

## Sperm features in turbot (*Scophthalmus maximus*): a comparison with other freshwater and marine fish species

Marc Suquet <sup>(1)</sup>, Roland Billard <sup>(2)</sup>, Jacky Cosson <sup>(3)</sup>, Germaine Dorange <sup>(5)</sup>,  
Laurent Chauvaud <sup>(1)</sup>, Chantal Mugnier <sup>(1)</sup> and Christian Fauvel <sup>(4)</sup>

<sup>(1)</sup> IFREMER, Laboratoire de Recherches aquacoles, BP 70, 29280 Plouzané, France.

<sup>(2)</sup> Muséum National d'Histoire Naturelle, 43, rue Cuvier, 75231 Paris, France.

<sup>(3)</sup> CNRS, Observatoire océanologique, URA 671, 06230 Villefranche-sur-Mer, France.

<sup>(4)</sup> IFREMER, Chemin de Maguelone, 34250 Palavas-les-Flots, France.

<sup>(5)</sup> Université de Bretagne Occidentale, Laboratoire de Biologie marine, 29287 Brest Cedex, France.

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### Abstract

This paper presents some features of turbot sperm and compares them with data reported in the literature on other freshwater and marine fish. In captivity, turbot spermiation period lasts 6 months. When compared to other fish species, turbot males are characterized by low values of maximum gonadosomatic index (0.6-0.8%) and are poor sperm producers in terms of volume (0.2-2.2 ml), concentration ( $0.7-11.0 \times 10^9$  spermatozoa/ml) and total sperm number released per stripping ( $0.2-12.0 \times 10^9$  spermatozoa). Compared to most freshwater species, the duration of cell movement is long (1-17 min). The spermatozoon of turbot can be considered to be of a primitive type. A very high protein content (8.8 mg/ml) is found in the seminal fluid of turbot. Increasing collection frequency of turbot males, from monthly to fortnightly and weekly stripping, results in the release of successive samples presenting decreasing semen volume and spermatozoa concentration. Similar spermatozoa concentration and total volume of semen produced per turbot, during a 2-month experimental period, are released from males submitted to an annual light and temperature cycle, compared to those receiving a 6 month contracted light and temperature schedule.

**Keywords:** *Scophthalmus maximus*, turbot, sperm biology, seminal fluid, sperm motility, sperm morphology, marine fish.

*Caractéristiques du sperme de turbot (Scophthalmus maximus): une comparaison avec d'autres espèces de poissons d'eau douce et marine.*

### Résumé

Les caractéristiques du sperme de turbot sont comparées aux données existantes de la littérature chez quelques poissons marins ou d'eau douce. En captivité, la période de spermiation dure 6 mois. Comparé à d'autres espèces de poisson, le turbot mâle présente des valeurs maximales peu élevées du rapport gonado-somatique (0,6-0,8%) et produit de faibles volumes de sperme (0,2-2,2 ml), concentration ( $0,7-11,0 \times 10^9$  spermatozoïdes/ml) et nombre total de spermatozoïdes récoltés par prélèvement ( $0,2-12,0 \times 10^9$  spermatozoïdes). Comparé à la plupart des poissons d'eau douce, la durée du mouvement des spermatozoïdes est élevée (1-17 min). Le spermatozoïde de turbot peut être considéré comme appartenant au type primitif. Une concentration élevée en protéines (8,8 mg/ml) est mesurée dans le liquide séminal de turbot. L'augmentation de la fréquence de prélèvement du sperme, d'une fois par mois à une fois tous les 15 jours ou une fois par semaine, affecte la concentration en spermatozoïdes et le volume de sperme récolté par prélèvement. La concentration en spermatozoïdes et le volume total de sperme récolté par turbot, durant une période expérimentale de 2 mois, ne sont pas modifiés, lorsque le cycle annuel de photopériode et température est contracté sur une période de 6 mois.

**Mots-clés :** *Scophthalmus maximus*, turbot, biologie du sperme, liquide séminal, mobilité du spermatozoïde, morphologie du spermatozoïde, poisson marin.

## INTRODUCTION

Sperm biology has been widely studied in domesticated freshwater fish (for review see Scott and Baynes, 1980; Stoss, 1983; Billard, 1986; Maisse, 1990; Billard, 1992; Billard *et al.*, 1986). The knowledge of different features of sperm physiology in these species has led to an improvement in gamete management, including handling, storage and insemination protocols (Billard, 1988). The standardization of these techniques has led to the development of genetic studies. As marine fish farming expands, there is an increasing need to improve the breeding process, *i.e.* artificial insemination, gamete handling and short-or long-term preservation. As a consequence, sperm has been recently studied in some marine fish species. These studies dealt with

spermatozoon morphology (for review see Jamieson, 1991), composition of the seminal fluid (Chambeyron and Zohar, 1990) and sperm concentration and motility (Billard *et al.*, 1977; Doi *et al.*, 1982; Gwo *et al.*, 1991; Methven and Crim, 1991; Lee *et al.*, 1992; Trippel and Neilson, 1992; Billard *et al.*, 1993). A good knowledge of sperm biology is especially useful in handstripped fish species for which insemination procedures have yet to be developed.

