June Sucker Milt Research – 2002

The June Sucker *Chasmistes liorus*, indigenous to Utah Lake (Utah County, Utah), is listed as endangered by the state and federal government. Since 1991 the Fisheries Experiment Station (Logan, Utah) has housed various age classes of the fish. Over the past decade, new year classes have been added from field-spawned progeny and light-trapped fry. Several years ago interest was raised in artificial spawning of the adult fish at the station. During the spawning seasons of 1998 and 1999, both females and males of certain age-classes were injected with human chorionic gonadotropin (HCG) to induce gamete maturation and spawning behavior. Variable results were obtained from both year's efforts, but it was evident that captive broodstock could be a valuable tool in reestablishing viable wild populations.

The quality and timing of eggs and sperm available during a spawning season greatly influence the success of a propagation program and may vary from year to year. To improve the success of fertilization, many artificial propagation programs rely on some sort of sperm extender. The purpose of an extender is to maintain the sperm in an inactivated state, under similar pH, chemical composition, and osmotic pressure as in seminal fluid. By diluting the milt with an extender, the milt can be stored for several days under refrigerated and oxygenated conditions. This allows the researcher alternatives such as holding milt until females are ripe, fertilization at alternative, convenient sites, and pooling multiple males collected over time to enhance genetic contributions. Extenders are commonly used on various fresh and marine species, but have not been investigated with the June Sucker.

Beginning in the spring of 2002, several attempts were made at characterizing June Sucker sperm, and evaluating various sperm activators and extenders that might benefit the program. During the first attempt at collecting milt (05-06-02) only one male of four available expressed any milt, but the quantity was very limited. A small sample of the milt was placed on a microscope slide and activated with a 0.7% lab-grade NaCl (Mallinckrodt AR® reagent grade) diluent but no sperm activity was noted, so it was assumed the sperm were not viable. During the next attempt of collecting milt (05-14-02), good quantities of milt were collected from three males. Once again samples of the collected milt were activated with 0.7% NaCl, but less than 1% of the sperm observed displayed any movement. Out of frustration a second sample was activated with well water from the June Sucker building and the sperm showed good activity. Subsequent samples were taken from each of the three milt samples, and three replicates from each were tested for activity. The average percent of sperm observed to be motile was 86%, and the duration of motility was 43 second. As a comparison, trout sperm may be motile for 1-2 minutes with generally >60% of sperm being motile. For each slide observed, 6.5 ΦL of milt was activated with 45 ΦL of diluent.

When it was established the sperm were viable, we checked to see what effect different diluents (activators) might have. Two types of salt were included: 0.7% lab-grade NaCl and 0.75% rock salt (Western Sun™ Solar Salt), which is the typical trout diluent. A commercial diluent, Diluer 532 (Sanofi Sante Animale), was included, as well deionized water, and well water. During the third hour after the milt was originally collected, each diluent was checked with respect to its ability to activate sperm. See Table 1 for these results as well as pH measurements of each, as high pH tends to keep

sperm deactivated. One slide viewed using lab salt as the diluent, which initially showed no activity, displayed good activity after a small amount of well water was placed around the margin of the cover slip. The extender, Ca^{++} HBSS, was included in this initial analysis to remove it as a possible activator. Rock salt was clearly the best diluent, followed by well and deionized water. It is worth noting that the three diluents with the best activation were also the three formulations with the lowest pH values.

Table 1. Average (N = 3) sperm motility and time of duration of June Sucker milt activated with various diluents approximately 4h after collection.

Diluent	*Motility (%)*	*Duration (s)*	*Diluent pH*
Dilueur 532	<1%	-	8.44
Rock salt, 0.75%	95%	103	7.61
Lab salt, 0.7%	<1%	-	7.90
Well water	48%	33	7.32
Deionized water	72%	40	6.08
Ca^{++} HBSS	0	0	7.25

Once it was established the sperm was viable, each sample was divided into two, with one being diluted on a 1:1 basis with Ca^{++} free Hank's Balanced Salt Solution (HBSS). This diluent had been used successfully to store razorback sucker for several days by Tiersch et al. (1997). The second portion of each sample was left untreated. All samples were placed in 50 mL plastic vials and kept at 5E C. These samples were then to be analyzed overtime to see if the extender enhanced storage time. After 24h, samples were again analyzed for quality using the above listed diluents. The results from this check are displayed in Table 2. Approximately twice daily each vial was uncapped and gently agitated to allow for gaseous exchange.

Table 2. Average (N = 3) sperm motility and time of duration of extended or un-extended June Sucker milt activated with various diluents approximately 24h after collection. E = extended, U = unextended.

Diluent		*Motility (%)*	*Duration (s)*
Dilueur 532	U	5*	67*
	E	52	64
Rock salt, 0.7%	U	<1*	115*
	E	67	107
Lab salt, 0.75%	U	<1*	52*
	E	18	62
Well water	U	0	0
	E	58	51
Deionized water	U	0	0
	E	80	38

* only one replicate (male #2) was useable, so means were not calculated.

