

Final Report to the
Ohio Lake Erie Commission

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Project title: Induced spermiation and characterization of spermatozoa of the sea lamprey (*Petromyzon marinus*).

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Introduction

The invasion of the sea lamprey in the Great Lakes had a devastating impact on the lake trout, but also on all fish assemblages (Coble et al. 1990). With the opening of the St. Lawrence Seaway, the non-indigenous sea lamprey (*Petromyzon marinus*) gained access to the Great Lakes in 1932. Current attempts to control lamprey are mainly 1) treatment of streams with lampricides to kill larvae and 2) production and release of sterile males to decrease spawning success.

Total reliance on a single control method is considered unwise-, therefore a search for alternative lamprey control methods is necessary to create an integrated control program. Quality and quantity of lamprey sperm are important components of reproductive success in this species and may be a potential target for control of its reproduction (Kobayashi and Yamamoto 1994).

Lamprey gametes are difficult to handle and basic information on this issue is lacking. Better understanding of this species gamete biology is important both for development of laboratory techniques for studies of reproduction and for future control of sea lamprey reproduction.

The recovery effort of the lake whitefish in all Great Lakes might be impaired by lamprey attack on the new host. Undoubtedly, efficient reproduction of lampreys contributed to the colonization success of this parasite. Females of the landlocked sea lamprey produce about 60,000 eggs and males are able to produce milt for several spawning events (Manion and Hanson 1980; Langille and Hall 1988). However, because of a lack of knowledge on the biology of fertilization in the sea lamprey, we do not have good understanding of the gamete interaction resulting in the enormous reproductive success of the sea lamprey. Understanding morphological changes in oocytes and sperm and the physiology of fertilization of lamprey might allow us to identify potential targets for preventing successful reproduction.

Objectives

1. To compare characteristics of sperm (volume, density, motility) from hormone-injected animals with semen obtained from lampreys spawned in the laboratory.
2. To describe ultrastructure of spermatozoa and basic composition of seminal plasma.

Induced spawning results

Lampreys were obtained from the Cheboygan River, Michigan (Mrs. Kim Fredricks), the St. Marys River, Michigan (Mr. Mike Twohey, Marquette Biological Station), and Cattaraugus Creek, Springville, New York (Lake Erie population). Animals were cooled with ice during transport (10-24 h) and then gradually transferred to 14°C on arrival. In the first phase of the study, animals were acclimated for 5 days and then weighed and individually tagged with plastic tags attached behind the dorsal fin. Males and females were divided to two subgroups, 8 animals of each sex were injected with 100 µg/kg of the luteinizing hormone releasing hormone (LHRH) and 10 mg/kg of pimozide (Sigma Chem., St Louis, MO) dissolved in 0.7% sodium chloride. Four animals were injected with a vehicle and four were not injected. The second injection of only 200 µg LHRH/kg was applied 8 days later. The first males spermiated 16 days after first injection and all of them at 22 days when water temperature already reached 19°C. The latter coincided with the first 25% of spermiating males in control groups (Fig. 1).

Sperm biochemical characteristics

Sperm volume, concentration, motility and amidase activity against trypsin- and chymotrypsinlike synthetic substrates were determined (Ciereszko and Dabrowski 1993; Ciereszko et al. 1994, 1996a,b). Milt volume in males of 264 (204-386) g was 3.3 ± 1.4 ml, sperm concentration was $1.03 \pm 0.4 \times 10^9 \text{cm}^{-3}$, and motility $52 \pm 25\%$ (n=15). No trypsin-like amidase activity was found, whereas chymotrypsin-like activity amounted to $1.12 \pm 0.74 \mu\text{U}/10^6$ spermatozoa. The preliminary characteristics of this activity have been established (Fig. 2). No hyaluronidase activity was found in lamprey sperm, in contrast to boar sperm used as control (Harrison 1988). Potassium (10.5 ± 0.6 mM) and sodium (106.3 ± 12.8 mM) were two major ions in the seminal plasma (Table 1), whereas protein concentration was 0.43 ± 0.18 g/L.

Preliminary data was collected on the effect of sperm concentration and time after sperm activation on the fertilization rate (Table 2).