In turbot (*Scophthalmus maximus*), the mean fertilization rate observed over a two-year period was about 70% (Omnes *et al.*, 1991) with high variations around this result. These variations may be due to gamete quality, but also to insemination conditions. Consequently, turbot gametes have recently received research attention. This work reviews some characteristics of sperm biology in this

**Table 1.** – Common and latin name of different fish species cited in the text and in the tables.

Environment	Species	Latin Name
Marine species	Atlantic cod	<i>Gadus morhua</i>
	Atlantic croaker	<i>Micropogonias undulatus</i>
	Atlantic halibut	<i>Hippoglossus hippoglossus</i>
	black seabream	<i>Spondyliosoma cantharus</i>
	blue cod	<i>Paraperis colias</i>
	bluefin tuna	<i>Thunnus thynnus</i>
	dab	<i>Limanda limanda</i>
	golden grey mullet	<i>Mugil auratus</i>
	Pacific herring	<i>Clupea harengus</i>
	plaice	<i>Pleuronectes platessa</i>
	puffer	<i>Fugu niphobles</i>
	seabass	<i>Dicentrarchus labrax</i>
	seabream	<i>Sparus aurata</i>
	sole	<i>Solea solea</i>
	stripped mullet	<i>Mugil cephalus</i>
	turbot	<i>Scophthalmus maximus</i>
Amphihaline species	Atlantic salmon	<i>Salmo salar</i>
	chum salmon	<i>Oncorhynchus keta</i>
	coho salmon	<i>Oncorhynchus kisutch</i>
	eel	<i>Anguilla anguilla</i>
	sturgeon	<i>Acipenser bearii</i>
Freshwater species	brook trout	<i>Salvelinus fontinalis</i>
	brown trout	<i>Salmo trutta</i>
	burbot	<i>Lota lota</i>
	carp	<i>Cyprinus carpio</i>
	catfish	<i>Ictalurus punctatus</i>
	<i>Coregonus</i> sp.	<i>Coregonus lavaretus</i>
		<i>Coregonus peled</i>
	goldfish	<i>Carassius auratus</i>
	grass carp	<i>Ctenopharyngodon idella</i>
	guppy	<i>Poecilia reticulata</i>
	perch	<i>Perca fluviatilis</i>
	pike	<i>Esox lucius</i>
	rainbow trout	<i>Oncorhynchus mykiss</i>
	roach	<i>Rutilus rutilus</i>
	tench	<i>Tinca tinca</i>
	<i>Tilapia</i> sp.	<i>Oreochromis aureus</i>
		<i>Oreochromis mossambicus</i>
		<i>Oreochromis niloticus</i>
		<i>Tilapia zillii</i>
	<i>Oreochromis niloticus</i> x <i>Oreochromis aureus</i> (hybrid)	
yellow perch	<i>Perca flavescens</i>	

**Table 2.** – Maximum gonadosomatic index reported in some fish species.

Environment	Species	GSI (%)	References
Marine species	turbot	0.6	Deniel, 1981
	turbot	0.8	Present study
	blue cod	0.8	Pankhurst and Conroy, 1987
	dab	2.3	Htun Han, 1978
	dab	2.5	Deniel, 1981
	plaice	2.5	Brule, 1987
	plaice	2	Deniel, 1981
	sole	0.2	Deniel, 1981
	seabass	4	Barnabé, 1976
	Atlantic cod	16	Trippel and Morgan, 1994
Freshwater species	rainbow trout	10	Billard, 1986
	brown trout	6	Billard, 1987b
	<i>Tilapia</i> sp.	0.4–2.7	Chao <i>et al.</i> , 1987
	catfish	0.3	Guest <i>et al.</i> , 1976
	guppy	5	Billard, 1986
	carp	7	Billard, 1986
	roach	9	Escaffre and Billard, 1976
	pike	2	Billard, 1986
	pike	2	Hoffmann <i>et al.</i> , 1980
	pike	3	Medford and Macay, 1978

species. In order to highlight some particular features of turbot sperm, data are compared with those reported in some fresh and seawater fish listed in *table 1*.