After 24 h, all three unextended milt samples had turned gelatinous, and two of the three extended ones were thickening noticeably. However the extended samples clearly exhibited superior sperm quality to the unextended milt. The 0.75% rock salt solution also exhibited better storage capabilities than the others. It is also worth noting that in its extended form, the sperm samples were activated to a high degree by lab salt and Dilueur 532, both of which exhibited little activation potential with 4 h unextended samples. Sperm quality samples taken at 48 h resulted in motility of 33% and duration of 56 sec for extended sperm activated with rock salt, and a motility of 27% and duration of 13 sec for extended sperm activated with well water. Unextended sperm was no longer viable at 48 h, and the other diluents were not tested further due to their limited success. Samples were evaluated again at 72 h but motility for extended sperm activated with rock salt or well water averaged <1%. No further viability assays were made beyond 72 h.

The results from the first test indicated that June Sucker sperm retained relatively good motility for 1-2 days when diluted 1:1 with Ca^{++} free HBSS and activated with rock salt. However the quickness with which the extended samples gelatinized suggested that the dilution ratio of milt: Ca^{++} free HBSS was probably not high enough. As a result, a second test was conducted (6-12-02) to determine the optimal dilution ratio to maintain viable sperm. For this test milt was collected from three males and each sample was diluted on a 1:1, 1:5, 1:10, and 1:20 basis; milt:Ca^{++} free HBSS. Rock salt was used again as an activator, and at the same time a new activator was tried; rock salt with urea. Urea is commonly used on eggs to help eliminate stickiness. This has been a problem in the past with June Suckers where large portions of a lot of eggs might be lost due the adhesion of the eggs forming a mass, and becoming more susceptible to fungus and resultant mortality. The formulation of the rock salt/urea diluent was 3 g urea, 4 g rock salt, and 1.0 L well water. Activation and sperm quality assays were conducted as mentioned previously.

Sperm quality assays taken 1-2 h after the milt was initially collected averaged 87% motile and 85s for rock salt, 72% and 93s for the rock salt/urea, and 47% and 38s for well water. The 24h sample (see Table 3) revealed a rapid drop in sperm viability one day after collection. As a comparison extended sperm from the first test activated with rock salt were 67% motile for over a minute compared to 34% motile for 57 sec from this test. This might indicate a drop in sperm viability as the spawning season reached its end or simply poorer quality among the males chosen for the second test. Sperm activate with the salt and urea mixture had a higher motility averaged across dilutions, 43%, compared with 21% for the sperm activated with salt only. A duration of 60 sec was also obtained from the urea mixture compared with 47 sec for the salt only.

Table 3. Average (N = 3) sperm motility and duration time of milt extended at different dilution ratios with Ca^{++} free HBSS extended June Sucker milt activated 24h after collection. For each dilution ratio treatment samples were activated with either rock salt or rock salt with urea.

	Rock Salt		Rock Salt with Urea	
Dilution ratio	*Motility (%)*	*Duration (s)*	*Motility (%)*	*Duration (s)*
1:1	34	57	55	57
1:5	24	39	53	63
1:10	9	41	27	65
1:20	15	49	38	53

When the milt samples were further assayed for quality at 48h most viability had been lost (Table 4). Sperm activated by rock salt alone exhibited less than 1% motility, while sperm diluted on a 1:1 basis and activated with salt and urea were 20% motile. The milt that was diluted at 1:1 had begun to gelatinize after 24 h and by 48h the 1:5 samples were gelatinizing also. The 1:10 and 1:20 samples maintained good fluidity, but this did not translate into an increase in sperm viability as indicated by the results. The samples were examined again after 72h, but no viable sperm were found.

Table 4. Average (N = 3) sperm motility and duration time of milt extended at different dilution ratios with Ca^{++} free HBSS extended June Sucker milt activated 48h after collection. For each dilution ratio treatment samples were activated with either rock salt or rock salt with urea.

	Rock Salt		Rock Salt with Urea	
Dilution ratio	*Motility (%)*	*Duration (s)*	*Motility (%)*	*Duration (s)*
1:1	<1	15	20	39
1:5	<1	24	2	48
1:10	<1	17	<1	13
1:20	<1	14	8	46

From these two examinations of June Sucker milt it appears possible to collect, extend, and store milt samples for 1-2 days and still retain viable sperm. The results from the first test are contradictory, where high quality lab salt inhibited sperm activation, while rock salt, at a similar concentration, proved to be the best diluent. The pH values of the diluents may be a factor although the difference between lab and rock salt was 0.31 units. Diluents of lower pH, less than 7.9, were capable of good activation. These results are opposite of what would be expected with other fish. In general, with trout, a pH of 8.0 or below and > 9.5 can prevent activation. We recommend continued use of a rock salt diluent of 0.75% for hatchery and field fertilization. The calcium free HBSS proved to be adequate as an extender when milt was stored for a day or less. Using dilution ratios of milt to extender of 1:1 or 1:5 appeared to be the best combinations. At higher dilutions the milt seemed nice and fluid after several days, although few or no viable sperm were retained. Some of these results are interesting and conflicting, and lead us to believe that more research is required to develop a good working extender. A good working extender would allow biologists to collect milt and hold it under oxygenated and refrigerated conditions for a week to ten days as is possible with other fish species.

Citations

Tiersch, T. R., Wayman, W. R., Figiel, C. R., Jr., Gorman, O. T., Williamson, J. H., and G. J. Carmichael. 1997. Filed collection, handling, and storage of sperm of the endangered razorback sucker. North American Journal of Fisheries Management 17:167-173.

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