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Reproduction of the round goby (*Neogobius melanostomus*) under controlled conditions

Rozród babki byczej, *Neogobius melanostomus*, w warunkach kontrolowanych

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Abstract: Learning mechanisms determining fish reproduction are particularly beneficial in the case of experimental work on juvenile forms of fish making research independent of the season of the year. This paper describes the reproduction method of the round goby, Neogobius melanostomus, under controlled conditions. This species is not native to the ichthyofauna of the Gulf of Gdańsk and was discovered here for the first time in 1990. In the case of pre-seasonal reproduction of the round goby, the first spawning took place 5 weeks after the beginning of thermal stimulation. On the other hand, artificial reproduction of the fish caught in the spring was possible after only a week of being kept in a temperature of 17–19°C. The spawn incubation period ranged from 17 to 22 days, depending on the temperature. For natural spawning, the period from spawning to hatching was 18–19 days, at constant temperature of 20°C.

Keywords: round goby, fish reproduction, alien species, artificial spawning

Streszczenie: Poznanie mechanizmów warunkujących rozród ryb jest szczególnie korzystne w przypadku prac doświadczalnych na formach juwenilnych ryb, gdyż uniezależnia prowadzenie badań od pory roku. Praca opisuje metodę rozrodu babki byczej N. melanostomus w warunkach kontrolowanych. Gatunek ten nie jest rodzimym składnikiem ichtiofauny Zatoki Gdańskiej i został tu po raz pierwszy stwierdzony w 1990 roku. W przypadku przedsezonowego rozrodu babki byczej pierwszego tarła doszło po 5 tygodniach od rozpoczęcia stymulacji termicznej. Sztuczny rozród ryb odłowionych na wiosnę był natomiast możliwy już po tygodniu przetrzymywania w temperaturze 17–19 °C. Okres inkubacji ikry wynosił od 17 do 22 dni w zależności od temperatury. Dla tarła naturalnego czas od tarła do wylęgu wynosił 18–19 dni przy stałej temperaturze 20°C.

Słowa kluczowe: babka bycza, rozród ryb, gatunki obce, sztuczne tarło

INTRODUCTION

Learning mechanisms determining fish reproduction and its management options are important from the point of view of both widely understood aquaculture and for purely cognitive reasons (Bieniarz, Epler 1991). Gaining control over reproductive biotechnology provides huge opportunity to acquire knowledge regarding factors influencing spawning and its process, reproductive behavior, and all stages of ontogenesis. Apart from widely understood aquaculture, it is particularly beneficial in the case of experimental work on juvenile forms of fish, as it makes the research independent of the season of the year and allows the researcher to produce research material on an ongoing basis. Environmental conditions have a significant impact on the reproductive system of fish and hence – on its functioning. Appropriate modifications of lighting and the temperature of water allow to manage the process of gonad maturation, making it possible to obtain spawn outside the natural reproduction period (Zakęś et al. 2006). Natural spawning is one of the methods where fish are provided with conditions appropriate for a specific species (photoperiod, temperature) and an adequate substrate for spawning. Another method – artificial "dry" spawning – involves stripping mature genital products, eggs and milt, into a dry dish, their mixing, and adding water afterwards (Bieniarz, Epler