### Gonadosomatic index

Turbot as well as sole and five of the six species of tilapia investigated, is characterized by low values of maximum gonadosomatic index, GSI (gonad weight/body weight)  $\times$  100) when compared with other fish (*table 2*). In species presenting seasonal reproductive cycles, GSI are indicators of the efficiency of spermatogenesis. A relationship has been noted between GSI and spawning behaviour (Billard, 1986): species presenting low GSI such as tilapias spawn generally in couples. On the other hand, species presenting high GSI such as trout and carp spawn in a group. Spawning behaviour is yet to be considered in turbot regarding such a hypothesis.

Variations in the gonadosomatic index are recorded during the reproductive cycle: changes with time of GSI are low in turbot (from 0.1 to 0.6%; Deniel, 1981), compared to rainbow trout (0.1–10%; Billard, 1986). In turbot (Deniel, 1981) as in other fish, maximum values of GSI are recorded in males one to three months prior to the spawning season of the females. Afterwards, testis weight decreases during the milting period, because of sperm release.

### Spermiation period

In captivity, the turbot spermiation period is long, lasting 6 months (*table 3*). However, according to Devauchelle *et al.* (1988), some males can release sperm for up to 9 months. In the wild, the turbot spermiation period lasts only four months (Jones, 1974). As commonly reported in other fish species,

the duration of the turbot reproduction period is longer in males than in females. The duration of the milting period may be related to the rhythm of stripping: the shortest milting period is recorded in weekly stripped males and the longest one in monthly stripped animals (Suquet *et al.*, 1992a). Similar observations are reported for sea bass (Zohar *et al.*, 1984). The percentage of turbot milters is maximum during the spawning season of the females, showing the synchronization of the male and female reproduction period. In contrast, ovulation is most often observed at the end of the milting period in pike (de Montalembert *et al.*, 1980).

### Sperm morphology

The structure of turbot spermatozoon described by Suquet *et al.* (1993) is similar to that reported in most teleost fish (for review see Mattei, 1991). The head is round (maximum diameter: 1.7  $\mu$ m) as in Cyprinidae (Baccetti *et al.*, 1984), tilapias *O. aureus* and *O. niloticus* (Bern and Avtalion, 1990) and golden-grey mullet (Bruslé, 1981). In contrast, an elongated head is noted in guppy (Billard, 1970). The depression observed in the anterior part of the head of turbot spermatozoon has not been reported in other fish. The absence of an acrosome and the reduced middle piece observed in turbot are commonly related in teleosts (Billard, 1970; Nicander, 1970). At the narrow posterior part of the middle piece, the flagellum (total length: 43  $\mu$ m) emerges from a muff like structure. The axoneme presents the typical "9+2" organization. Similarly to trout and guppy (Billard, 1970), lateral ridges are observed in turbot. The rounded nucleus, the reduced middle piece and the structure of the flagellum mean that turbot spermatozoon can be considered to be of a primitive type as described by Franzen (1970).

**Table 3.** – Duration of the spermiation period recorded in some fishes.

Environment	Species	Spermiation period (months)	References
Marine species	turbot	6	Suquet, 1992
	dab	3	Htun Han, 1978
	seabass	4	Barnabé, 1976
	Atlantic halibut	6	Methven and Crim, 1991
Freshwater species	rainbow trout	5	Schmidt-Baulain and Holtz, 1991
	rainbow trout	6	Munkittrick and Moccia, 1987
	brown trout	3	Billard, 1983
	pike	2	de Montalembert <i>et al.</i> , 1980
	roach	2	Escaffre and Billard, 1976
	carp	3	Billard, 1987a

According to Jamieson (1991), turbot spermatozoon can also be classified as an aquasperm since it is released into water by fish presenting external fertilization.

### Semen volume

Scott *et al.* (1991) suggested that the handstripped milt of herring and plaice was most often contaminated by urine. This can result in the variation of many of the sperm parameters as suggested by Rana (pers. comm.) for Atlantic salmon. Consequently, care was taken when collecting turbot milt to discard urine and faeces from extruding sperm and drying the genital area.

In turbot, sperm volumes released per stripping are low when compared to salmonids, carp and Atlantic halibut (*table 4*). Collected sperm volumes may be affected by many parameters. In turbot, the effect of the sampling method, the weight of the milts, stripping frequency and environmental factors have already been studied. No significant correlation is observed between sperm volume and the weight of turbot milts in the range of 1.4 to 3.2 kg (Suquet, 1992). In contrast, the mean volume per stripping is three times higher in three-year-old rainbow trout milts compared with two-year-old specimens (Büyükhatoğlu and Holtz, 1984). In Atlantic salmon,

the ejaculate volume increases with the weight of the fish (Kazakov, 1981).

