevance of motility in low sodium media is unknown. Although Ca⁺⁺ is required for SMIF-induced motility initiation, high concentrations of Ca⁺⁺ or Mg⁺⁺ can inactivate or remove SMIF from eggs (Yanagimachi and Kanoh, 1953; Yanagimachi, 1957).

Effect of Salinity on Sperm Activation

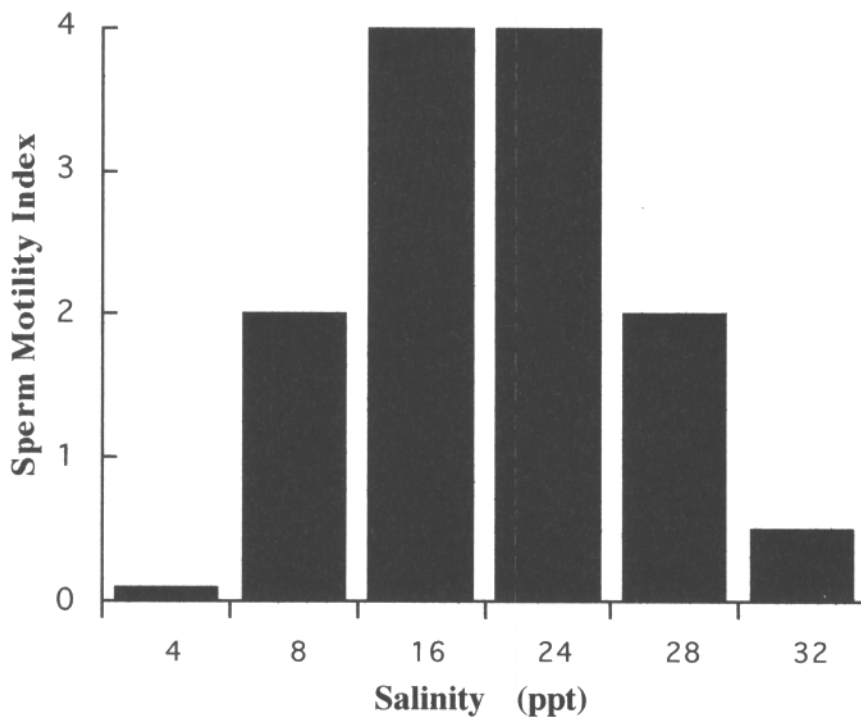


Figure 3. Graph illustrating the effect of varying salinities on SMIF induced herring sperm motility *in vitro*. Motility was qualitatively graded on a scale of 0 to 4; 0 = no motility; 4 = 4+ motility.

Since low sodium sea water induced sperm motility *in vitro*, we investigated a role for sodium in SMIF-induced activation. Amiloride, a sodium channel blocker, was found to inhibit SMIF-induced motility. In addition, elevated extracellular sodium inhibited SMIF-induced motility in a dose dependent manner, similar to the one observed for fertilization. Using the fluorescent sodium indicator Sodium Green, we have recently found a decrease in intracellular sodium upon

SMIF activation of sperm (not shown). This finding is consistent with the hypothesis that there is an efflux of sodium from *C. pallasii* sperm following interaction with SMIF, and that elevated salinities (such as those experienced during the extended drought of the late 1980s) at the time of spawning may adversely affect the ability of sperm to initiate motility.

CONCLUSIONS

The role of SMIF in initiating herring sperm motility may reflect the spawning behavior of herring. Most fish sperm have a limited, very short lifespan once released and diluted into the aqueous environment (reviewed by Morisawa, 1994). Since male herring typically spawn prior to females, it may be advantageous for the sperm to remain immotile, conserving metabolic energy until eggs are actually present in the vicinity of the sperm. Interaction of the sperm with the chorion factor SMIF, possibly through a receptor mediated mechanism, may trigger processes that initiate motility. The ability of SMIF to activate sperm appears to be dependent upon a reduction in salinity of the surrounding medium, with sodium potentially playing a key role in this regulation. Other ions such as calcium and potassium undoubtedly also have roles, perhaps facilitating SMIF binding to a sperm receptor or acting as counter ions during sodium efflux. Our current research efforts involve these and other related issues.

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