

The effect of temperature on round goby (*Neogobius melanostomus*) embryo development

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Abstract

The round goby (*Neogobius melanostomus*) has expanded its range from the Ponto-Caspian region to new habitats in Europe and North America. It is an invasive species in many areas that has a significant impact on new environments. The round goby inhabits various ecosystems with different environmental conditions. Here, we investigated the optimal temperatures for round goby reproduction. Our experimental study on the development of round goby embryos demonstrated a high tolerance to different temperatures at this stage of ontogenesis. The development of round goby embryos was highly successful (over 90% of larvae hatching) at temperatures ranging from 12°C to 20°C. In contrast, embryo development was less successful at 25°C, while no effective embryo development was observed in temperatures below 12°C. We found that larvae hatching at temperatures between 12°C and 20°C have yolk remnants, which provide an additional supply of energy in the first days after hatching. A wide range of temperature tolerance, along with tolerance to other changing factors, are the features that contribute to successful population growth. Thus, temperature should not be a factor limiting the expansion of round goby in the temperate climate zone.

Keywords:

Climate change; Fish early life stages; Ontogenesis; Temperature

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1. Introduction

The round goby (*Neogobius melanostomus*) is one of the most prominent and wide-ranging invasive fish on Earth (Corkum et al., 2004; Sapota, 2004; Kornis et al., 2012; Oesterwind et al., 2017; van Deurs et al., 2021). It is reported as one of the 100 most invasive species in Europe (Hirsch, 2015; DAISIE, 2025). The round goby is a generalist species, and many of its characteristics facilitate the colonisation of new areas, i.e., opportunistic feeding and a wide thermal tolerance (Erős et al., 2005; Kornis et al., 2012), high fecundity, multiple batch spawning and nest guarding (Janáč et al., 2019), and also tolerance to low dissolved oxygen levels (Charlebois et al., 1997). This species, native to the Ponto-Caspian region, has become an important component of many ecosystems in Europe and North America. One of the most important features determining the success of a round goby invasion is its eurythermal nature. The round goby inhabits waters with temperatures ranging from 0°C to 30°C (Skóra et al., 1999; Bahrens et al., 2022). In the temperate climate zone, the monthly mean air temperatures above 10°C persist for 4

to 7 months per year (Belda et al. 2014). In the Baltic Sea, a clear seasonal migration of round goby between shallow coastal areas and deeper offshore regions has been observed, mirroring patterns found in North American freshwater environments (Behrens et al., 2022).

Fish in different ontogenetic stages generally display varying tolerance to environmental conditions. Early stages of development, including embryos, are most vulnerable as their ability to adapt to environmental conditions is limited (Pankhurst and Munday, 2011; Dahlke et al., 2020). Embryonic development occurs under suitable environmental conditions. In its native range, the round goby reproduces at temperatures of 9–26°C (Kovtun, 1979; Moskal'kova, 1996) or 10–30°C (Makeyeva et al., 2011). Exposure to extreme temperatures, both high and low, results in smaller hatching sizes, deformities, and finally reduced survival. Embryonic fish also show cardiovascular and aerobic metabolic responses to temperature. Heart rate and oxygen consumption rate increase with higher temperature (Melendez and Mueller, 2021).

The proper temperatures minimize the duration of the yolk-sac stage and allow for maximum growth per unit of yolk absorbed (Hart and Pursles, 1995). In the round goby, not only the typical embryonic stages, but also most lar-

val stages proceed under the protection of the embryonic envelope. Eggs are laid in nests (Charlebois et al., 1997). After hatching, the individuals already have definitive organs of locomotion, vision, respiration, and digestion (Moiseyeva, 1983). They are capable of active movement and feed on plankton. Negative photoreactions keep them in the nest until the yolk sac reduces in size (Moskal'kova, 1978, 2007).

