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THE EFFECT OF TIME AND TEMPERATURE ON MOTILITY OF SPERMATOZOA OF COMMON AND GRASS CARP

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ABSTRACT

The sperm collected from common and grass carp males was stored at 5°C, and activated with water of various temperatures (20, 26, and 30°C). Time of spermatozoa motility was measured. Motility decreased with time after milt collection. Common carp spermatozoa were active longer, up to 70–80 s. In most series their activity was reduced after 24 hours. Spermatozoa of grass carp were active up to 30–55 s, and their motility shortened already in 8 hours post collection. After 24 hours they were motile less than 10 s. The effect of temperature of activation was observed – the spermatozoa were active for the longest time at 20°C. Spermatozoa motility time was also affected by temperature of storage. Even short–term (15 min) keeping spermatozoa at 20°C shortened their motility time in both species, and after 2 hour storage common carp sperm motility was reduced by about 50%. Storage or acclimation at high temperature reduced also spermatozoa viability (ability to undertake movements after activation). The results of present study indicate the possibility of milt storage in refrigerator (5°C) – up to 24 hours for common carp, and 8 hours for grass carp, without considerable reduction of sperm quality.

Key words: common carp, grass carp, spermatozoa motility, temperature.

INTRODUCTION

Spermatozoa motility – time of their motion after activation – is very important for successful fertilization of the eggs, so together with morphologic characteristics, is considered an indicator of milt quality. Sperm motility, and their ability to fertilize eggs is highest just after stripping. In case of artificial spawning, storage of milt is sometimes necessary.

Possibility of milt storage at about 0°C was proved by Babiak and Glogowski [1], Blaxter [4], Hulata and Rothbard [10] and Malczewski [14]. The authors observed that sperm stored at that temperature was able to fertilize eggs. It is interesting, however, how storage affects sperm motility time. It seems that temperature of activating medium may affect activity of spermatozoa. Goodall et. al [9], and Babiak and Glogowski [1] showed that at lower temperatures time of motility is longer.

The aim of present study was an evaluation of the effect of temperature of storage, acclimation, and activation on motility time of spermatozoa of comon and grass carp over time post stripping.

MATERIALS AND METHODS

Sperm of common and grass carp obtained from the hatchery of Inland Fisheries Institute in Zabieniec was used in the study. Experimental pattern is shown in Table 1. The milt was stored at 5°C, except common carp sperm in series 4 which was initially (2 hrs) kept at about 20°C. Time of spermatozoa motility, after their activation at 20, 26, or 30°C was measured, after 15 minutes of acclimation, or without acclimation. A drop of milt (about 5 mm^3) was placed on a slide, and activated with 50 mm^3 of water, and covered with a cover glass. Spermatozoa motion was observed using microscope (10 × 20 magnification), and time of motility was measured using stopwatch, until progressive movement of about 80% of spermatozoa ceased. Time of sperm motility was measured repeatedly, in 12 hrs intervals (only in series 2 for grass carp the measurements were done every 4 hrs). Five series of measurements were done for common carp (Fig. 1), and 2 for grass carp (Fig. 2). The results are shown in graphs.

Table 1. Experimental pattern

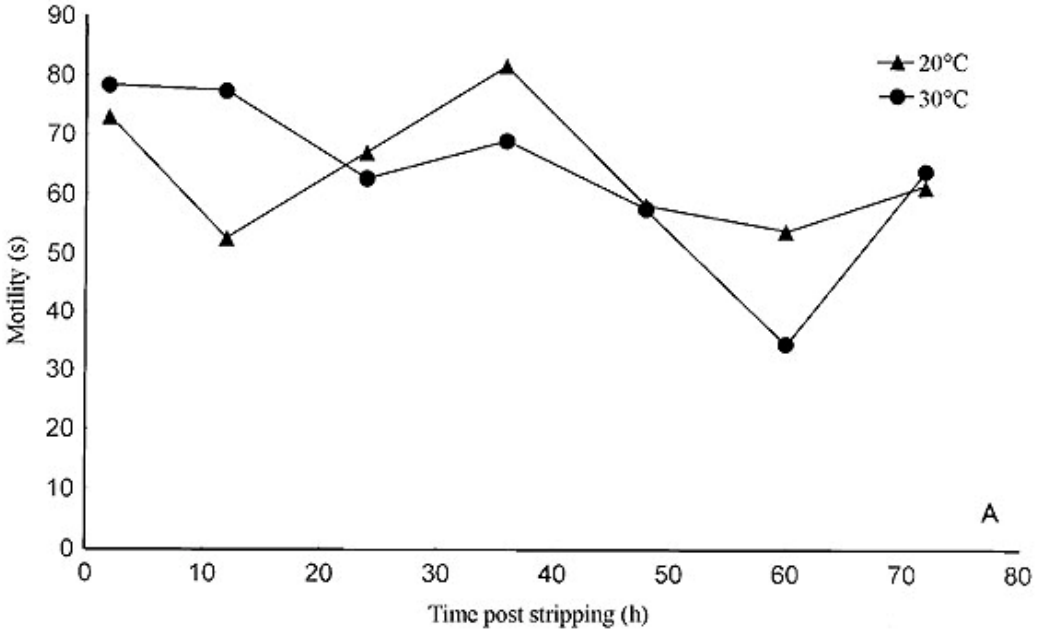
Species	Series	Activation temperature °C	Acclimation	Number of males (n)
Common carp	1	20	+	3
		30	+	3
	2	20	+	3
		30	+	3
	3	20	+	5
		20	-	5
		30	+	5
	4*	20	+	5
		30	+	5
	5	20	-	2
		26	-	2