Stripping frequency has a major effect on sperm volume released per fish. In turbot (Suquet *et al.*, 1992a) but also in rainbow trout (Büyükhatoğlu and Holtz, 1984), in Atlantic salmon (Gjerde, 1984) and in sea bass (Zohar *et al.*, 1984), fortnightly and weekly stripping result in the release of decreasing sperm volumes per stripping as a function of time. However, increasing stripping frequency from monthly to weekly in rainbow trout allows 57% greater total volume to be collected (Büyükhatoğlu and Holtz, 1984). A similar increase in collection frequency in turbot had no effect on total volume collected during a 2-month period.

In turbot, similar total volumes of semen produced per fish during a 2-month experimental period are released from males submitted to an annual light and temperature cycle compared to those receiving a 6-month contracted schedule. Equivalent observations are recorded in rainbow trout (Pohl-Branscheid and Holtz, 1990).

### Spermatozoa concentration

According to Chereguini (pers. comm.), spermatozoa concentration is low in turbot, when compared with fish other than sturgeon and tilapias (*table 5*).

**Table 4.** – Individual extreme values of sperm volume released per stripping (minimum-maximum).

Environment	Species	Sperm volume (ml)	Calculated from
Marine species	turbot	0.2-2.2	Suquet <i>et al.</i> , 1992a
	Atlantic halibut	1-60	Methven and Crim, 1991
	Atlantic halibut	2-92	Edwardes, pers. comm.
Amphihaline species	Atlantic salmon	10.3-17.8	Kazakov, 1981
	Atlantic salmon	15-50	Piironen, 1985
Freshwater species	rainbow trout	0.1-17.2	Sanchez Rodriguez <i>et al.</i> , 1978
	rainbow trout	1.2-4.6	Büyükhatoğlu and Holtz, 1984
	pike	0.1-1.5	de Montalembert <i>et al.</i> , 1980
	tench	0.2-2.0	Linhart and Krasnicka, 1992
	grass carp	1.0-9.0	Belova, 1981
	<i>Coregonus</i> sp.	0.02-2.5	Hochman <i>et al.</i> , 1974
	perch	0.5-1.5	Piironen and Hyvärinen, 1983
	burbot	0.5-2.5	Piironen and Hyvärinen, 1983
	<i>Tilapia</i> sp.	0.3-3	Chao <i>et al.</i> , 1987

Table 5. – Extreme values of sperm concentration recorded in some fish species (minimum-maximum).

Environment	Species	Sperm concentration ( $\times 10^9$ spz/ml)	Calculated from
Marine species	turbot	0.7-11.0	Fauvel <i>et al.</i> , 1993a
	Atlantic halibut	11.9-37.2	Edwardes, pers. comm.
	seabass	10-40	Villani and Catena, 1991
	Atlantic croaker bluefin tuna	23.4-36.4 49.6-64.8	Gwo <i>et al.</i> , 1991 Doi <i>et al.</i> , 1982
Amphihaline species	Atlantic salmon	13.8-16.5	Kazakov, 1981
	Atlantic salmon	3.5-17.9	Aas <i>et al.</i> , 1991
	chum salmon	21.6-25.6	Doi <i>et al.</i> , 1982
	sturgeon	0.05-0.5	Gallis <i>et al.</i> , 1991
Freshwater species	rainbow trout	10-25	Billard <i>et al.</i> , 1971
	rainbow trout	1.9-20.6	Ciereszko and Dabrowski, 1993
	rainbow trout	4.8-25.4	Piironen and Hyvärinen, 1983
	brown trout	5.0-25.7	Piironen and Hyvärinen, 1983
	yellow perch	11.4-30.2	Koenig <i>et al.</i> , 1978
	yellow perch	37.0-47.4	Ciereszko and Dabrowski, 1993
	perch	37.0-127.4	Piironen and Hyvärinen, 1983
	perch	40-130	Goubier, 1990
	<i>Tilapia</i> sp.	0.8-27.4	Chao <i>et al.</i> , 1987
	pike	17.8-25	de Montalembert <i>et al.</i> , 1980
	tench	4.2-34.6	Linhart and Krasnicka, 1992
	burbot	23.3-59.3	Piironen and Hyvärinen, 1983

The effects of the sampling method, the weight of the milters, stripping frequency and environmental factors on sperm concentration have been studied in turbot. Spermatozoa concentration of 10 turbot males stripped once during the spawning season does not differ significantly between different fractions of the volume collected. In contrast, spermatozoa concentration decreases in rainbow trout from the anterior part to the posterior part of the efferent duct (Billard *et al.*, 1971). This decrease was suggested to be due to the dilution phenomena of gametes in the sperm duct (Morisawa and Morisawa, 1988).