Considering metabolism, the round goby maintains a high aerobic scope from 15°C to 28°C, and its capacity to increase aerobic metabolic rate above maintenance metabolism remains high across a broad thermal range (Christensen et al., 2021). Projections of global climate change indicate a general and progressive warming (Kiehl and Trenberth, 1997; Lindzen, et al., 2001; Lean and Rind, 2009). Given the potential of the round goby to colonize new areas, it is reasonable to ask whether climate change could facilitate the invasion of new regions. It is assumed that the establishment of a population in any area requires a suitable water temperature for at least the time required for complete incubation of the eggs (from fertilization to hatching). Other factors that may influence fish population growth are, e.g., diseases, predation, oxygen concentration, salinity, competition for food, or presence of dams (Kornis et al., 2012; Matern et al. 2021; Backström and Winkelmann, 2022). In this work, we focused on the influence of temperature and hypothesized that it is a limiting factor for the successful development of round goby embryos.

2. Material and methods

The study was carried out using round goby from the Gulf of Gdańsk in the south-eastern Baltic Sea. Fish in spawning condition (200 individuals) were collected in the shallow, inshore waters of the Gulf of Gdańsk, using fyke nets, between June 22 and August 28, 2015, when the water temperature was around 16–19°C. This is the time when most males and females have ripe gonads and are ready to reproduce. The fish were transported to the laboratory and maintained for one week in four 1 m³ water tanks (2 tanks each for females and males) in recirculated system, with well aerated (100% oxygenation), saline artificial water (7 PSU – salinity typical of inshore waters of the Gulf of Gdańsk), and at temperature of 17°C, until the experiment was conducted.

After collecting the fish, their sex was determined by the shape of their urogenital papilla (Charlebois et al., 1997; Marentette et al., 2009). The range of females' total length was 8.0–14.0 cm and males 9.0–18.0 cm. For the experiment in each temperature, five males and five females were put separately in two 60 dm³ thermostatic recirculated aquatic systems. The temperature was gradually changed from 17°C to the specific target (acclimation) temperature (5, 10, 12, 15, 16, 20, or 25°C) by 1°C day⁻¹. The fish were kept for 3 days at a specific acclimatisation temperature before starting the experiments.

To determine whether fish were in spawning condition, the maturity of gametes was checked. For females, the shape of the body was the first criterion. Then, some eggs were collected by pressing gently the abdominal side and examined under a stereomicroscope. Eggs should be transparent with a well-developed yolk. For males, the dark color of fish was the first criterion. Then drop of sperm in the Billard solution was put on a microscopic slide and after the addition of water from the experimental tank, spermatozoon shape and motility were checked. Females in spawning condition were thoroughly dried with a paper towel. The abdominal side was gently pressed. Eggs released through the urogenital papilla were collected into a plastic strainer (mesh size 1 mm). At least 50 eggs were placed into one strainer. Males were killed by decapitation. Testes were removed from the abdominal cavity and placed between two Petri dishes. They were smashed and the sperm was extracted into a 2 ml syringe using 1 ml of Billard solution (Cosson et al., 2008; Kowalski et al., 2018) as the dilution fluid. Gametes from one female (eggs) and one male (sperm) were used for each trial (testing embryo survival at one temperature). Females were released in the place of their original capture.

The experiment was repeated five times at each temperature, using a total of 9980 eggs. In total, we used gametes from 35 females and 35 males.

Strainers with eggs were placed in 500 ml glass desiccators.

Sperm was added to eggs and gently mixed with a sterilized goose feather (Woynárovich and van Anrooy, 2019). Then, 10 ml of water (salinity of 7 PSU, 100% oxygenation, and temperature according to respective incubation conditions) was added to each desiccator (eggs were covered with water). After 1 minute, the remaining sperm dilution was rinsed with water from the incubation tanks, and the strainers were mounted on floats in constant-temperature water circulating systems. Eggs remained on the mesh, 1 cm below the water surface.