Sperm ultrastructure

The data collected in 1996 is the first information on the basic characteristic of sea lamprey sperm (Fig. 3). In our ultrastructural studies we utilized experience gained in similar studies on the acrosomal sperm of the lake sturgeon (*Acipenser fulvescens*) (Ciereszko et al. 1994) and the anacrosomal sperm of muskellunge (*Esox masquinongy*) (Lin et al. 1996). In accordance with earlier findings of Jaana and Yamamoto (1981) and Kobayashi and Yamamoto (1994) for spermatozoa of *Lampetra japonica*, we described changes in the sperm head. We identified the dynamic changes in the acrosomal vesicle of activated spermatozoas, but were unable to observe the discharge of acrosomal filament (AFD) (Fig. 3a,b). Kobayashi (personal communication, 1997) was also unable to control the AFD process and associated it with the maturity of individual males. The treatment with "egg water" (substances washed out from ovulated eggs) used to activate spermatozoa, resulted in significant changes in the duration of sperm motility and swimming characteristics (Fig. 4), but did not result in AFD in our preliminary studies with the sea lamprey.

Sperm characteristics from other lamprey species have been studied in non-parasitic lampreys *Lampetra planeri* and *Mordacia mordoxas* as well as in parasitic, *L. fluviatilis*, *L. japonica*, and *M. praecox* (Jamieson 1991). Longitudinal sections of the sea lamprey sperm head revealed the content of the acrosomal vesicle attached to the inner surface of the vesicle membrane, however, it was distinct from the subacrosomal ring (Fig. 3c,d). The acrosomal vesicle of the sea lamprey sperm seemed to differ from the subovoidal shape of *L. planeri* (Stanley 1967) and the flattened shape of *L. japonica* (Jaana and Yamamoto 1981). The latter authors, who described the testicular sperm discharging the acrosomal filament (central fiber) suggested that this may occur at the time of fixation. Kille [1960; including observations by Ballowitz (1905)] concluded that the most of spermatozoa extrude the filament when they come into contact with the surface of egg chorion or simply glass.

Sperm motility

Prolonged sperm motility may be important in determining the reproductive advantage of lamprey over other aquatic animals. Spermatozoa of freshwater fish have a very short duration of motility as compared to marine organisms. For example, rainbow trout spermatozoa are motile for only about 30 s (Billard 1992). Kobayashi (1993) reported that about 40% of spermatozoa of *L. japonica* were motile for 5 minutes after activation, which is almost as long as in the lake sturgeon (Ciereszko et al. 1996a). The prolonged motility may contribute to an enormous fertilization success in lampreys.

We have observed that indeed the spermatozoa of the sea lamprey remained motile for up to 7 min after activation (unpublished). Furthermore, with the help of the computer-assisted

sperm motion analysis (CASA), we demonstrated that the motility is significantly extended and the swimming velocity increased when the sperm-activating protein (or peptide) (SAP-1) was present (Fig. 4).

Current understanding of fertilization process

The acrosome is a structure that serves a role in the spermatozoon's (1) species specific recognition of egg, (2) its further penetration through the egg envelopes, and (3) its fusion with the egg nucleus. We observed a gradual expansion of the acrosomal vesicle in lamprey spermatozoa, however, the process was not related to the presence of "egg water" factor (Fig. 3). The acrosome is characterized by heterogenous contents of the acrosomal vesicle and, in some species of fish (sturgeon, Ciereszko et al. 1994; lamprey, Jaana and Yamamoto 1981), a penetration of perforatorium, i.e. acrosomal filament, also known as central fiber discharge (Jamieson 1991). The disruption of the apical plasma membrane and the membrane of the acrosomal vesicles, is called the "acrosomal reaction" (AR). These processes are associated with an influx of Ca and Na, and cause a membrane depolarization and intracellular alkalization. The AR, usually triggered at the egg surface, leads to the release or exposure of a number of hydrolytic enzymes associated with the acrosome. The spermatozoon's penetration of egg vitelline envelopes without distinguished micropyle, when flagellum activity ceased (Kobayashi and Yamamoto 1994) in lamprey, must be facilitated by the acrosomal hydrolyzing enzymes (Fig. 5).