No significant correlation can be noted in turbot between spermatozoa concentration and the weight of the milters, in the range of 1.4 to 3.2 kg (Suquet, 1992). The age of rainbow trout milters has been shown to have no effect on spermatozoa concentration (Billard, 1974; Büyükhatoğlu and Holtz, 1984).

After a regular sampling period of two months, spermatozoa concentration observed at the last stripping was significantly lower in weekly and fortnightly stripped turbot when compared to monthly sampled turbot (Suquet *et al.*, 1992a). In rainbow trout, a similar decrease has previously been described using the same sampling frequencies (Büyükhatoğlu and Holtz, 1984).

A similar spermatozoa concentration was assessed for turbot submitted to an annual light and temperature cycle compared to those receiving a 6-month contracted schedule. Rainbow trout subjected either to 6, 9 or 12-month light cycle showed no significant difference in sperm concentration (Pohl-Branscheid and Holtz, 1990).

Significant correlations between spermatozoa density and spermatocrit (packed cell volume  $\times 100$ /total

semen volume) are reported in rainbow trout (Baynes and Scott, 1985; Munkittrick and Moccia, 1987), Atlantic salmon (Piironen, 1985; Aas *et al.*, 1991), coho salmon (Bouck and Jacobson, 1976) and pike (de Montalembert *et al.*, 1980). In contrast, no significant correlation is observed in turbot between these two parameters. Spermatids, which have a higher volume than spermatozoa, are often observed in turbot sperm samples at the beginning of the milting period. Furthermore, morphological alterations in turbot spermatozoa can be noted at the end of the spermiating period. These two facts can bring about a change in spermatocrit values in turbot. Compared to salmonids, high spermatocrits are reported in turbot (table 6), but these values are lower than those reported in other marine fish species. High individual variations are observed in turbot as is the case in most fish species.

### Sperm cell production

Total production of spermatozoa (volume  $\times$  concentration) released per stripping is low in turbot, when compared to other fish studied up to now (table 7). This observation is in part a result of the low GSI assessed in this species (table 2). Sperm cell production is affected by the same parameters and follows the same trend as sperm volume or concentration.

In captivity, the total number of spermatozoa collected during the spawning period by handstripping is generally lower than spermatogenic production. Only 5% of the spermatozoa can be collected in pike (de Montalembert *et al.*, 1980), 15% in brown trout (Billard, 1983) and 20 to 100% in rainbow

**Table 6.** – Spermocrit reported in some fish species (mean  $\pm$  standard error). \* minimum-maximum.

Environment	Species	Spermocrit (%)	Calculated from
Marine species	turbot	40.4 $\pm$ 2.8	Suquet <i>et al.</i> , 1992b
	Atlantic halibut	47-100*	Edwardes, pers. comm.
	Atlantic halibut seabass	40-100*	Methven and Crim, 1991
	Atlantic cod	65-90*	Zohar <i>et al.</i> , 1984
		59.4 $\pm$ 9.7	Trippel and Neilson, 1992
Amphihaline species	Atlantic salmon	23.4 $\pm$ 1.6	Aas <i>et al.</i> , 1991
	coho salmon	25.6 $\pm$ 1.4	Bouck and Jacobson, 1976
Freshwater species	rainbow trout	11.2 $\pm$ 0.5	Munkittrick and Moccia, 1987
	rainbow trout	25.8 $\pm$ 3.1	Ciereszko and Dabrowski, 1993
	rainbow trout	17.8 $\pm$ 0.6	Piironen and Hyvärinen, 1983
	rainbow trout	27.1 $\pm$ 1.2	Bouck and Jacobson, 1976
	brown trout	33.5 $\pm$ 2.0	Piironen and Hyvärinen, 1983
	perch	70.8 $\pm$ 3.3	Piironen and Hyvärinen, 1983
	yellow perch	64.7 $\pm$ 3.3	Ciereszko and Dabrowski, 1993
	pike	38.7 $\pm$ 6.6	de Montalembert <i>et al.</i> , 1980
	burbot	66.5 $\pm$ 6.5	Piironen and Hyvärinen, 1983

trout (Billard, 1987a). However, this percentage can be increased using frequent sampling in trout (Büyükhatoğlu and Holtz, 1984), or hormonal stimulation in pike (Billard, 1987a). During the post-spawning period, the process of resorption of spermatozoa remaining in the genital tract and phagocytosis by Sertoli cells have been described in rainbow trout (Billard and Takashima, 1983). Macrophagic function cells have also been observed in turbot sperm.

