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A Study on the Milt Quality of *Oncorhynchus mykiss* (Walbaum, 1972) and *Carasobarbus luteus* (Heckel, 1843) in Atatürk Dam Lake, Southeastern Turkey

Faruk Aral[1], Erdinç Şahinöz[2], Zafer Dogu[2,*]
[1] University of Harran, Veterinarian Faculty, Department of Artificial Insemination and Reproduction, Şanlıurfa, Turkey.
[2] University of Harran, Bozova Vocational School, Department of Fisheries, Bozova, Şanlıurfa, Turkey.

* Corresponding Author: Tel: +90. 414 7116206; Fax: +90. 414 7116499;
E-mail: zaferdogu@harran.edu.tr -zafer_dogu@yahoo.com

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Abstract

The aim of this study was to determine the milt quality of *Oncorhynchus mykiss* (Walbaum, 1972) and *Carasobarbus luteus* (Heckel, 1843). Milt was collected twice by abdominal massage during the spawning seasons of the species. Volume, forward motility, duration of motility, milt concentration and pH were determined in milt collected. Body weights and lengths of brood fish were also measured. Milt volume (ml), forward motility (%), the duration of motility (sec), concentration (x10^9cell/ml) and pH values were 1.22±0.22 and 0.80±0.06, 73.25±5.15 and 55.50±4.59, 90.80±10.40 and 175.80±17.00, 6.06±0.90 and 11.29±1.46, 7.99±0.03 and 8.07±0.12 for *O. mykiss* and *C. luteus*, respectively. The spermatozoa concentration of *O. mykiss*, and duration of motility, spermatozoa concentration and sperm pH of *C. luteus* were affected significantly by milt stripping dates ($P<0.05$, $P<0.01$). The results suggested that the spermatozoa concentration of *O. mykiss*, and duration of motility, spermatozoa concentration and sperm pH of *C. luteus* were affected by stripping dates.

Key words: *Oncorhynchus mykiss*, *Carasobarbus luteus,* milt quality, sperm motility, sperm concentration.

Introduction

Rainbow trout (*Oncorhynchus mykiss*; Walbaum, 1972) is one of the most important cultured fish species with significant economic value. Similarly, himri (*Carasobarbus luteus*) (Heckel, 1843) has delicious meat and are consumed considerably by local people. The species is found in Tigris and Euphrates River Systems (Geldiay and Balık, 1999) and they are one of the major economic fish species in Atatürk Dam Lake (Şevik and Yüksel, 1997).

Both species have seasonal reproductive cycles. Spawning season of *O. mykiss* is between January and February at water temperatures of around 9–12ºC, while the spawning season of *C. luteus* is between June and July (22–25ºC) (Şevik and Yüksel, 1997).

Spermatozoa motility, milt volume and the spermatozoa concentration are good indicators for milt quality (Cabrita *et al.*, 2001; Tekin *et al.*, 2003). Milt volume is one of the features reflecting the milt yield and spermatozoa concentration. Likewise, Moon *et al.* (2003) reported positive correlation between milt volume and spermatozoa concentration in male starry flounder, *Platichtys stellatus*.

Spermatozoa are immotile immediately after collection (Morisawa and Suzuki, 1980; Morisawa *et al.*, 1988). Changes in the osmotic pressure (0–300 mosmol/l) could start motility in most of the fish species (Morisawa and Suzuki, 1980). The spermatozoa motility and its duration have great influence on successful fertilization. Spermatozoa acquire the motility during the transition from the testis to sperm duct. Their values were high in good quality sperm (Babiak *et al.*, 1999; Tekin *et al.*, 2003).

Spermatozoa concentration may also influence the rate of fertilization (Aas *et al.*, 1991; Pool and Dillanea, 1998). For this reason, determination of the spermatozoa concentration is important in fertilization studies. The milt pH effects the spermatozoa motility and maturation (Billard *et al.*, 1995; Liley *et al.*, 2002). Thus fertility is markedly increased with increasing milt pH from 8.0 to 8.2 in *O. mykiss* (Lahnsteiner *et al.*, 1998). So, determination of variation in milt pH, could provide information on fertilization capacity spermatozoa.

