Investigation of the effects of age and length traits on sperm cell size and egg microphyll aperture in rainbow trout (*Oncorhynchus mykiss*)

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Abstract

This review study examines the effects of biometric characteristics such as age and length on sperm size and egg microphyll aperture in rainbow trout. Understanding the biological factors affecting fertilization success in trout will contribute to the development of reproductive biology and increasing efficiency in fish farming. Previous studies suggest that fish age and size play decisive roles in sperm morphology and egg structure, which directly affects fertilization rates. In this review, the relationships between age and length and sperm and egg characteristics are evaluated in the light of current studies and the findings obtained from different studies are brought together to identify knowledge gaps in the field. The study aims to provide a biologically based perspective to increase successful fertilization rates in trout farming.

Keywords: Rainbow trout, Sperm, Egg, Biometric, Fertilization

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Introduction

Rainbow trout (*Oncorhynchus mykiss*) has an important place in the aquaculture sector worldwide and is largely preferred in the aquaculture industry. Trout farming is growing rapidly due to its local and global economic contributions and its importance in food security. Turkey has become one of the prominent countries worldwide, especially in recent years, with the increase in trout production and its success (Atasever and Bozkurt, 2011; D'Agaro *et al*., 2022). However, in order to increase the sustainability and efficiency of farming, various factors affecting the reproductive biology and fertilization success of trout need to be examined. One of the most critical biological factors affecting reproductive success is the biometric characteristics of fish, such as age and length. These characteristics directly affect the structure and function of reproductive cells and determine the fertilization rate (Bondarenko *et al*., 2018; Chen et al., 2022).

The biological structures of sperm and egg cells play an important role in the fertilization process in trout. Features such as size, shape and movement speed of sperm are factors that directly affect the capacity to reach the egg cell and perform fertilization. For example, it has been observed that sperm cells produced by younger and smaller trout are shorter but faster; whereas sperm length increases with age but movement speed decreases (Risopatrón *et al*., 2018; Kowalski and Cejko., 2019). These differences show that the biological age of trout changes the structure and function of reproductive cells. In addition, the microphyll opening of the egg enables fertilization by providing sperm entry. The size of the microphyll opening may vary depending on age and individual biometric variables and plays a decisive role in the efficiency of the fertilization process (Ahti *et al*., 2020; Gao *et al.*, 2021; Permana *et al.*, 2023). Studies examining the effects of biometric features on reproductive cells clearly reveal the contribution of these factors to fertilization success. For example, Kurta *et al.*, (2023) and Bondarenko *et al*. (2018) reported that sperm produced by younger individuals had higher swimming speeds by comparing sperm characteristics of trout in different age and size groups. This shows that young individuals have more advantages in the fertilization process and that fertilization rates may be higher. However, the increase in sperm size with age may negatively affect the swimming speed of the sperm, which may lead to a decrease in fertilization rates (Tan *et al*., 2019). The effect of these biometric changes on the fertilization process enables the understanding of the basic mechanisms of reproductive biology and the development of more efficient production strategies in trout farming (Thorstad *et al*., 2008; Paul *et al*., 2023).

The differences observed in the reproductive cells depending on the biometric characteristics of trout are factors that should be taken into consideration to increase fertilization efficiency in farming. In the farming

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environment, higher fertilization rates and therefore more offspring can be obtained with selective production practices according to the age and size of individuals. In this context, detailed examination of biometric factors related to age and length provides important clues in terms of reproductive biology in trout farming. In this study, the effects of biometric characteristics on fertilization success in trout will be discussed with an extensive literature review. In particular, the contribution of sperm characteristics such as size and swimming speed and the microphyll structure of the egg to the fertilization rate will be examined. This study, based on an extensive literature review, aims to compile information that will contribute to the development of selective production strategies based on biometric characteristics in trout farming. Increasing fertilization rates in trout farming is of critical importance for sustainable production, and every study conducted on this subject offers more efficient methods to producers (Araujo *et al.*, 2022).

The aim of this review is to develop suggestions to increase reproductive efficiency in fish farming by examining the effects of biometric characteristics such as age and length on fertilization biology in trout. In this direction, in the light of the studies in the existing literature, features such as sperm morphology, sperm dimensions, and egg microphyll aperture of trout will be evaluated in detail. In this context, understanding the biological basis for improving fertilization rates according to the biometric characteristics of trout will contribute to increasing the success rates in fish farming.

Age and size factors

The age and size of fish have significant effects on reproductive biology. Generally, older and larger individuals have higher reproductive efficiency. This is because mature individuals have more developed reproductive organs and therefore have an increased potential to produce higher quality sperm and eggs (Chen *et al.*, 2022; Ohlberger *et al.*, 2022; Yıldırım and Çantaş, 2022). In particular, there is a noticeable improvement in the size and motility of sperm cells with increasing age in trout. Studies show that the sperm cells of mature trout are longer and more active (Shamspour and Khara., 2016; Danis and Samplaski., 2019).