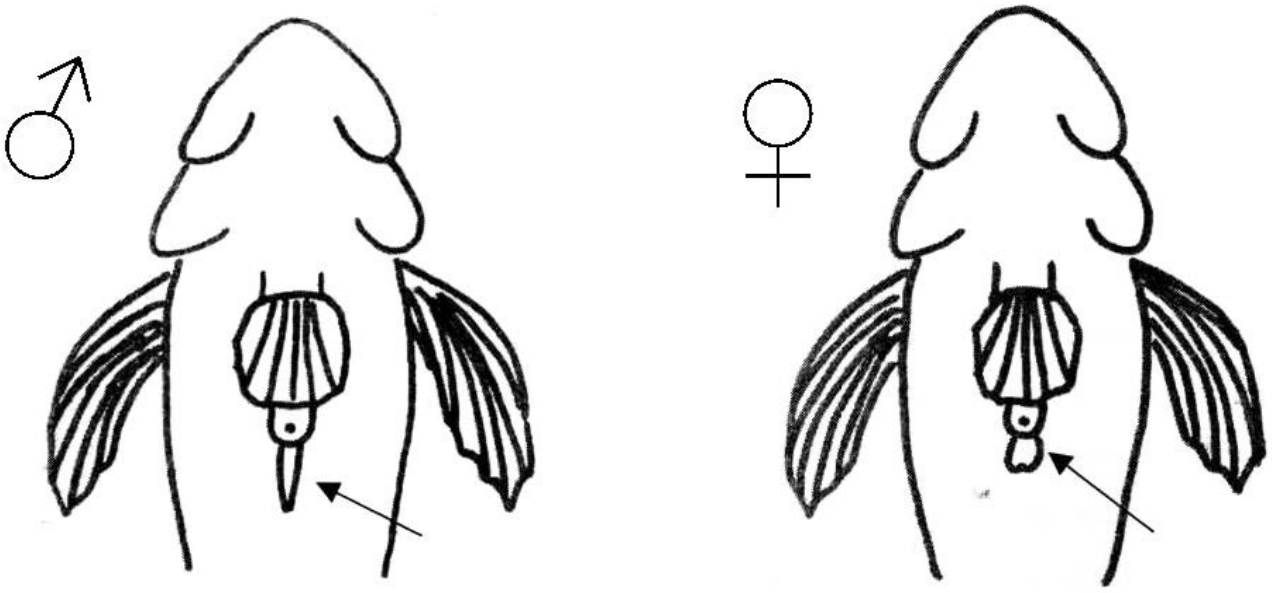

Fig 1. Genitourinary papilla of the round goby (Charlebois et al. 1997).

1991). This allows the mortality rate of incubated eggs to be decreased through isolating it from pathogenic factors occurring in a natural environment. It also enables the researcher to explore the influence of a chosen factors on the incubation process. The round goby Neogobius melanostomus (Pallas 1811) is not native to the ichthyofauna of the Gulf of Gdańsk. It was probably transported here with ballast waters from the Pontic-Caspian region where it occurs naturally (Sapota 2005). For the first time, the presence of this species in the Baltic Sea waters was recorded in the region of Hel, in 1990 (Skóra, Stolarski 1996). Since then, its population has exhibited a stable growth in terms of its abundance and occupied area; areas with rocky and an artificially strengthened seabed are this population's main habitat (Sapota 2005). The round goby is a batch spawner and its spawning in the Gulf of Gdańsk falls in the period between April and September (Sapota 2005) in waters with temperatures above 9°C. This indicates that during the reproductive season, females spawn more than once. The spawn is usually stuck to a hard substrate in crevices between stones where it is guarded by the male throughout the incubation period. The development period of a fertilized egg, depending on the temperature, ranges from 14 to 20 days (Berg 1949; Miller 1986; Moskalkova 1996; Charlebois et al. 1997; Kozik 1999).

MATERIALS AND METHODS

Fish for the study were caught in the Gulf of Gdańsk in a fishing harbor in Hel, in autumn, November 2006 (temperature of water: around 8°C) and in a yacht harbor in Gdynia, in the spring of 2007 (temperature of water: 8–10°C) with the use of fyke nets. The fish caught were transported to the laboratory of the Institute of Oceanography of the University of Gdańsk where they were placed in tanks with cooled water. The fish were acclimatized in a temperature of 8°C for a week.

Natural spawning under controlled conditions

Heating of water was commenced after the acclimatization period; the water was heated up by 1.5°C/day on average. After a week, based on morphological features, "spawning groups" were selected; each group consisted of 2 males and 3 females of similar size. Their sex was determined based on the genitourinary papilla (Fig. 1.).

Next, the selected fish were placed in aquaria with a capacity of 100 liters, in water with a salinity of 6.7 PSU and a temperature of 20°C, and with the light on for 24 hours a day. "Nests" made of ceramics and PVC pipes were placed in the aquaria, one per male. The fish were fed once a day with mussels and fragmented fish. After spawning was observed, the stock was transferred to another tank, leaving only the male guarding the eggs. The male and the other fish were still fed with fine mussels once a day. After the spawn incubation period, the hatched eggs were sucked out with the use of a gravitation method with a rubber hose of 5 mm in diameter and transferred to a separate aquarium. After isolating the hatched eggs, the females were placed back in the aquarium with the male.