Five repetitions were performed at each temperature (5, 10, 12, 15, 16, 20, or 25°C). Eggs were observed every 24 h under a stereomicroscope. They were counted (live and dead separately). Dead eggs were removed. They were treated as dead when they were pale or deformed. Eggs were observed in transmitted and reflected light using an Olympus SZX7 stereomicroscope and photographed with an Olympus SP-350 digital camera integrated with the stereomicroscope. The stages of embryo development (Nikolski, 1963; Tomczak and Sapota, 2006) were noted.

For eggs incubated at 15°C and 20°C, the yolk sac was measured (two axes) from the images to determine its volume. Fifty yolk sacs were measured per day at each temperature. It was done to check differences in embryo energy demands at various temperatures. The formula for the spheroid volume was used (Williams et al., 2004):

$$V = \frac{4}{3}\pi abc$$

where:

V – volume of the spheroid

a, b, c – half axes (the two shorter axes were assumed to be equal, $b = c^*$)

The hatching time was determined on the day when at least 50% of the larvae hatched in each repetition.

The ratio of hatched larvae was treated as an indicator of embryo development success. The lifespan of embryos at each temperature was presented in days and degree-days (number of days multiplied by temperature of water in which development took place; in the experiment described here, the temperature was constant for each treatment). Kaplan-Meier statistics (Kaplan and Meier, 1958; Smith et al., 1994; Blažek et al., 2013; Chen et al., 2020) were used to establish the survival rate.

Microsoft Excel 365 and Statsoft Statistica 12 were used for calculations.

Experiments were conducted under permission given by the Local Ethics Committee for animal experiments of the Medical Academy in Gdansk (opinion no 29/05).

3. Results

No viable hatch was observed in temperatures below 12°C. The development time of embryos varied from 36 days in water at 12°C to 11 days in water at 25°C. This gives a range of 432 to 275 degree-days (Table 1, Figure 1).

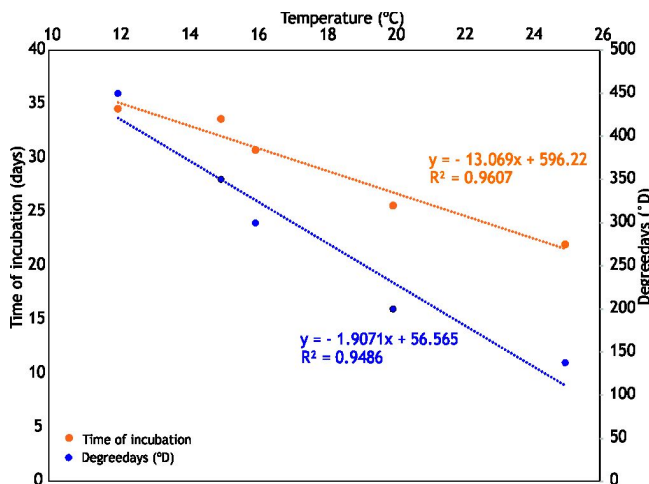


Figure 1. Comparison of the development time of round goby embryos at different temperatures.

*Regardless of the angle of observation, both axes on all photos had the same measurements.

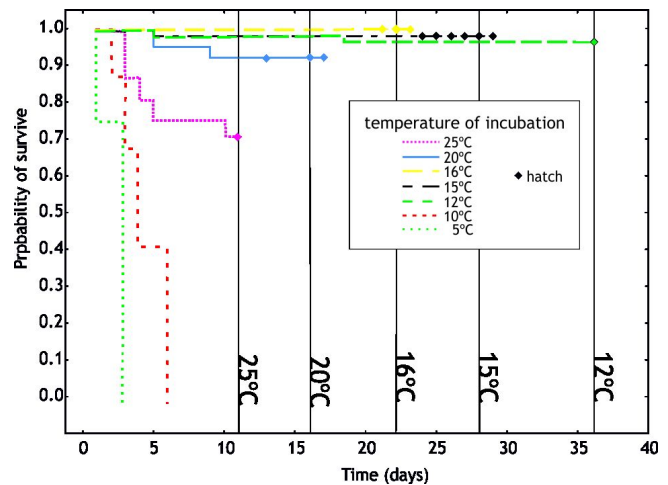


Figure 2. Survival rate of round goby embryos developing at different temperatures (Kaplan-Meier statistics, standard error less than 0.02).