The size of fish also has a similar effect on reproductive biology. In general, larger individuals have larger sperm sizes. This is related to the shape and morphology of sperm cells. Changes in the morphological characteristics of sperm cells have been observed as trout grow taller (Temple-Smith *et al*., 2018; Gao *et al.*, 2021). This may increase fertilization rates by affecting the interaction between sperm and eggs during fertilization (Cheng *et al*., 2021).

However, the interaction of age and length factors also plays an important role in fertilization success rates. It has been observed that fertilization success rates generally increase as the age and length of trout increase (Liljedal *et al*., 2008). However, this may not always be the case; some studies have shown that excessive aging and large size can negatively affect sperm quality (Mylonas *et al*., 2017).

Studies on the effects of age and length contribute to the development of reproductive strategies in trout. In this context, selection according to age and length criteria may have the potential to increase fertilization success rates in trout farming (McBride *et al.,* 2015; Everson *et al*., 2021).

Sperm sizes

Sperm size is a critical factor affecting reproductive success in trout. Sperm morphology and size are closely related to the age and size factors of the fish. Larger individuals are generally capable of producing larger and higher quality sperm cells (Balshine *et al*., 2001). Sperm size has a direct impact on motility and fertilization success; larger and healthier sperm cells increase the chances of reaching and fertilizing eggs (Geffen and Evans., 2000; Liljedal *et al*., 2008).

Sperm cell size varies depending on various external and internal factors. Studies have revealed the effects of environmental factors and nutrition on sperm quality. For example, adequate nutrient intake can positively affect the sperm size and morphological characteristics of trout (Salas-Huetos *et al*., 2018). In addition, environmental conditions such as water temperature and chemical composition are also important factors affecting sperm morphology (Mylonas *et al*., 2017).

Age and size factors lead to significant changes in sperm morphology. Sperm cells of young individuals are generally smaller and less motile. This leads to increased fertilization rates, with the production of larger and more functional sperm cells in mature individuals (Alavi *et al*., 2013; Yang *et al*., 2021). In particular, as male trout grow, the size of sperm cells also increases, which positively affects fertilization success rates (Gallego *et al*., 2013; Ahti *et al*., 2020). These relationships on sperm size and morphology should be taken into account to develop important strategies in trout farming. Selection practices aimed at increasing sperm size and quality among selected individuals can increase fertilization efficiency (Everson *et al*., 2021; Weber *et al*., 2021). In particular, such practices may provide economic benefits, given that sperm size and morphology are associated with higher fertilization rates (Everson *et al*., 2021; Merino, *et al*., 2023).

The effect of sperm size on fertilization success is an important area of research for understanding the reproductive biology of trout (Pitnick *et al*., 2009; Fitzpatrick., 2020). The literature on this topic contributes to the development of approaches to optimize sperm size in trout farming (Balshine *et al*., 2001; Temple-Smith *et al*., 2018; Gao *et al.*, 2021)

The sizes of sperm samples taken from different fish species vary

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according to the age and weight of individuals. For example, trout sperm cells are generally 20-30 micrometers long and 1.5-2.5 micrometers in diameter. However, it has been observed that sperm samples taken from larger individuals can reach sizes of up to 30-50 micrometers (Islam and Akhter, 2011; Alavi *et al*., 2013). Figure 1 shows trout sperm milking, and b and c show its microscopic appearance.

The weights of the fish also showed that there are differences in sperm sizes. In comparisons made between sperm samples taken from fish, the average length of sperm samples obtained from trout weighing 1 kg was around 20-25 micrometers, while sperm samples taken from individuals weighing 3 kg reached an average length of 30-35 micrometers (Rurangwa *et al*., 2004). This shows that the growth processes of the fish affect sperm sizes.

