Changes of Selected Features of Turbot *Scophthalmus maximus* (L.) Spermatozoa During Spawning Season with Potential Effect of Butyltin Xenobiotics

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The Baltic Sea is a semi-enclosed sea with regional differences in its abiotic characteristics. The physicochemical agents and temporal variability in the environmental conditions have created several unique ecosystems within this basin. For example, the Pomeranian Bay is a highly dynamic environment with higher salinity levels and different seasonal temperature variations than the Gulf of Gdańsk. In contrast to the Pomeranian Bay, the Gulf of Gdańsk is more affected by chemical pollution, e.g., butyltin (BT) contamination, and by eutrophication. These environmental conditions determine, to a certain extent, the environmental quality of both locations and, consequently, have the potential to affect fish populations in various ways.

The turbot *Scophthalmus maximus* is adapted to the brackish waters of the Baltic Sea, which is most likely due to phenotypic plasticity rather than genetic adaptation. This fish is a bottom-dweller with stationary behavior that is also characterized by spawning site fidelity. The turbot is a highly prized food fish and a commercially important flatfish species in the Baltic Sea. Turbot catches have been decreasing in the Baltic Sea, and a reduction in population biomass indices has been observed in recent years. The exact reasons for these declines are not known. However, the abundance and distribution of the species is related to its reproductive success. Hence, in the light of environmental and fishery pressures on the turbot from the Baltic Sea, factors that affect reproductive success and thus recruitment of this species must be examined.

One of the limiting factors of reproductive success is the quality of male and female gametes. Egg quality can be defined as the ability of the egg to be fertilized and to subsequently develop into a normal embryo. Similarly, spermatozoon quality can be defined as its ability to successfully fertilize an egg and subsequently allow the development of a normal embryo. However, studies concerning fish gametes are more focused on the quality of eggs rather than that of spermatozoa, even though poor sperm quality may be important in reducing the apparent population size and genotype diversity.

The factors affecting spermatozoon quality are diverse and are dependent on complex interactions between genetic, physiological and environmental units. Laboratory experiments

provide essential concepts in the understanding of processes that affect sperm function, but they involve only one or two levels of organization. Thus, in vitro assays or studies performed on cultivated fish do not consider the ecosystem processes and neglect environmental factors. In the light of the above findings, the quality of fish spermatozoa may be affected differently by the same factor, depending on experiment design. For this reason, laboratory results must be confirmed in the natural environment.

One of the factors that affects spermatozoal quality but that remains poorly understood is an aging phenomenon. The aging of fish spermatozoa is illustrated by variations in the chromatin condensation and the decreasing quantity of the midpiece and nucleus vesicles. In addition, an effect of aging on the decline of the morphometric parameters describing the area, perimeter, length and width of the spermatozoal head was observed in wild and cultivated fish species at the end of the spawning period. However, data describing changes in spermatozoal size throughout the spawning period in the natural environment, especially those changes that are related to the metabolism of the cell, are scarce.

Spermatozoal morphometry, an important determinant of male reproductive success, can be a phenotypically plastic trait. Variations in spermatozoal morphometry, including the size of the head, midpiece and tail, are found within specimens of the same species. For example, significant spermatozoal variation in terms of morphometry are found among fish population. The phenotypic plasticity of spermatozoa may be related to social, e.g., sperm competition, genetic or assorted environmental factors. However, the associations of these factors and spermatozoal morphometry are still far less than clear.

Xenobiotics cause reproductive dysfunction by disrupting the reproductive endocrine system and resulting in the production of poorer quality spermatozoa. An example of a xenobiotic that affects fish spermatozoa is tributyltin (TBT). This xenobiotic leads to reduced fish spermatozoal motility, induces the production of abnormal spermatozoa, causes the direct inhibition of spermatozoal lactate dehydrogenase, and leads to spermatozoal membrane degradation and cell death. TBT is considered the most harmful of all tin compounds and is still found at considerable concentrations in sediments and marine organisms along the Polish coast.

The overall objective of the thesis was to determine the changes in selected features of turbot spermatozoa in relation to the spawning period, study locations and BT xenobiotic contamination. The specific objectives were to investigate turbot spermatozoal size (defined as the measurements of the morphometric parameters of the head, midpiece and tail) and energy metabolism (defined as the measurements of the enzymatic activities of NAD+- and

NADP- dependent dehydrogenases and creatine kinase) in relation to both the spawning day and the study location, analyze the tissue-specific accumulation of BT xenobiotic with a focus on the male reproductive organs, and examine the relationships between both spermatozoal morphometry and enzymatic activity and the BT concentration in turbot reproductive organs.