Although evaluation of milt quality and the characteristics of the seminal plasma have been studied in many fish species, there are no available data on *C. luteus* from Southeastern Turkey. Similarly, there are limited data on the rainbow trout farmed in this region. *C. luteus* and *O. mykiss* have also economic importance for aquaculture, inhabiting in the Atatürk Dam Lake, Euphrates River System. Therefore, investigation of the possible influence of environmental factors on reproductive activity of fish in Atatürk Dam Lake was aimed by measuring spermatological characteristics in these species.

The main aim of this study was to examine the milt volume, the forward motility, duration of motility, spermatozoa concentration and sperm pH of *O. mykiss* and *C. luteus* in natural spawning seasons.

Materials and Methods

O. mykiss reared in floating cages and native species *C. luteus* captured with fishing nets (18–20–

28–32 mm mesh size) in Atatürk Dam Lake (37º23'29" N, 38º34'38" E) Southeastern Turkey were used in 2004. Scales and otoliths of *C. luteus* are used for age determination according to method suggested by Ricker (1975). Physico-chemical parameters (temperature, dissolved oxygen and pH) of the water were measured by YSI Environmental (YSI 85). Fish samples obtained were transported to the laboratory in tanks containing lake water and for age determination, scales and otoliths were examined under a stereo microscope (Nikon SMZ 2T stereo).

During the study, twenty male *O. mykiss* were selected randomly and were kept separately under natural water temperature (11±0.04ºC) in floating cages (4x4x4 m).

Fish were fed with crude pellets containing 91.6% dry matter, 45.8% crude protein, 20.0% crude lipid and 12.0% crude ash. Feed was provided twice a day at a daily feeding rate of about 1% of the fish biomass.

Milt collections were carried out on February 26, 2004 and on March 11 for *O. mykiss* and, on June 25 and July 09 for *C. luteus*. Ten fish from each species at each date were stripped after weighing and measuring. Milt samples were taken into different sperm collection tubes by applying gentle manual pressure to the abdomen in both species. Milt was collected once from each fish in the field. After collection, milt samples were transported to the laboratory under cold conditions (7–10ºC). All fish were in the middle of the spermiation (milt could be brought out by gentle pressure on the abdomen) in sperm collection dates.

Volume, forward motility, duration of motility, spermatozoa concentration and pH were then determined.

Milt volume was determined by measuring with a pipette and expressed as ml. Forward motility was assessed according to Tekin *et al.* (2003) and expressed as a percent of motile spermatozoa at 10–11 ºC for *O. mykiss* and at 24ºC (natural lake water temperature) for *C. luteus*. One drop of milt (0.5 µl) and one drop of activating solution (0.03% NaCl) (10 µl) were placed close to each other under a microscope (x 400). Then, two drops were mixed and the motility of spermatozoa was observed. The duration of motility was timed by chronometer from the initial contact between the solution and milt until cessation of motility and expressed as seconds. Spermatozoa concentration was determined using haemocytometer and expressed as number of cells x 10^9 cell/ml. Sperm pH was determined with a pH indicator strips (pH:0–14; Merck, Germany).

In both species, two sample t-test was used to compare semen volume, motility duration, milt pH, body weight, and total length at two milt collection dates. Correlation analysis was performed among sperm characteristics. Data were analyzed in Minitab 12.0 Statistical Program.

Results

Mean water temperatures were 11ºC during stripping *O. mykiss* and 24-25ºC for *C. luteus*, while dissolved oxygen and pH values ranged around 8.3-9.5 mg/l and 8.5, respectively. *O. mykiss* specimens were 11–13 months and *C. luteus* were 2 years old. Body weight (BW), total length (TL) and milt properties were summarized in Table 1 and 2 for *O. mykiss* and. *C. luteus*. Total length and body weight of males of *C. luteus* were lower ($P<0.01$) in the first collection date than those in the second collection, but not in *O. mykiss*.