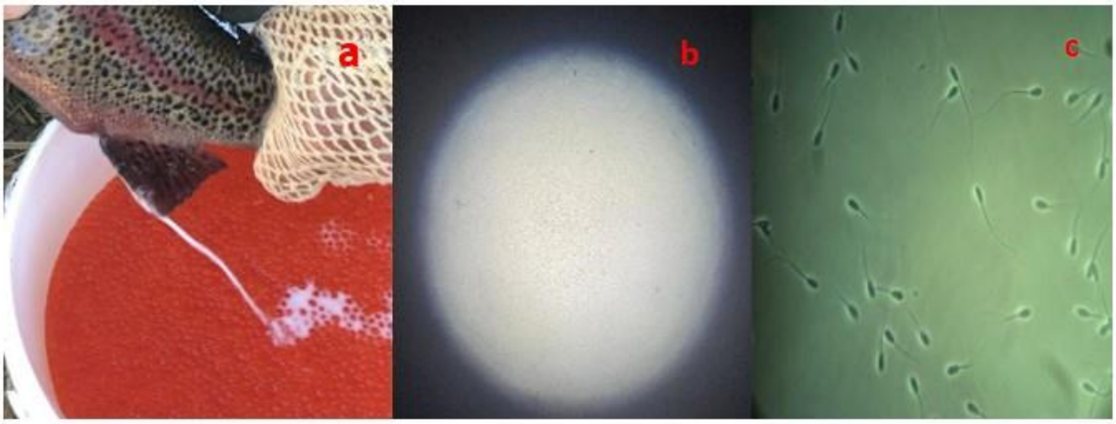

Figure 1: Trout sperm milking and microscope view; a, b (Original, 2023, c (Keskenler, 2019).

However, there are also significant differences between sperm sizes in different species. While salmon sperm cells are 40-60 micrometers long and 1.8-2.2 micrometers in diameter, sea bass sperm cells can be shorter, with an average length of 30-45 micrometers and a diameter of 1.2-1.8 micrometers (Temple-Smith *et al*., 2018; Cheng *et al*., 2021). These differences show the effects of age, length and weight factors of the fish on sperm morphology.

Egg microphyll sperture

Egg microphyll aperture is an important component in the reproductive biology and fertilization process of fish. Microphyll aperture refers to a series of microstructures in the internal structure of the egg that facilitate the entry of sperm cells into the egg. This structure plays a critical role in allowing sperm cells to reach the egg and perform the fertilization process (Zhou *et al*., 2019).

Egg diameter varies depending on the age, size, and species of fish. Generally, eggs taken from larger and more mature individuals have larger microphyll diameters than those taken from smaller and younger individuals (Geffen and Evans., 2000; Liljedal *et al*., 2008; Temple-Smith *et al*., 2018). For

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example, while the average microphyll diameter of eggs obtained from 1-2-year-old trout varies between 5-10 micrometers, eggs obtained from 3-4-year-old individuals can be 10-15 micrometers in diameter (Alavi *et al*., 2013). This situation shows that the egg structure and therefore the microphyll diameter develop as the fish age.

The effect of the egg microphyll aperture diameter on fertilization has also been demonstrated by studies. Wider microphyll apertures can increase fertilization rates by facilitating sperm cells to enter the egg (Cheng et al., 2021; Weber *et al*., 2021). In addition, the size of the microphyll diameter is also related to the motility of sperm cells; sperm cells with high motility can more easily pass through wider microphyll apertures (Liljedal *et al*., 2008; Bondarenko *et al*., 2018; Alavi et al., 2021). Therefore, an optimum egg microphyll diameter is essential for successful fertilization.

Significant differences have been observed between microphyll diameter and fertilization success in different fish species. In salmon, the average microphyll diameter is found to be between 12-15 micrometers, while in sea bass this value is around 8-10 micrometers (Rurangwa et al., 2004). Figure 2 shows the microscopic appearance of egg milking from broodstock trout in (a) before fertilization and in (c) 1 week after fertilization. In addition, the width of the microphyll aperture is also affected by genetic variation and environmental factors (Merino, *et al*., 2023). Determination of egg microphyll diameter is considered an important parameter in terms of the reproductive biology of fish. In particular, optimizing egg microphyll aperture in fish farming can be a strategic approach to increase fertilization rates. This increases the reproductive success of fish and contributes to the development of more economically efficient production methods (Temple-Smith *et al*., 2018; Everson et al., 2021).

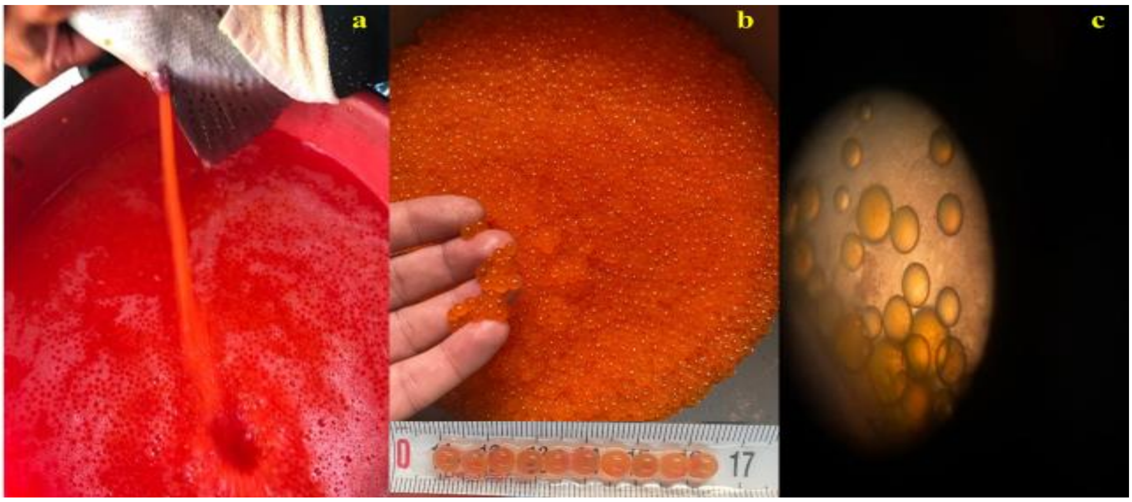

Figure 2: Trout egg milking and egg diameter a, b, c (Orginal, 2024).