This thesis provides, for the first time, information concerning certain physiological aspects of spermatozoal aging [1, 3], potential phenotypic plasticity of spermatozoa [1], and the vulnerability of male gametes to BT xenobiotic [2, 3] in the wild turbot population form the Baltic Sea. This field-based work, supported by chemical and biochemical assays, is an initial attempt to connect and understand the different factors that have the potential to affect the quality of spermatozoa in a wild fish population.

The size and the energy metabolism decreased in turbot spermatozoa in relation to the sampling day throughout the spawning period [1, 3]. The rates of the decrease in the morphometric parameters were found to be similar at each study location [1]. The enzymatic activity showed a decline that was also considered to be similar at both locations and that amounted to 1.2-2.6% per day for each enzyme [3]. The observed differences in spermatozoal features between the locations were not related to the total length of the fish, age, semen volume, weight of the testes or gonadosomatic index [1, 3]. Thus, the decline in the size and energy metabolism was interpreted as the results of the aging phenomenon of turbot spermatozoa.

Significant differences were found in the size of the turbot spermatozoa between fish collected at the two study locations, the Gulf of Gdańsk and the Pomeranian Bay. The spermatozoa of males from the Gulf of Gdańsk were characterized by lower values of the morphometric parameters related to the head and midpiece than the spermatozoa of males from the Pomeranian Bay, and no significant difference was detected in the tail length. Even when controlling for the effect of the aging, the spermatozoa of males from the Gulf of Gdańsk still presented significantly lower values of the morphometric parameters related to the head and midpiece than the males from the Pomeranian Bay. The differences in spermatozoal size did not appear to be related to the male characteristics, e.g., the total length, semen volume, weight of the testes, gonadosomatic index. Thus, the observed variations in size that were significantly related to the study location [1] were considered to represent the phenotypic plasticity of the turbot spermatozoa. However, this trait should be precisely studied in the future.

Males of the turbot form the Baltic Sea appeared to be vulnerable to BT xenobiotic contamination [2]. Fish caught in the location contaminated by BT (the Gulf of Gdańsk) had

higher concentrations of TBT and its break-down products, di- (DBT) and mono-butyltins (MTB) in measured tissues than the males from the reference location (the Pomeranian Bay). The ripe testes of the species appeared to be a target tissue for TBT accumulation [2, 3]. TBT was shown to be extensively eliminated at the specific whole-body level, and the males from the TBT-contaminated location reached this specific level at 23–24 cm in total length. The testes-specific accumulation of TBT and the significant decrease of this xenobiotic in fish with higher total length (>23-24) was likely related to gamete-based elimination. Based on the amount of TBT eliminated in the males from the Gulf of Gdańsk, the potential sperm-based elimination may significantly support the eliminatory functions of the liver in decreasing the whole-body concentration of TBT [2]. However, the high concentrations of TBT in the reproductive organs of turbot males may render the spermatozoa vulnerable to TBT intoxication [2, 3].

The activity of spermatozoal lactate dehydrogenase, malate dehydrogenase, glucose-6-phosphate dehydrogenase and creatine kinase increased significantly with BT concentration in the turbot testes. In the light of the above, the studied enzymes appeared to be selectively hyperactivated by the BT xenobiotic [2]. This effect suggested that the spermatozoal enzymes are reliable biomarkers of BT contamination. However, because of high variations of turbot spermatozoa features, definite causality between BT contamination and hyperactivation of the enzymes could not be established.

The selected features of the turbot spermatozoa, morphometry and energy metabolism, appeared to be highly variable, and this variability was related to spermatozoal aging, phenotypic plasticity and BT xenobiotic contamination. The specific conclusions were as follows: I) the turbot spermatozoa age as the spawning period progresses, and this process results in decreasing spermatozoal size and energy metabolism, II) the turbot spermatozoa are assumed to be phenotypically plastic, III) the ripe testes of turbot appear to be a target tissue for TBT accumulation, and the potential sperm-based elimination of the BT xenobiotic was found, IV) a positive correlation between the activity of spermatozoal enzymes and BT burdens in fish testes was found, but definite causality between the BT contamination and the hyperactivation of the enzymes was not established.

References

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