In both species, no significant differences in milt volumes were observed between the collection dates in the milt volumes.

The mean spermatozoa motility rates were 73.25±5.15% in *O. mykiss* and 55.50±4.59% in *C. luteus*, respectively (Table 1 and Table 2) and no significant differences were observed between sperm collection dates.

The motility duration of *C. luteus* was significantly higher in the second collection date than that of first collection ($P<0.05$), but there was no difference in *O. mykiss*.

Spermatozoa concentrations of both species were significantly lower in second collection compared to the first collection ($P<0.05$ for *O. mykiss* and $P<0.01$ for *C. luteus*).

Sperm pH of *C. luteus* in the first collecting date was significantly higher than that of second collection date, while no differences were observed in *O. mykiss* (Table 1 and 2).

When correlation analysis was performed among sperm characteristics, sperm concentration was negatively correlated with pH in *O. mykiss* ($r=-0.53$, $P<0.05$), but positively correlated with pH in *C. luteus* ($r=0.58$, $P<0.01$).

Discussion

The mean milt volume of 1-2 years old *O. mykiss* reported by Tekin *et al.* (2003) was considerably higher than that obtained in current study. Low milt volume could be related to differences in husbandry, feeding, size and age of the fish.

The mean milt volume of *C. luteus* (0.80 ml) was higher than that reported by Yeuehi and Chang (1997) for *C. carpio* (0.30 ml). Besides environmental factors, these differences of milt volume may be due to spawning season of *C. luteus*. There were no differences in milt volumes of both species between their milt collection dates (Table 1 and 2).

The mean spermatozoa motility in *O. mykiss* was just within the limits of motility rates suggested by Morisawa *et al.* (1988) (70–90%). Spermatozoa motility of *C. luteus* was less than those reported for this species (75-85%) (Linhart *et al.*, 2000; Hovårth *et al.*, 2003; Basavaraja and Hegde, 2004). Spermatozoa

Table 1. Mean values of spermatological traits and size of *O. mykiss* broods at the first and second stripping

Traits	First collection	Second collection	Mean± S.E	Min.	Max.
Body weight (g)	271.23±23.12	267.40±23.48	269.32±16.04	178.00	422.00
Total lenght (cm)	16.53±1.02	16.65±0.98	16.59±0.69	11.90	21.30
Milt volume (ml)	1.61±0.31	0.84±0.25	1.22±0.22	0.10	3.30
Motility (%)	76.50±6.83	70.00±7.92	73.25±5.15	30.00	95.00
Duration (s)	93.80±18.55	87.80±10.61	90.80±10.40	50.00	240.00
Spermatozoa concentration ($x10^9$cell/ml)	8.38±0.98[a]	3.73±0.11[b]	6.06±0.90	4.50	13.55
pH	7.96±0.07	8.02±0.02	7.99±0.03	7.70	8.40

Different superscript letters (a, b) in the same row indicate significant differences, from each other (P<0.05)

Table 2. Mean values of spermatological traits and size of *C. luteus* at the first and second stripping dates

Traits	First collection	Second collection	Mean± S.E	Min.	Max.
Body weight (g)	66.41±4.36[a]	43.53±4.86[b]	54.92±4.13	16.50	97.50
Total lenght (cm)	17.01±0.43[a]	13.53±0.83[b]	15.27±0.60	10.40	19.60
Milt volume (ml)	0.86±0.09[a]	0.74±0.79[a]	0.80±0.06	0.40	1.40
Motility (%)	59.00±5.47[a]	52.00±7.49[a]	55.50±4.59	30.00	95.00
Duration (s)	136.40±24.48[a]	215.20±16.60[b]	175.80±17.00	40.00	310.00
Spermatozoa concentration ($x10^9$cell/ml)	14.59±1.97[a]	7.99±1.64[b]	11.29±1.46	3.65	30.12
pH	8.47±0.15[a]	7.67±0.59[b]	8.07±0.12	7.50	9.00