Artificial spawning with a "dry" method

After the acclimatization period, the fish were placed in pools with a capacity of 1 m³ and salinity of 6.5 PSU in an aquatic room with a temperature of around 15–17°C and with the light on for 24 hours. Males and females were kept together. The fish were fed with mussels once a day. After 7–11 days, based on the morphological appearance (coloration and body shape), an appropriate number of females and males was selected and gonads maturity inspections were performed. Spawn samples were taken from the females through squeezing and transferring onto a Petri dish. Maturity of oocytes was checked under

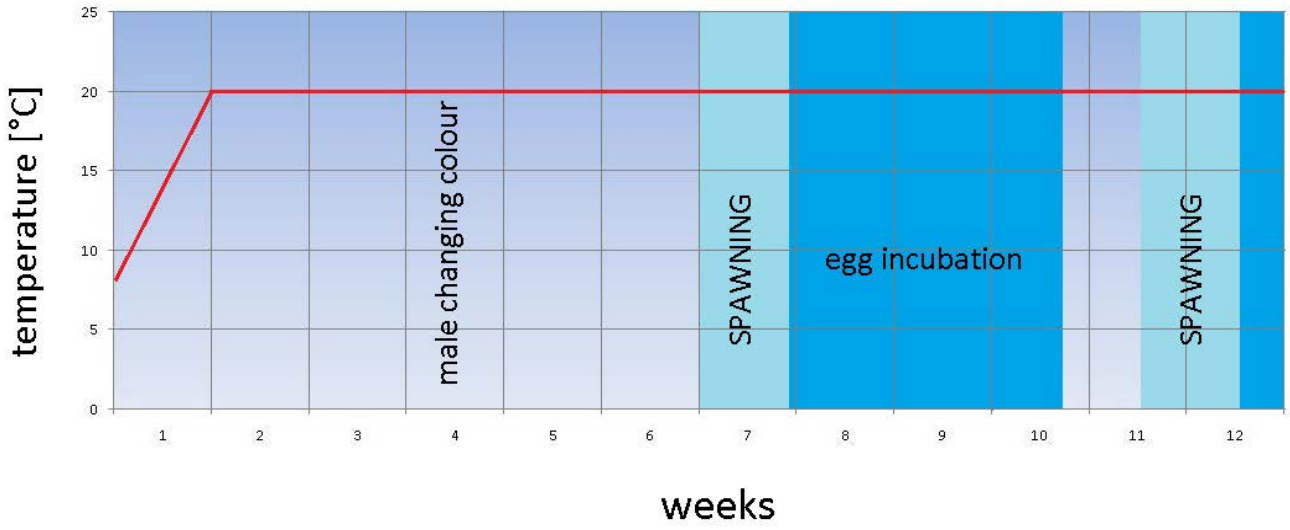

Fig 2. Thermal stimulation process.

a binocular with the use of the SERRS SCANNING SOLUTION. The degree of polarization of the nucleus and, additionally, oil droplet dispersion (Zakęś at al. 2006), as well as the egg shape and size of the "frill" on the vegetal pole, used for attaching the eggs were considered the main criteria of oocyte maturity classification. Maturity of males was determined based on the analysis of sperm motility with the use of a microscope. Then, a spawner was dried by wrapping it in a paper towel and squeezing the gametes out into a dry dish by applying pressure to the fish's abdominal cavity. Sperm samples were collected with the use of two methods. The male was dried; then with the use of a syringe, the sperm flowing out during the massage of the male's genitourinary papilla was stripped. Cutting out the testes and their homogenization was another method. A homogenate of testes was stripped with a syringe. The sperm or the testes homogenate was added to the spawn and mixed, followed by adding water with the same temperature as the spawn's . The spawn was left for 2 min. and then it was washed several times with water in order to remove stickiness and remains of the homogenate. Batches of fertilized spawn were placed on mesh nets floating on the surface in an aquarium with visible water circulation. The spawn was incubated at temperatures of 17°C and 26°C. After hatching, the clutch was transferred from the nets to the aquarium.