### Spermatozoa motility

Variations in osmotic pressure induce spermatozoa motility in many fish species. Hypotonic media trigger the motility of spermatozoa in cyprinids such as goldfish and carp (Morisawa *et al.*, 1983). Dilution in hypertonic solution initiates sperm movement in marine fish such as cod (Morisawa, 1985), sea bass (Billard, 1978), sea bream (Chambeyron and Zohar, 1990) and stripped mullet (Lee *et al.*, 1992).

Sperm motility is triggered in turbot by dilution in hypertonic and isotonic media. However, the duration of movement is lower in isotonic diluents. In agreement with Chambeyron and Zohar (1990), this observation suggests the involvement of factors other than osmotic pressure in sperm activation. A high potassium concentration inhibits sperm motility

in salmonids (Schlenk and Kahman, 1938), but not in cyprinids (Morisawa *et al.*, 1983). In marine fish such as cod, high concentration of potassium does not seem to interfere with sperm motility (Morisawa, 1985).

Turbot spermatozoa can be activated by dilution in a solution of sucrose isotonic to seawater, so that the presence of ions in the diluent does not appear to be necessary to trigger sperm motility in this species. Activation of motility in stripped mullet sperm was also obtained using a non-ionic diluent (Lee *et al.*, 1992). However, motility initiation of herring spermatozoa in the micropyle area requires extracellular potassium (Yanagimachi *et al.*, 1992).

Sperm motility can be expressed by the duration of spermatozoa movement observed after dilution in an activation medium. In marine fish, the duration of sperm motility is generally higher than that reported in salmonids, pike and carp (table 8). The spermatozoa of guppy which undergoes internal fertilization have a longer duration of movement. The effect of the weight of the milters, stripping frequency and environmental factors on the duration of sperm movement has been studied in turbot.

As in rainbow trout (Büyükhatoğlu and Holtz, 1984), no correlation is observed in turbot between the duration of sperm movement and the weight of the milters, in a range of 1.4 to 3.2 kg (Suquet, 1992).

**Table 7.** – Individual extreme values of sperm production released per stripping (minimum-maximum).

Environment	Species	Sperm number ( $\times 10^9$ spz)	Calculated from
Marine species	turbot	0.2-12.0	Suquet <i>et al.</i> , 1992a
Amphihaline species	Atlantic salmon	65-681	Piironen, 1985
Freshwater species	rainbow trout	8-35	Büyükhatoğlu and Holtz, 1984
	rainbow trout	20-80	Schmidt-Baulain and Holtz, 1991
	rainbow trout	10-350	Sanchez Rodriguez <i>et al.</i> , 1978

**Table 8.** – Extreme values of sperm motility assessed in some fishes (duration of cell movement: minimum-maximum).

Environment	Species	Sperm motility (min s)	References
Marine species	turbot	1.00-17.00	Fauvel <i>et al.</i> , 1993a
	Atlantic halibut	0.10-3.00	Methven and Crim, 1991
	sea bass	3.40-30.00	Billard <i>et al.</i> , 1977
	bluefin tuna	13-15	Doi <i>et al.</i> , 1982
	Atlantic cod	0.1-120	Trippel and Morgan, 1984
Amphihaline species	Atlantic salmon	0.13-1.02	Kazakov, 1981
	eel	1.02-3.14	Moczarski and Koldras, 1979
Freshwater species	rainbow trout	0.13-0.20	Büyükhapıoglu and Holtz, 1984
	rainbow trout	0.15-0.30	Billard, 1986
	<i>Coregonus</i> sp.	1.14-2.00	Hochman <i>et al.</i> , 1974
	pike	0.30-1.00	Billard, 1986
	carp	0.43-2.50	Kruger <i>et al.</i> , 1984
	carp	0.15-2.00	Belova, 1981
	carp	0.40-1.00	Billard <i>et al.</i> , 1986
	grass carp	0.12-0.56	Belova, 1981
	guppy	60.0-120.0	Billard, 1986
	<i>Tilapia</i> sp.	4.0-5.0	Chao <i>et al.</i> , 1987

The duration of spermatozoa movement in turbot is not affected by increasing stripping frequency from monthly to weekly sampling (Suquet *et al.*, 1992a). On the other hand, the duration and intensity of sperm movement were reduced in rainbow trout with increasing stripping frequency (Büyükhapıoglu and Holtz, 1984). Sperm motility in turbot males subjected to an annual light and temperature cycle compared to those receiving a 6-month contracted programme is not significantly different.