motility might be affected by osmotic pressure of the activation solution or high air temperature peculiar to the region at the time of sperma collection (38–40ºC). Because, osmotic pressure plays an important role in starting spermatozoa motility and the suitable range is around 0–300 mosmol/L (Morisawa *et al.*, 1988).

The duration of motility was 90.80 seconds in *O. mykiss*. This value complies with that of motility duration reported by some authors (75-155 seconds) for same species (Büyükhatipoğlu and Holtz, 1984; Babiak *et al.*, 1999; Tekin *et al.*, 2003). Similarly average motility duration for *C. luteus* was within the limits (90-180 seconds) of reported values in previous studies (Billard *et al.*, 1995; Percheca *et al.*, 1995; Suzuki, 1995; Linhart *et al.*, 2000; Basavaraja and Hegde, 2004) for this species. While motility duration of *O. mykiss* spermatozoa showed slight decrease in terms of sperm collection dates, the motility duration of *C. luteus* spermatozoa rose significantly on the second sperm collection (P<0.05). It has been suggested that it may result from sperm pH or the change in spermatozoa internal pH (Lahnsteiner *et al.*, 1998; Krasznai *et al.*, 1995). In fact, milt pH dropped significantly on the same day compared with the first day of milt collection (Table 2).

In the present study, the mean spermatozoa concentration for *O. mykiss* was considerably lower than those obtained in previous studies (8.97 - 17.9 x 10^9 cell/ml) (Ciereszko and Dabrowski, 1993; Bloom and Ottobre, 2001; Babiak *et al.*, 2002; Tekin *et al.*, 2003). This might be due to various factors including husbandry procedures and age of the fish. Average spermatozoa concentration of *C. luteus* was similar to spermatozoa concentration values of Salmonidae and Cyprinidae species (12.0–35.0 x 10^9 cell/ml) (Babiak et al., 1997; Babiak et al., 2002; Tekin et al., 2003).

Spermatozoa concentrations dropped significantly in the second sperm collection in *O. mykiss* and *C. luteus* (P<0.05, P<0.01). In *O. mykiss*, spermatozoa concentration dropped towards to the end of spawning season (Munkittrick and Moccia, 1987). As a result, it can be suggested that spermatozoa concentration in *O. mykiss* may have been lower due to the fact that they were approaching to the end of the spawning season on the second date of sperm collection. It is quite possible that a similar situation is valid for *C. luteus* or this decrease may have resulted from the fact that in the second date the fish strripped were smaller (Table 1 and 2).

In most fish species (e.g. *Hippoglossus hippoglossus, O. mykiss, Polyodon spathula)* sperm pH is one of the major factors activating spermatozoa (Billard, 1983; Billard *et al.*, 1993; Linhart *et al.*, 1995). The mean milt pH in *O. mykiss* was similar to that found in previous studies (7.2-7.9) (Tekin *et al.*, 2003; Aral *et al.*, 2004). But, in *C. luteus* no comparison could be made, because there were no data available related to that parameter. Considering the milt collection dates, sperm pH of *O. mykiss* was similar to that of previous studies while *C. luteus* was significantly lower on the second milt collection date (P<0.01).

The correlation between milt pH and spermatozoa concentration in *O. mykiss* and *C. luteus* could be resulted from changes in the osmolality of seminal plasma, concentration of sodium and potassium in collected milt (Lahnsteiner *et al.*, 1998).

In conclusion, the results suggested that the

spermatozoa concentration of *O. mykiss*, and duration of motility, spermatozoa concentration and sperm pH of *C. luteus* were affected by spawning dates.

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