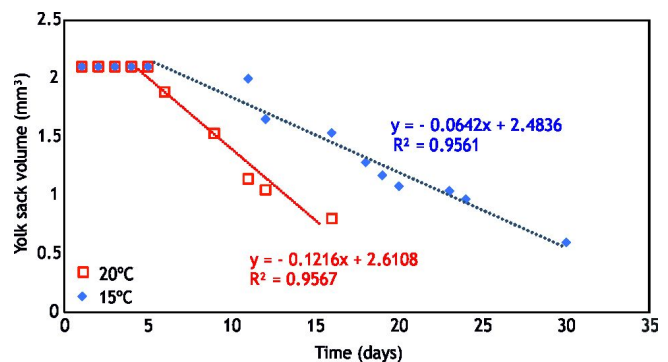


Figure 3. Changes in yolk volume of round goby embryos (50 eggs per day at each temperature) during embryo development.

The slopes of the linear relationships describe the reduction of incubation time with increasing temperature (Figure 1).

In water at 5°C, all eggs were pale or deformed three days later than in water at 10°C (Figure 2). This was most likely due to the decomposition time of undeveloped eggs – decomposition is slower in lower temperatures.

High survival rates, over 90% (Kaplan-Meier statistics, standard error lower than 0.02), were observed for embryos incubated at water temperatures ranging from 12°C to 20°C (Figure 2). Embryos incubated at 25°C hatched after 11 days, but the survival rate was much lower – about 70%.

The larvae hatched by breaking the egg capsule at the distal end. The entire clutch of larvae hatched within 24 h, at all temperature regimes.

The spherical yolk was located at the distal end of the egg. The initial volume of the yolk was about 2 mm³. It remained almost intact during the first days of embryo development (up to 11 days at 15°C and approximately 5

Table 1. Development time of round goby embryos at different temperatures.

Temperature [°C]	Time of incubation [days]	Number of degree-days [°D]	Number of observed eggs [n]	Rate of survival [%]
5	–	–	1348	0
10	–	–	1452	0
12	36	432	1312	96
15	28	420	1414	98
16	24	384	1564	100
20	16	320	1492	91
25	11	275	1398	70

days at 20°C). After this initial period of embryo development, the reduction in yolk sac volume was twice as fast at 20°C as at 15°C. Ultimately, the hatching larvae had a yolk volume of about 0.6 mm³, regardless of the incubation temperature (Figure 3). The differences in yolk sac measurement (both axes) for each individual, on each day at both temperatures were less than 0.1 mm.

4. Discussion

Fish are ectothermic organisms, and the level of their metabolism as well as the rate of growth and development depend on the temperature of the water in which they live (Haesemeyer, 2020; Volkoff and Rønnestad, 2020). The round goby has a wide range of thermal tolerance, which means that it is an eurythermal organism. Sensitivity to environmental conditions varies at different stages of ontogenetic stages. Individuals at the earliest stages of development are the most sensitive. Adaptation to environmental conditions at these stages is one of the most important factors affecting the species' distribution range (Vagner et al., 2019). It is currently unknown how, in the long-term scenario, global warming will affect the ability of species to colonise new areas or expand in already inhabited regions. The study was conducted on individuals of round goby from the Gulf of Gdańsk (Baltic Sea), originating from a non-native but already established population – the first appearance of round goby in this region was observed in 1990 (Skóra and Stolarski, 1993). It is not known whether the results would have differed if the experiments had been conducted on representatives of other populations (from areas of native occurrence or those where the round goby is a non-native species).