Some problems remain in the assessment of sperm motility in fish. A high dilution rate is necessary to initiate synchronous motility in 100% of spermatozoa (Billard and Cosson, 1992). However, increasing dilution seems to decrease the percentage of motile spermatozoa and the duration of the movement in turbot. Sperm motility can be maintained by adding Bovine

Serum Albumin as a source of protein in the extender (Fauvel *et al.*, 1993a). Significant progress has been made in the assessment of fish spermatozoa motility such as video recording and analysis from a video monitor (Trippel and Neilson, 1992; Iwamatsu *et al.*, 1993) or stroboscopic illumination and dark field microscopy (Cosson *et al.*, 1985). This last technique has been used in salmonids to measure finer parameters such as flagellar beat frequency, cell velocity and distance covered by spermatozoa. In order to characterize precisely turbot sperm motility, these fine parameters of sperm movement have to be investigated.

In turbot, no significant correlations were observed among sperm volume, concentration, motility and total sperm number released per stripping. In the literature, correlations between these parameters

**Table 9.** – Osmotic pressure assessed in the seminal fluid of some fishes (mean  $\pm$  standard error).

Environment	Species	Osmotic pressure (mOsmol/l)	Calculated from
Marine species	turbot	306 $\pm$ 3	Suquet <i>et al.</i> , 1993
	Atlantic halibut	340 $\pm$ 1	Edwardes, pers. comm.
	seabream	365 $\pm$ 1	Chambeyron and Zohar, 1990
	black seabream	359 $\pm$ 17	Morisawa, 1985
	cod	400	Hwang and Idler, 1969
	seabass	400	Villani and Catena, 1991
Amphihaline species	Atlantic salmon	245 $\pm$ 11	Aas <i>et al.</i> , 1991
	Atlantic salmon	232 $\pm$ 13	Hwang and Idler, 1969
	chum salmon	401 $\pm$ 19	Morisawa, 1985
Freshwater species	rainbow trout	297 $\pm$ 15	Morisawa, 1985
	brook trout	300 $\pm$ 11	Marshall, 1986
	goldfish	317 $\pm$ 11	Morisawa, 1985
	goldfish	290 $\pm$ 3	Grant <i>et al.</i> , 1969
	carp	302 $\pm$ 5	Morisawa <i>et al.</i> , 1983
	carp	286 $\pm$ 3	Plouidy and Billard, 1982
	yellow perch	317 $\pm$ 13	Koenig <i>et al.</i> , 1978

**Table 10.** – pH assessed in the seminal fluid of some fishes (mean ± standard error).

Environment	Species	pH	Calculated from
Marine species	turbot	7.31 ± 0.03	Suquet <i>et al.</i> , 1993
	seabream	7.83 ± 0.04	Chambeyron and Zohar, 1990
Amphihaline species	Atlantic salmon	8.25 ± 0.03	Hwang and Idler, 1969
	chum salmon	8.15 ± 0.07	Morisawa and Morisawa, 1988
Freshwater species	rainbow trout	7.96 ± 0.04	Inaba <i>et al.</i> , 1958
	rainbow trout	7.99 ± 0.06	Morisawa and Morisawa, 1988
	carp	7.96 ± 0.03	Plouidy and Billard, 1982
	yellow perch	8.50 ± 0.00	Koenig <i>et al.</i> , 1978

**Table 11.** – Total protein content assessed in the seminal fluid of some fishes (mean ± standard error).

Environment	Species	Protein mg/ml	Calculated from
Marine species	turbot	8.8 ± 0.8	Suquet <i>et al.</i> , 1993
Freshwater species	rainbow trout	1.7 ± 0.1	Loir <i>et al.</i> , 1990
	rainbow trout	1.3 ± 0.2	Ciereszko and Dabrowski, 1993
	carp	1.2	Plouidy and Billard, 1982

are rarely observed except sperm volume and concentration which were positively correlated in carp (Belova, 1981).

### Composition of the seminal fluid

The seminal fluid represents around 60% of the total sperm volume in turbot. The mean characteristics of the seminal fluid assessed during the spawning period (salinity = 35‰, T = 13°C) are presented in tables 9, 10, 11 and 12 in terms of osmotic pressure, pH, total protein and potassium contents. Data are compared to those collected in other fish species.

Osmotic pressure of the seminal fluid is generally slightly higher in marine fish than in freshwater fish. When compared with other marine fish, the osmolality of the seminal fluid of turbot is low. Turbot blood plasma (Gaumet, 1994) is almost isotonic to the seminal plasma, as reported in salmonid or cyprinid

fishes (Morisawa, 1985) and in seabream (Chambeyron and Zohar, 1990). Osmolality of the seminal fluid of turbot does not vary during the milting period. Similar observations have been reported in Atlantic salmon (Aas *et al.*, 1991) and Atlantic halibut (Edwardes, pers. comm.). In contrast, seminal osmolality was found to increase in landlocked salmon at the end of the spawning period (Piironen, 1985) and decrease in rainbow trout (Munkittrick and Moccia, 1987).

pH of the seminal fluid of turbot is low when compared to values recorded in freshwater species. Values of pH recorded in turbot blood plasma (Fauvel *et al.*, 1993b) are similar to those assessed in the seminal plasma. Low individual variation of pH of the seminal plasma is generally observed within a species (table 10). On the other hand, during the milting period pH variations have been reported in landlocked salmon (Piironen, 1985) and tilapia (Kruger *et al.*, 1984).