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Effects on egg fertilization

The fertilization success of fish is affected by many factors. These factors include morphological and physiological characteristics such as age, length, sperm size, and egg microfil opening. In this section, we will summarize the findings in the current literature by discussing the effects of these factors on the fertilization process.

Fertilization is the process by which the sperm cell reaches the egg and fuses with it. In this process, the morphological characteristics of sperm cells are directly related to the structural characteristics of the egg. Studies show that sperm size has a significant effect on fertilization success. For example, it has been reported that larger sperm cells have a higher chance of reaching the egg and thus increase fertilization rates (Ochokwu *et al*., 2015; Fitzpatrick., 2020). In addition, sperm motility also has a significant effect on fertilization rates. Individuals with high sperm motility have higher fertilization rates than individuals with low motility (Alavi *et al*., 2021).

Age and length factors are other important parameters affecting the reproductive potential of fish. It has been observed that sperm and eggs obtained from larger and older individuals increase fertilization rates (Bondarenko *et al*., 2018). For example, sperm cells obtained from 3-4 year old trout have been found to have higher motility and larger sizes, which increases fertilization success rates (Geffen and Evans., 2000; Liljedal *et al*., 2008).

The size of the egg microfil diameter also has a critical effect on fertilization. Eggs with larger microfil openings can increase fertilization success by facilitating the passage of sperm cells (Cheng *et al.*, 2021). In particular, it has been determined that larger microfil openings provide better orientation of sperm cells, thus increasing fertilization rates (Cheng *et al*., 2021; Merino, *et al*., 2023).

However, environmental factors also play an important role in the fertilization process. Environmental factors such as water temperature, pH level, and oxygen content can affect sperm motility and egg quality. For example, it has been observed that sperm motility increases when water temperature is high, but extreme temperatures negatively affect egg quality (Rurangwa et al., 2004). Taking such environmental variables into account can contribute to increasing fertilization success rates in fish farming. In general, there are many factors that affect the fertilization success of fish.

Variables such as age, size, sperm morphology and egg microfil opening are the main components of the fertilization process. Evaluating these factors together can help develop more effective strategies in the field of fish reproductive biology and reproduction. In hatcheries with water temperatures of 8-11 degrees, the egg fertilization rate is approximately 92-95% and the hatching rate is 97-99%, and quite successful results can be obtained. Fertilization of the egg in Firuge 3 (a), trout egg with eye spots (b) and newly hatched marsupial fry are seen.

Figure 3: Egg and marsupial hatchling with prominent eyes, a, b, c (Original, 2024).

Discussion and conclusions

In this review, the effects of age and length of fish, sperm size and egg microfil opening on fertilization were examined. The findings show that these factors significantly affect fertilization success. First of all, it was concluded that age and length factors play a decisive role in the reproductive biology of fish. It was determined that sperm and eggs obtained from larger and older individuals increased fertilization rates. This shows that the developmental process of fish has a direct effect on reproductive success (Geffen and Evans., 2000; Bondarenko *et al*., 2018).

Sperm size and morphological characteristics have an important place in the success of the fertilization process. Studies have shown that larger and more motile sperm cells increase the chance of reaching the egg and thus increase fertilization rates (Fitzpatrick., 2020; Alavi *et al*., 2021). These findings support that sperm quality is a critical factor in the reproductive success of fish.

The effects of egg microfil opening on fertilization are also remarkable. It has been observed that large microfil openings provide better orientation of sperm cells and thus increase fertilization rates (Cheng *et al*., 2021). This shows that the egg structure of fish plays an important role in their interaction with sperm.

Environmental factors also affect the fertilization process. Variables such as water temperature, pH level and oxygen content have been determined to have an effect on sperm motility and egg quality (Rurangwa *et al.*, 2004). Taking these environmental variables into account can contribute to increasing fertilization success rates in fish farming practices. In particular, optimizing these factors in the culture environment has the potential to increase the reproductive performance of fish.

In conclusion, this review study has comprehensively examined the effects of age and length of fish on sperm size and egg microfil opening and fertilization rates. The findings constitute an important resource for understanding the reproductive biology of fish and developing more effective culture strategies. It is thought that

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future research can develop new methods to increase efficiency in fish farming by examining these relationships in more detail.

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