Grass carp	1	20	+	6
		20	-	6
		30	+	6
	2	20	+	6
		30	+	6

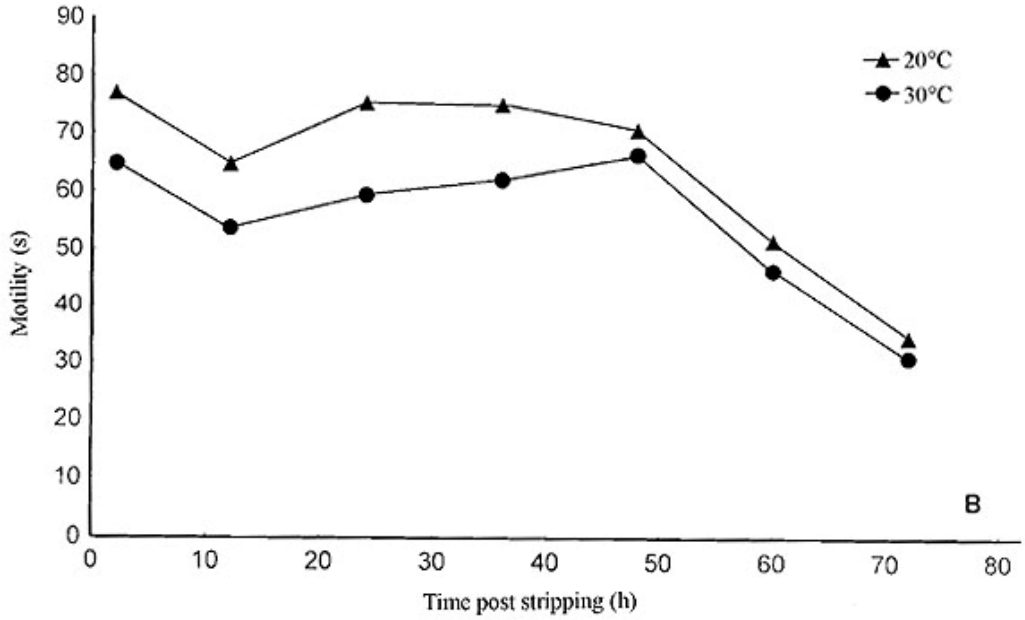
4* - milt kept at 20°C for first 2 hours post stripping, then at 5°C.

Figure 1. The effect of time and temperature on common carp spermatzoa motility (A – series 1, B – series 2, C – series 3, D – series 4, E – series 5)