**Table 12.** – Potassium content assessed in the seminal fluid of some fishes (mean ± standard error).

Environment	Species	K <sup>+</sup> (mmol/l)	Calculated from
Marine species	turbot	3.8 ± 0.3	Suquet <i>et al.</i> , 1993
	black seabream	2.0 ± 0.1	Morisawa, 1985
	puffer	5.3 ± 0.7	Gwo <i>et al.</i> , 1993
Amphihaline species	Atlantic salmon	27.5 ± 1.0	Aas <i>et al.</i> , 1991
	Atlantic salmon	22.0 ± 1.3	Hwang and Idler, 1969
	chum salmon	86.5 ± 2.7	Morisawa, 1985
Freshwater species	rainbow trout	32.5 ± 2.1	Morisawa and Morisawa, 1988
	rainbow trout	34.5 ± 4.5	Gwo <i>et al.</i> , 1993
	brook trout	25.1 ± 6.1	Marshall, 1986
	carp	82.4 ± 3.3	Gwo <i>et al.</i> , 1993
	carp	43.5	Plouidy and Billard, 1982
	goldfish	70.2 ± 1.7	Morisawa, 1985
	goldfish	55.2 ± 1.4	Grant <i>et al.</i> , 1969

Total protein content of the seminal fluid, assessed during the female spawning period, is very high in turbot (up to 8.8 mg/ml), when compared to values reported in other fish (<2 mg/ml). In rainbow trout, the highest total protein concentration is reported at the onset of spermiation (Sanchez-Rodriguez *et al.*, 1978). In salmonids, proteins of the seminal liquid are suggested to play a protective role for spermatozoa (Billard, 1982). A similar role may be suspected in turbot since the percentage of motile spermatozoa and the duration of movement are depressed at high dilution rates using mineral extenders, while it is maintained in a Bovine Serum Albumin supplemented media (Fauvel *et al.*, 1993a).

Potassium content is very low in marine fish when compared with freshwater ones. Among freshwater fish, high values are mainly recorded in cyprinids. The seminal plasma of salmonids and cyprinids contains a concentration of potassium higher than blood plasma. In contrast, the potassium content of blood (Gaumet, 1994) and seminal plasma is similar in turbot.

## CONCLUSION

Some characteristics of turbot sperm production are commonly observed in other fish species: sperm morphology is simple since there is a reduced middle piece, a flagellum presenting a "9+2" pattern of tubules and no acrosome. Osmotic pressure induces sperm motility. The reproduction period is longer in males than in females. Increasing stripping frequency decreases sperm volume and concentration. Turbot males can be submitted to a 6-month contracted light and temperature schedule without changes in sperm sample characteristics. No relationship is observed between the weight of the milters and sperm characteristics.

However, when compared to other fish species studied up to now, turbot sperm presents some

particular features. Sperm production is low in terms of sperm volume, concentration and total production of spermatozoa. Furthermore, a low gonadosomatic index is recorded in turbot. However, turbot spermiation period is long in captivity. No relationship can be observed between sperm concentration and spermatocrit. Increasing stripping frequency does not affect the duration of sperm movement. The total protein concentration assessed in the seminal plasma is high. Proteins seem to protect spermatozoa against the effect of dilution. Finally, the head of the spermatozoon presents an anterior depression, the role of which remains unknown.

These particular features of turbot sperm must be taken into account for aquacultural purposes. The low sperm production is compensated by a low sperm requirement for turbot eggs during artificial insemination. Since the spermiation period is long in captivity, sperm can be collected before and after the spawning period of the females. However, the possibility of an ageing phenomenon of sperm and its consequences on sperm morphology and fertilization capacity remain to be studied. Insemination or storage diluents can be defined by modifying the osmotic pressure of these media. In order to protect spermatozoa against dilution, the addition of proteins is necessary in all diluents.

Many aspects of sperm biology remain to be explored in turbot, *i.e.* the existence of a phenomenon of sperm maturation in the genital tract, the study of factors inhibiting and triggering sperm motility, a description of spermatozoa motility using fine parameters, energy resources of spermatozoa during the resting and motility phases. These studies will help to suggest a quality criterion for turbot sperm. Using this knowledge, it will be possible to define preservation and insemination media which are well adapted to this species.

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