References

- Billard, R. 1992. Reproduction in rainbow trout: sex differentiation, dynamics of gametogenesis, biology and preservation of gametes. *Aquaculture* 100:263-298.
- Ciereszko, A. and K. Dabrowski. 1993. Estimation of sperm concentration of rainbow trout, whitefish and yellow perch using spectrophotometric technique. *Aquaculture* 109:367-373.
- Ciereszko, A., K. Dabrowski, F. Lin and S.I. Doroshov. 1994. Identification of trypsin-like activity in sturgeon spermatozoa. *J. Exp. Zool.* 268:486-491.
- Ciereszko, A., G.P. Toth, S.A. Christ and K. Dabrowski. 1996a. Effect of cryopreservation and theophylline on motility characteristics of lake sturgeon (*Acipenser fulvescens*) spermatozoa. *Theriogenology* 45:665-672.
- Ciereszko, A., K. Dabrowski and S.I. Ochkur. 1996b. Characterization of acrosin-like activity of lake sturgeon (*Acipenser fulvescens*) spermatozoa. *Molecular Reprod Develop.* 45:72-77.
- Coble, D.W., R.E. Bruesewitz, T.W. Fratt and J.W. Scheirer. 1990. Lake trout, sea lampreys, and overfishing in the Upper Great Lakes: a review and reanalysis. *Trans. Am. Fish. Soc.* 119:985-995.
- Lin, F., A. Ciereszko and K. Dabrowski, 1996. Sperm production and cryopreservation in muskellunge after carp pituitary extract and human chorionic gonadotropin injection. *Prog. Fish Cult.* 58:32-37.
- Jaana, H. and T.S. Yamamoto. 1981. The ultrastructure of spermatozoa with a note on the formation of the acrosomal filament in the lamprey, *Lampetra japonica*. *Jap. J. Ichthyol.* 28:135-147.

- Jamieson, B.G.M. 1991. Fish evolution and systematics: Evidence from spermatozoa. Cambridge University Press, Cambridge, New York, Port Chester, Melbourne, Sydney.
- Kille, R.A. 1960. Fertilization of the lamprey egg. *Exp. Cell Res.* 20:12-27.
- Kobayashi, W. 1993. Effect of osmolality on the motility of sperm from lamprey, *Lampetra japonica*. *Zool Sci.* 10:281-285.
- Kobayashi, W. and T.S. Yamamoto. 1994. Fertilization in lamprey, *Lampetra japonica* eggs: implications of the presence of fast and permanent blocks against polyspermy. *J Exp. Zool.* 269:166-167.
- Langille, R.M. and B.K. Hall. 1988. Artificial fertilization, rearing, and timing of stages of embryonic development of the andromonus sea lamprey, *Petromyzon marinus* L. *Can. J Zool.* 66:549-554.
- Manion, P.J. and L.H. Hanson. 1980. Spawning behavior and fecundity of lampreys from the upper three Great Lakes. *Can. J Fish. Aquat. Sci.* 37:1635-1640.
- Stanley, H.P. 1967. The fine structure of spermatozoa in the lamprey *Lampetra planeri*. *J Ultrastruct. Res.* 19:84-99.
- Toth, G., A. Ciereszko, S.A. Christ and K. Dabrowski. 1997. Objective analysis of sperm motility in the lake sturgeon, *Acipenser fulvescens*: Activation and inhibition conditions. *Aquaculture* 154:337-348.

Table 1 Osmolality (miliosmomole/kg) and ionic concentrations (mM) in seminal plasma of two teleost fish, lake sturgeon (*Chondrostei*) and sea lamprey (1996). Data are presented as a mean \pm SD.

	Species			
	<i>Oncorhynchus mykiss</i> ¹	<i>Esox masquinongy</i> ²	<i>Acipenser fulvescens</i> ³	<i>Petromyzon marinus</i>
Osmolality	220.8 \pm 69.7	284.3 \pm 10.0	---	249.0 \pm 20.0
P	2.66 \pm 2.92	5.57 \pm 5.57	3.90 \pm 0.38	0.66 \pm 0.28
K	20.01 \pm 4.54	23.69 \pm 1.69	5.82 \pm 0.49	10.48 \pm 2.58
Na	67.61 \pm 13.64	131.96 \pm 2.96	25.65 \pm 2.79	106.3 \pm 12.8
Ca	1.39 \pm 0.47	2.11 \pm 0.19	0.16 \pm 0.05	0.57 \pm 0.08
Mg	1.01 \pm 0.27	1.45 \pm 0.61	0.25 \pm 0.02	1.24 \pm 0.28
Cl	43.07 \pm 11.62	132.32 \pm 7.02	5.41 \pm 2.79	---

¹Ciereszko and Dabrowski, unpublished data for 2 year old males of Mount Shasta Strain.