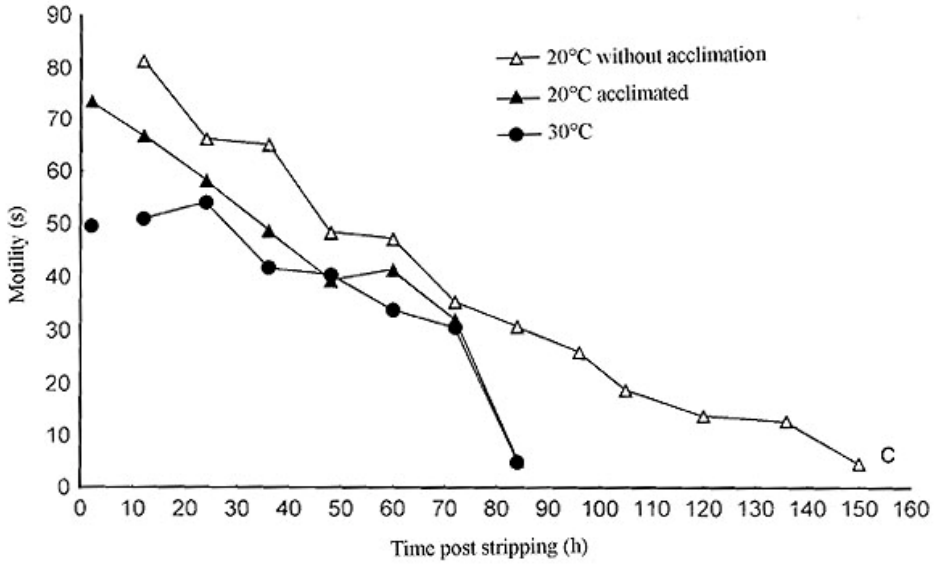
A



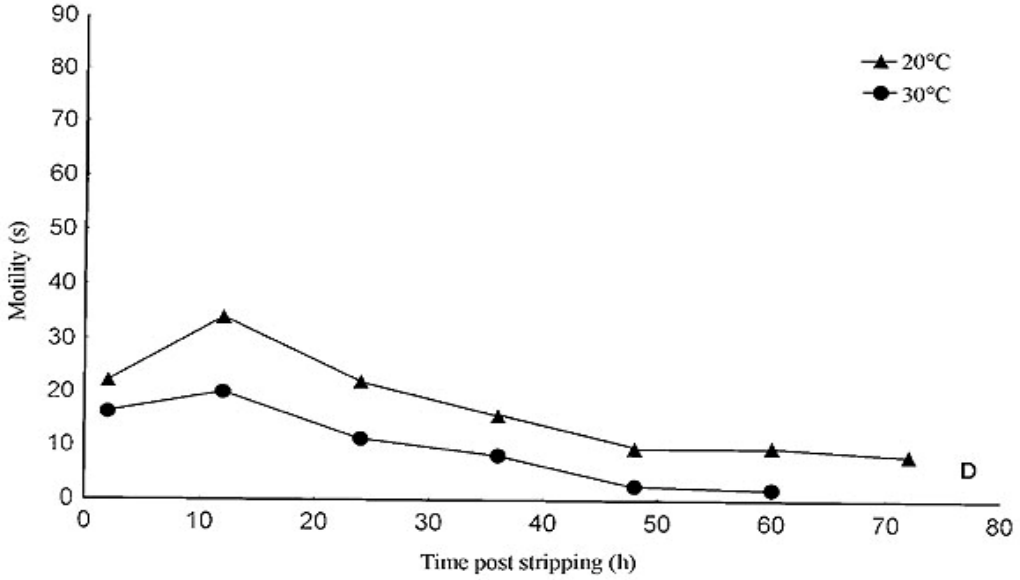
B



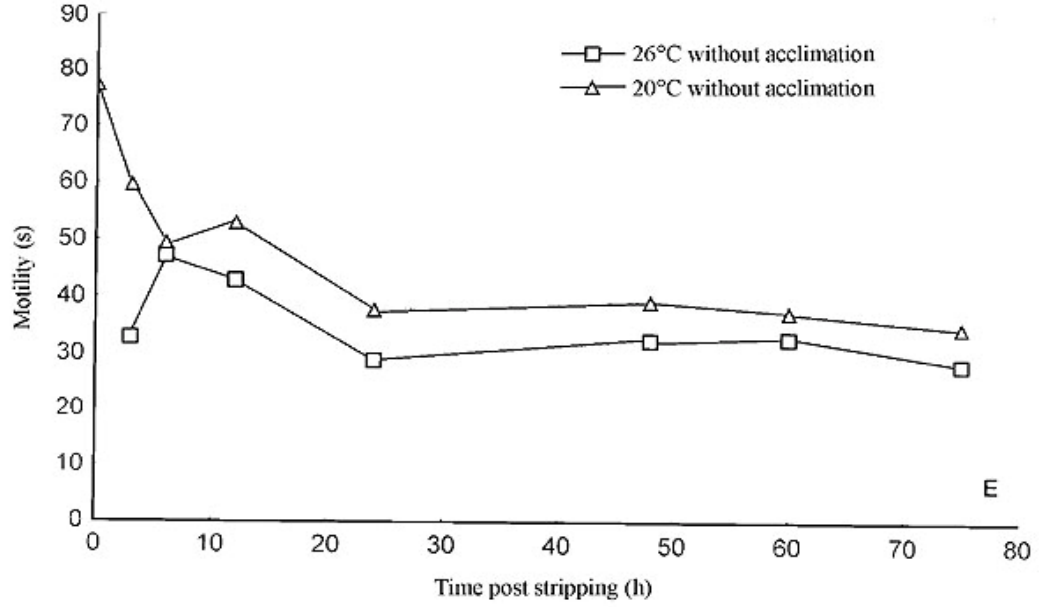
C


20°C without acclimation
20°C acclimated
30°C
Motility (s)
Time post stripping (h)
C

D


20°C
30°C
Motility (s)
Time post stripping (h)
D

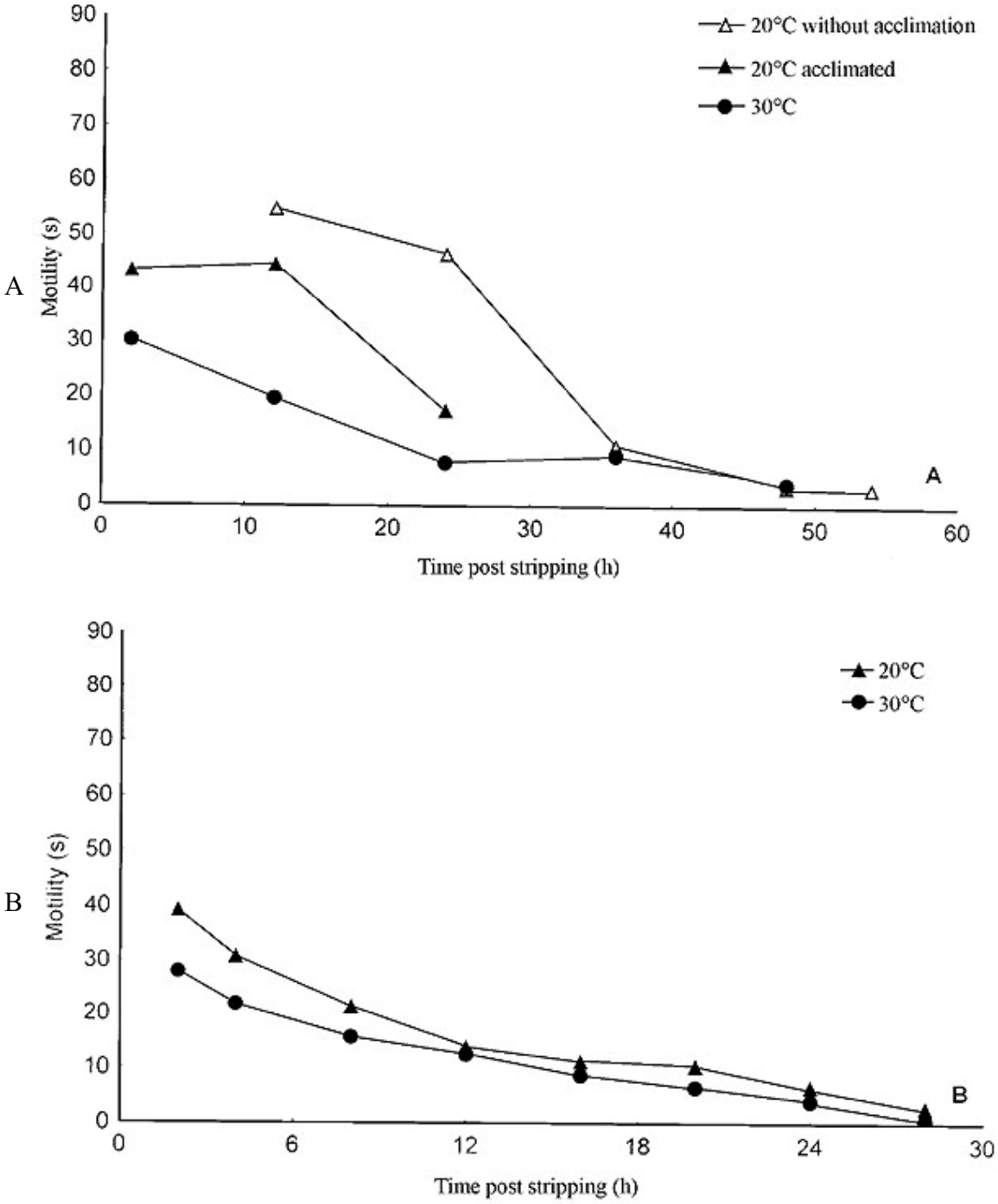
E


26°C without acclimation
20°C without acclimation
Motility (s)
Time post stripping (h)
E

DISCUSSION OF RESULTS AND CONCLUSIONS

Figures 1A–E (common carp), and 2A, B (grass carp) show that spermatozoa activity in various series considerably differed. Such variability might have resulted from individual differences among the spawners, and pre–spawning conditions [2, 5, 6, 7, 13].