In our study, we found that the effective embryogenesis of round goby can occur at temperatures ranging from 12°C to 25°C (Figure 2). The finding that incubation at 16°C and 20°C takes 24 and 16 days is consistent with the approximate data from literature, according to which incubation at 19°C and 26°C takes 20 to 14 days (Kalinina, 1976 cited in Bogutskaya et al., 2004). In both cases, higher temperatures resulted in shorter development time.

The success of embryonic development, estimated as the proportion of hatched larvae, significantly decreased at temperatures above 20°C. Similar to other fish species (Ojanguren, and Braña, 2003; Melendez and Mueller, 2021;

Godoy et al., 2023), it was observed that the duration of embryonic development, expressed both in days and degree-days, decreased with increasing temperature. The embryonic development lasted 36 days at low water temperature (12°C). This may indicate a lower thermal barrier to the colonisation potential of the species. It cannot be disregarded that the round goby has batch spawning to ensure that at least some of the eggs develop under optimal conditions (Charlebois et al., 1997). The boundary of the region for successful embryonic development should be set at a location where the water temperature does not drop below 12°C for at least 36 days (this period must be continuous). An open question remains as to what would happen in case the temperature temporarily drops below this critical value. The available literature reports that reproduction of round goby occurs at water temperatures of 9°C or higher (Sea of Azov – Kovtun, 1979; Caspian Sea – Moskal'kova, 1996). It should be noted that at different stages of embryogenesis, developing embryos have varying sensitivity to changes in environmental conditions, making it difficult to answer such a question. Therefore, the specified threshold of 12°C for at least 36 days should be regarded as an approximate value. As for the upper limit of effective embryogenesis, it can be assumed based on the conducted experiments that there are virtually no regions with natural environments where this limit is exceeded. The only doubts may concern tropical regions.

It is important to note that above 20°C, the percentage of hatching larvae decreases. There is no evidence of the effect of such high temperatures on the development and survival of the round goby at subsequent ontogenetic stages. However, data on other fish species suggest a high probability of various disruptions. Nevertheless, high temperatures, within the range recorded in the natural environment, should not limit the reproductive potential of the round goby. It is worth mentioning that Makeyeva et al. (2011) report that 30°C is the upper temperature limit of the reproduction of round goby in the Caspian Sea.

The observed reduction in yolk volume of the round goby confirms the higher metabolic rate and faster development at higher temperatures. It is worth noting that at temperatures of 15°C and 20°C, the hatched larva has a similar amount of energy stored in the yolk for the first days of development outside the egg.

We have shown that the survival and development of round goby embryos the most successful at water temperatures between 12°C and 20°C. After development in this range of temperature, the hatching larvae have a supply of energy accumulated in the remaining part of the yolk. Successful development of embryos has not been observed at lower temperatures in this study. At higher temperatures, the survival rate decreases significantly.

Quattrocchi et al. (2023) stated that during the past decades, the climatic conditions have favoured the round goby population inhabiting the Baltic Sea. The rising temperatures will probably not mitigate their performance and dispersal potential in the future until 2100. Considering the predicted rise in sea surface temperature (from 1°C to 5°C per 100 years, depending on the model and region; Bilgili et al., 2024; Marshall et al., 2015; Yang and Wu, 2024) and the range of efficient reproduction temperatures for the round goby (from 12°C to 20°C, based on our study), in temperate climate zone, temperature should not be considered a limiting factor. However, in regions where the sea surface temperature (SST) already exceeds 20°C, reproduction of this species may no longer be efficient. On the other hand, the colder regions, where SST is below 12°C, may become favorable for round goby. Temperature appears to be one of the first factors influencing efficient embryo development. Out of the proper temperature range, there will be no embryo development. However, further studies should also include other environmental factors, i.e. salinity.

We hypothesised that temperature is a limiting factor in the efficient development of round goby embryos. Although the optimal temperature range for embryo development is reported to be 12–20°C, we conclude that temperature cannot be considered a significant factor limiting the round goby expansion range in the temperate zone, i.e. in areas where the monthly average air temperature is above 10°C for a period of 4 to 7 months.

Conflict of interest

None declared.

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