²Lin et al. (1996); saline injected group.

³Toth et al. (1997).

Table 2 Sperm and egg viability tests.

A. Test for egg quality in lamprey (June 29, 1996).

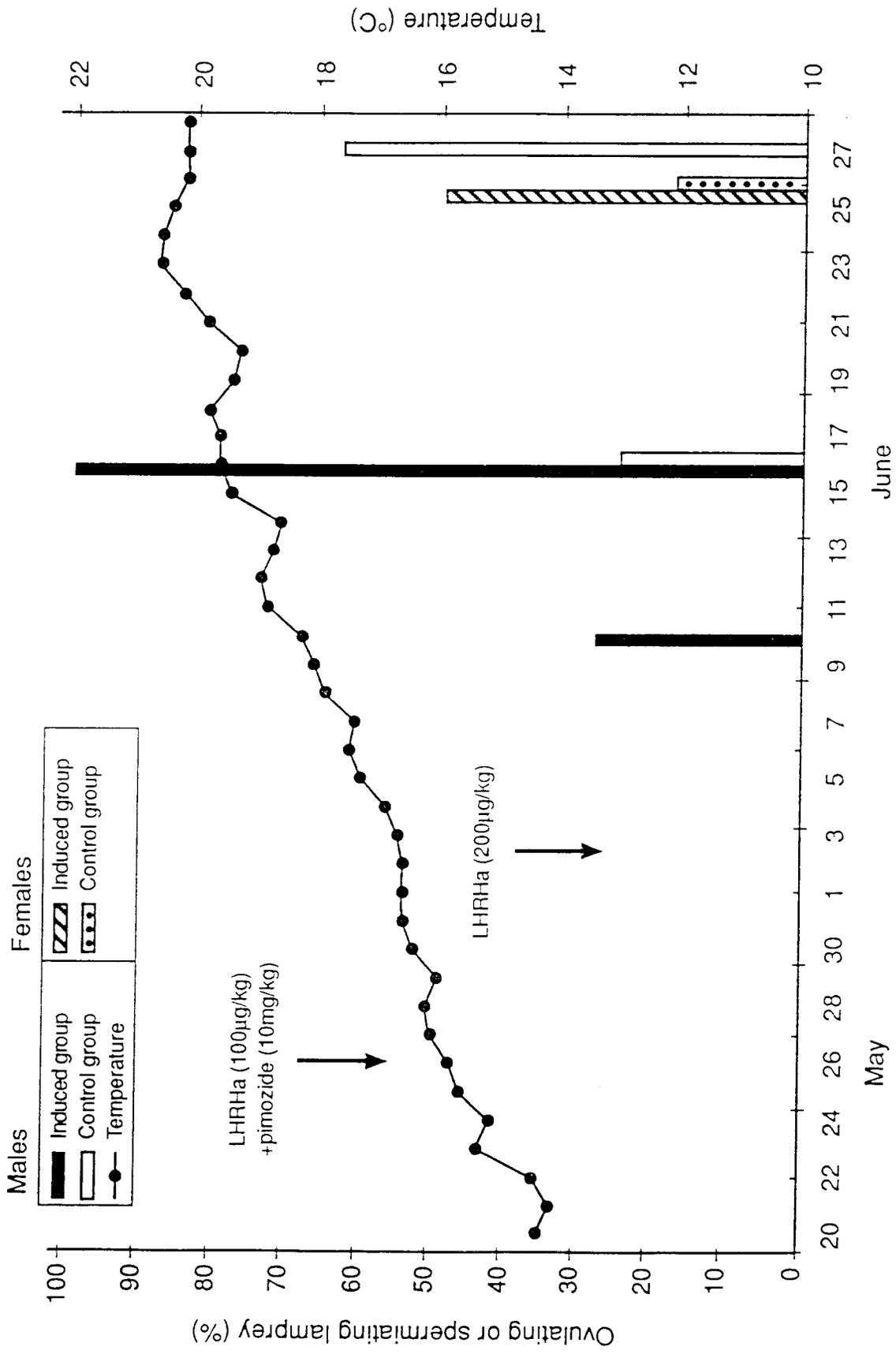
No.	Female wt. (g)	Eggs (%)	Fertilization (%) (duplicate)
1			16.4
2	207	20.7	33.7
3	330	29.3	0
4	318	24.2	44.1
5			14.2