Figure 2. The effect of time and temperature on grass spermatzoa motility (A – series 1, B – series 2)



Maximum time of common carp spermatozoa motility observed in present study ranged from 70 to 80 s, only in series 4 was considerably shorter. The results confirm the data obtained by other authors. Krüger et al. [13] demonstrated that average time of carp spermatozoa motility

depended on the season and ranged from 1.2 to 1.6 minutes. According to Gluchowska and Jezierska [7] and Jezierska et al. [11] maximum motility time of carp sperm was 60 s, and according to Koldras and Moczarski [12] – even over 2 minutes.

Time of grass carp sperm motility was shorter – 30–55 s. That is also consistent with the data of other authors who obtained 30–45 s [17, 18, 19]. According to Belova [3] grass carp spermatozoa were active for 56 s, and according to Zukinski and Aleksenko [20] – for 30–100 s.

Irrespective of the species, and temperature of activation, all measurements showed that motility time decreased with time post stripping. Reduction was, however, different in each species, and in the series.

Motility time of common carp sperm in series 1 and 2 was slightly reduced after 60 hours (Fig. 1A, B). In other series it was considerably shortened already after 24 hrs post stripping (Fig. 1C, D, E).

Storage even more affected milt activity of grass carp (Fig. 2A, B). In series 1 time of motility was considerably reduced in 24 hrs, and in 36 hrs post stripping did not exceed 10 s. In series 2 time of sperm motility was reduced already in 8 hrs, and in 24 hrs almost ceased.

Reduction of spermatozoa quality (their ability to fertilize eggs) with time is well known, but there are little detailed data on motility time reduction. Goodall et al. [9], Gluchowska and Jezierska [7], and Jezierska et al. [11] showed the effect of storage on motility time, and Ravinder et al [15] – on the percentage of spermatozoa motile after activation. According to Sarnowski et al. [17], however, storage of sperm for 5 h in the refrigerator did not adversely affect fertilization rate. Present study showed that common carp sperm may be stored in refrigerator for about 24 hours, and grass carp sperm – 8 hours without considerable reduction of motility time.

It is generally thought that spermatozoa are active longer at lower temperatures. There are, however, little data on that. Goodall et al. [9] showed the effect of temperature of milt itself, and of activating medium on sperm motility of *Sillago ciliata*. The activity was longest when milt kept at 0°C was activated with the medium of the same temperature, and shortest – when milt and medium were at room temperature. In present study spermatozoa were motile longer at 20°C compared to 26 or 30°C (common carp), or to 30°C (grass carp).

Also the comparison of motility of acclimated and non–acclimated sperm revealed the effect of temperature (Fig. 1C, 2B). Time of motility of non–acclimated spermatozoa was always longer, and longer was their viability (ability to movement after activation). The effect of temperature of storage on spermatozoa viability was also observed by Ravinder et al. [15] – carp spermatozoa stored at 2 or 5°C were much longer able to undertake movement after activation than ones kept at 22°C.

Adverse effect of high storage temperature is also obvious from the results obtained in 4 series for common carp (Fig. 1D). The milt was kept for first 2 hrs at 20°C which considerably reduced its motility time comparing to other series.

Short–term storage of milt is sometimes necessary in hatcheries where artificial spawning is done. Babiak and Glogowski [1] concluded, basing on the data of various authors, that

refrigerated sperm is able to fertilize eggs in several hours, or even days after stripping. Hulata and Rothbard [10] recommend temperature range 0–5°C for freshwater fish milt storage. Malczewski [14] observed that storing common carp sperm at 0–4°C up to 12 hours did not reduce fertilization.

The results of present study confirmed the possibility of milt storage at about 5°C, and also showed adverse effect of storage at temperature 20°C upon milt activity and viability. Assuming that sparmatozoa motility time is an indicator of its quality and fertilization ability, it seems that common carp milt may be stored in refrigerator up to 24 hours, and grass carp milt – to 8 hours post stripping.

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