Combined sperm from 3 males; motility $60 \pm 10\%$; sperm density $2.10^6/\text{egg}$.

B. Test for "delayed" fertilization of eggs released to fresh-water (n=3)

Time (min)	0	10'	60'
Fertilization (%)	17.2 ± 8.7	0.92 ± 0.27	0

*Test on July 15, 1996.



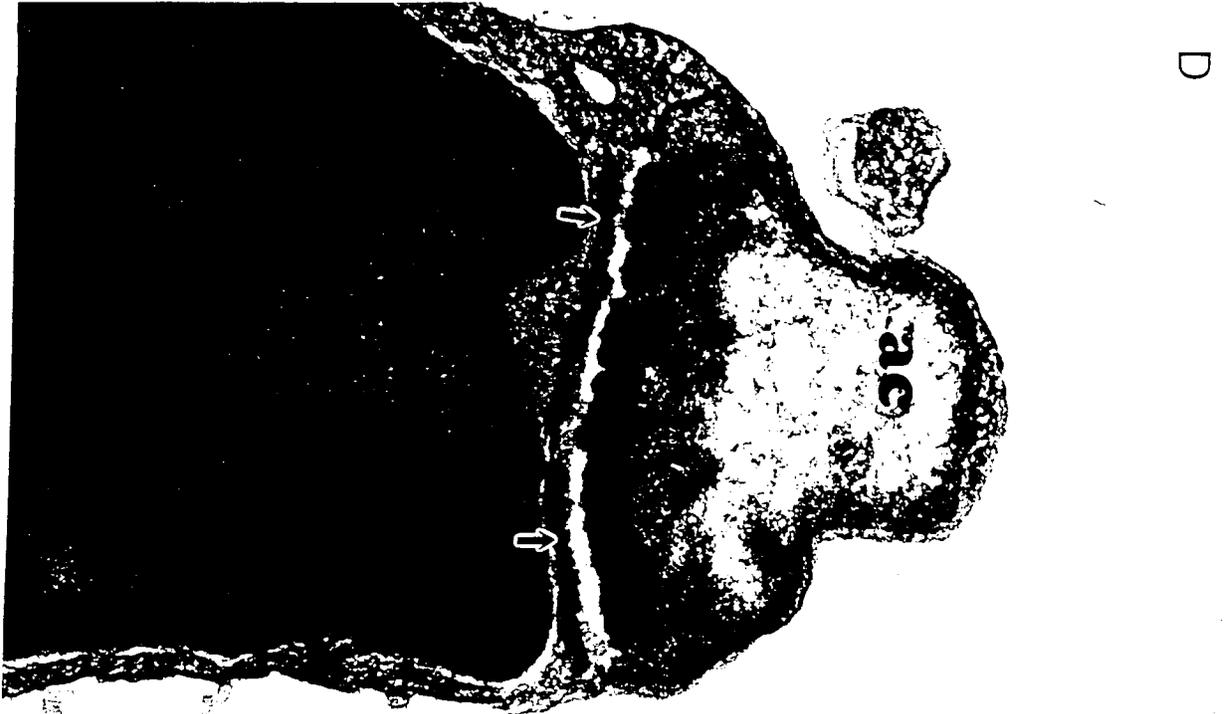
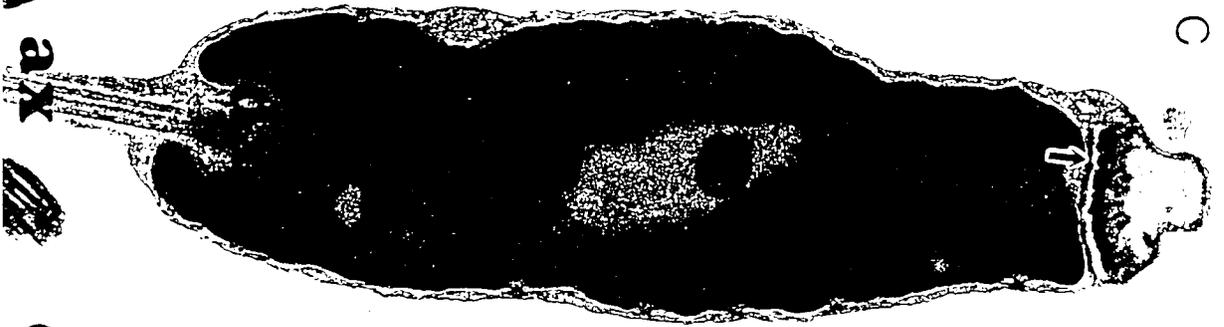
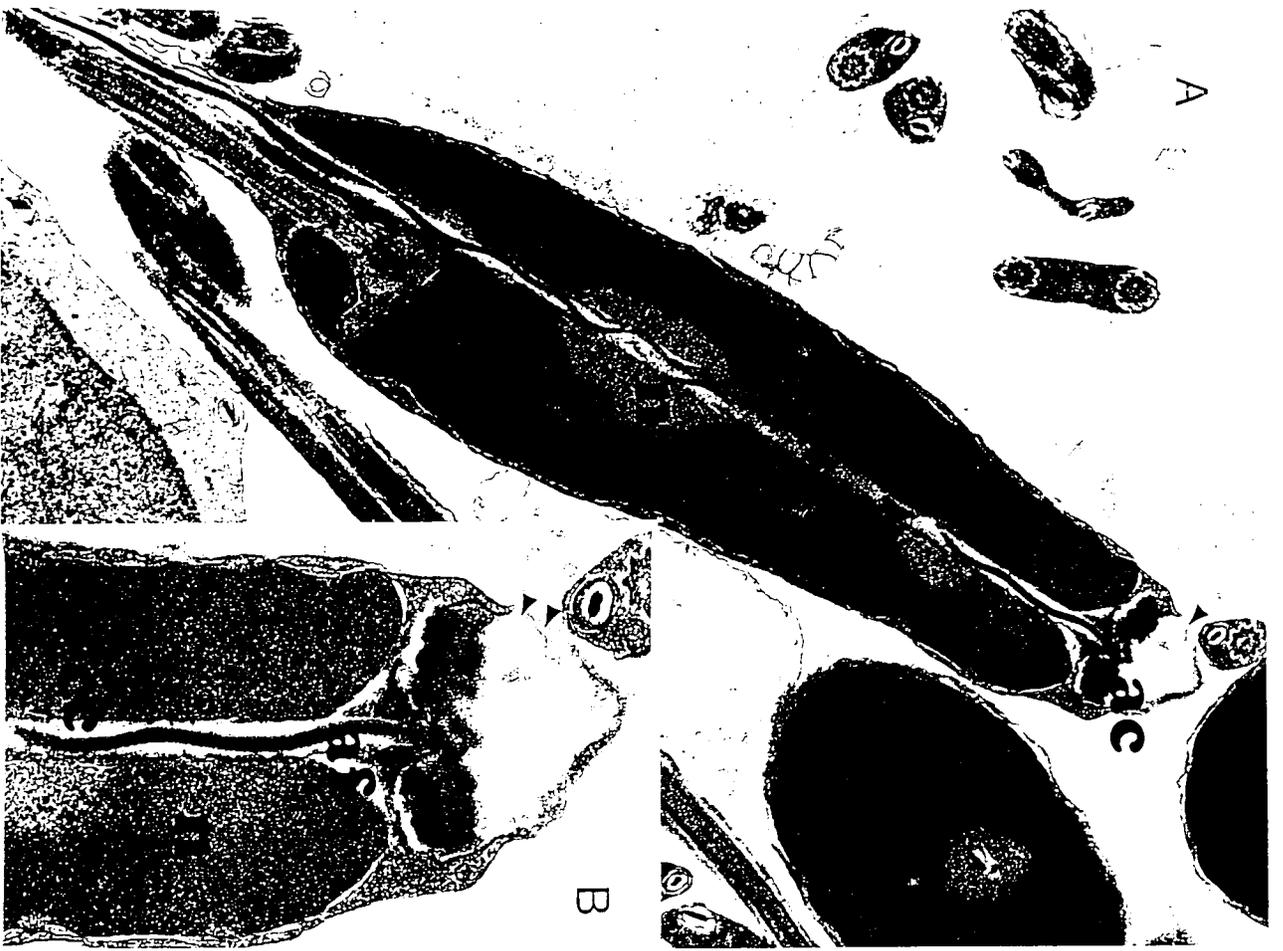
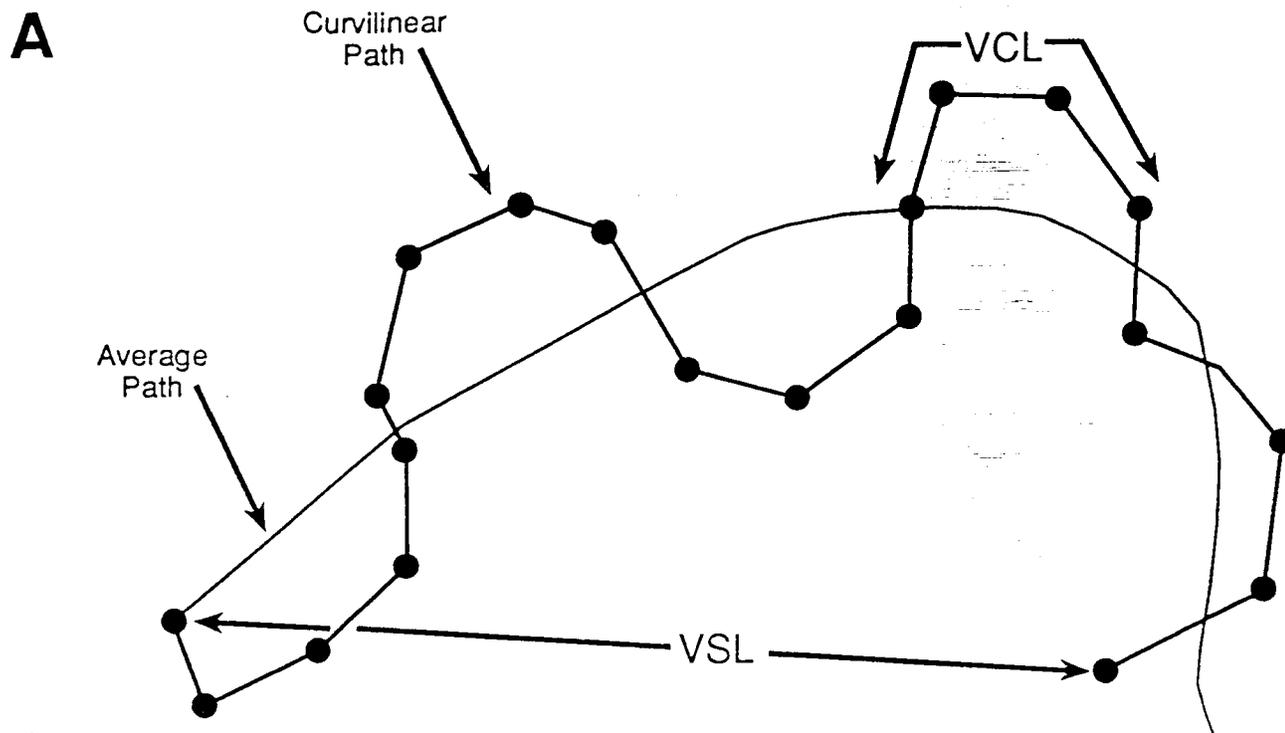


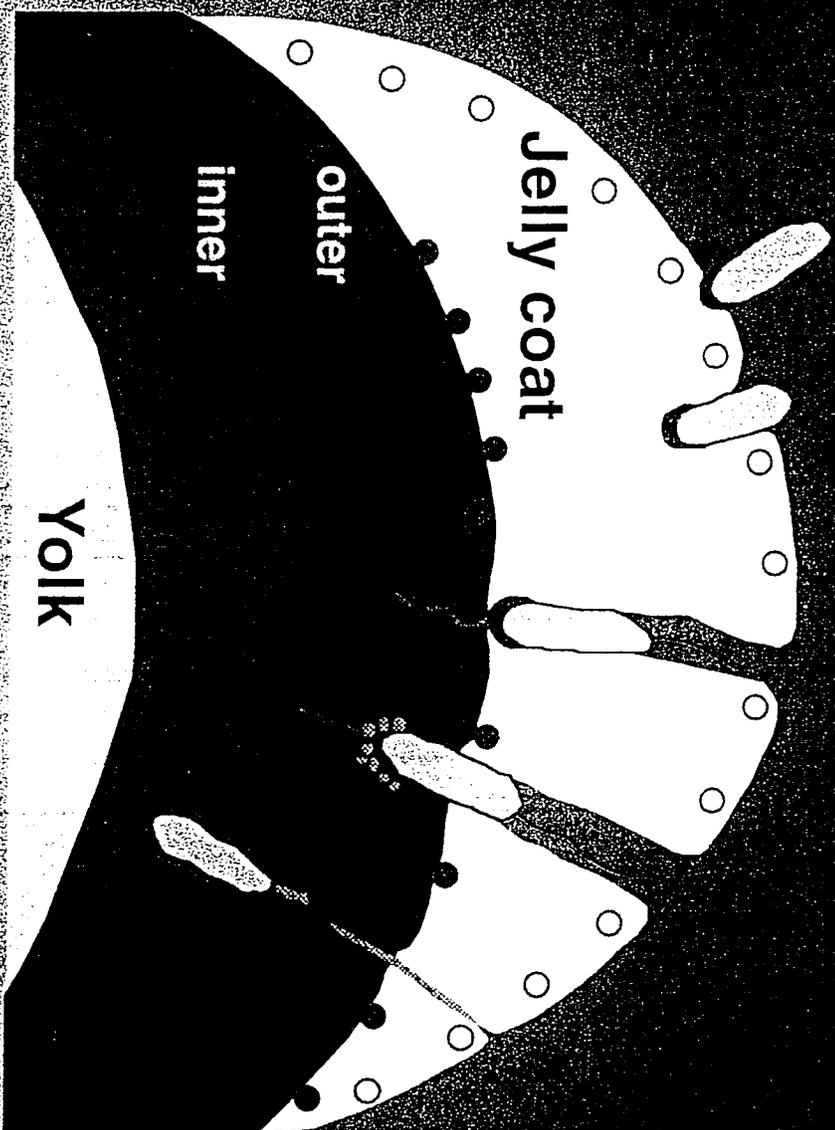
Fig. 3 Electron micrographs of spermatozoa of Petromyzon marinus.
A, B - fresh sperm fixed; C, D - sperm fixed 1 min after
activation in "egg water".
ac - acrosomal vesicle
af - acrosomal filament
ax - axoneme
cc - central (nuclear) canal
dx - distal centrioles
n - nucleus
arrows - postacrosomal ring
arrow heads - exocytosis of acrosome



B Sea lamprey sperm motility filmed at 200 Hz and assessed on a minimum 100 cells ($n = 3-4$)

Activation medium	Diluted Ringer (LR)	"Egg water"
Motile cells (%)		
0 min	35.0 \pm 20.9	28.6 \pm 15.4
3 min	7.8 \pm 3.1	44.2 \pm 13.9
Curvilinear velocity ($\mu\text{m/s}$)		
0 min	276 \pm 41	321 \pm 34
3 min	308 \pm 34	306 \pm 51
Straight line velocity ($\mu\text{m/s}$)		
0 min	40.9 \pm 7.5	39.3 \pm 2.4
3 min	35.4 \pm 1.6	39.5 \pm 5.0

Fig. 4 Using CASA for sperm motion studies. (A) An example of path of the spermatozoon; VSL-straight line velocity; VCL-curvilinear velocity. (B) Observation of VCL and VSL of the sea lamprey spermatozoa activated in the diluted Ringer solution (Kobayashi 1993) and "egg water" containing the sperm activating factor or the "gynoamone."



- channel (vesiculus) (cytogeninone)
- acrosomal filament discharge trigger

Fig. 5 Schematic representation of a spermatozoa penetrating oocyte membranes in lamprey. (February 1997).