The round goby clutch was kept in the aquaria with a capacity of 60 l, where the water surface level was 5–7 cm; the was maintained on this same level as temperature of eggs incubation and continuous airing was carried out. For the first three days after hatching the clutch was fed with nauplii of brine shrimp and a small amount of trout starter food. In the further stage of pre-rearing, the water level in the aquarium was raised and a bucket filter with a suction hose placed 20 cm from the bottom of the aquarium was installed. Juvenile fish was fed exclusively with the trout starter food or with the ground aquarium fish food flakes. Every third day, the water was changed in a quantity of a 1/3 of the aquarium's capacity.

RESULTS

Natural spawning under controlled conditions

In the case of pre-seasonal reproduction of the round goby (winter), the external features of spawning readiness became visible after 3 weeks of keeping a constant temperature of 20°C (Fig. 2.). The male became completely black (Fig. 3.) and performed a "mating dance" upon the appearance of a female in the vicinity of the nest. In the 3rd week, females showed evident roundness of the abdominal integuments. The first spawning took place 5 weeks after the beginning of the experiment (Fig. 2.). Only one male and almost all females (at least two) in each aquarium were spawning.

After a short period of changed color, the second male either lost its black undertone or died. It was also noticed that spawning by one female constituted positive stimulation for the rest of them to lay eggs in the nest. Eggs incubation lasted for 18–19 days at a temperature of 20°C, and hatching of all the larvae lasted up to three days. Larvae of the round goby (Fig.4.) developed all fins, partial body pigmentation and almost completely resorbed the yolk-sac.

After guarding the nest with deposited eggs for the first week, the male ignored the food provided; in the subsequent period he would only take it when it was in the direct vicinity of the nest. The next spawning occurred 3–4 weeks after the first one.

Spring reproduction of the round goby, attempted between March and April 2007 was unsuccessful. When caught, females had already clearly rounded abdomens, on the other hand, only some males displayed pre-spawning colors. After being placed in the aquaria, at a temperature of 20°C, the females lost eggs or released it (unfertilized) in the nest within two weeks. The males usually died or did not spawn. Attempts to obtain the spawn with the use of the males that were kept at a higher temperature for longer were also unsuccessful.

Fig 3. The male – visual courtship display.

Fig 4. A round goby 24 hours after hatching.

Fig 5. Mature oocytes of the round goby in SERRS solution.

Artificial spawning with a "dry" method

Round goby females caught in the spring had oocytes in developmental stages III and IV. After the fish were kept in a temperature of 17–19°C for a week, a majority of them reached full maturity (Fig. 5.).

Some mature females, after collection of oocytes samples by squeezing, released the rest of the spawn after being placed back in the aquarium. Attempts to collect oocytes with the use of a catheter were unsuccessful. Due to the structure of the genitourinary papilla, inserting the right diameter catheter into the body cavity was unsuccessful. Obtaining mature spawn for spawning with the "dry" method did not cause problems.

Obtained males had testes in stage 3–4 (Nikolski 1963, Tomczak 2001). In both studies living sperm was found in the testes but small quantities of the sperm could only be obtained from the fish with gonads in stage 4 through massaging the genitourinary papilla area. This action often led to the fish's death due to eversion of the gastrointestinal tract to the mouth cavity. The most effective method of obtaining sperm was cutting out and homogenization of the testes.

There was no evident effect of week-long thermal stimulation on testes development. In water with a salinity of 6.7 PSU and a temperature of 20°C, the sperm kept viability for 1–1.5 minutes. The eggs showed slight stickiness, easily removed by washing several times. The fertilization effect was visible an hour after fertilization. Fertilized eggs had a visibly elongated shape in relation to the unfertilized ones, the yolk-sac and amniotic fluid were visible (Fig. 6.).

Eggs incubation at a temperature of 17–20 °C lasted 22 days, and at a temperature of 20–21°C – 17 days; larvae hatching lasted up to 3 days. Young round gobies were fed on the very first day after hatching. The fish were keen to feed on both the nauplii of brine shrimp and trout starter food.

DISCUSSION

In order to obtain pre-seasonal spawning, it is necessary to carry out full photothermal stimulation (Zakęś et al. 2006). However, as author's observations and the studies of Kolat (2006) indicate that temperature is the most important factor in the case of the round goby. Round gobies show a good tolerance to temperature increases even up to 10°C/day (author's observations) but it was concluded that a temperature increase by a maximum 2°C/day would be optimal. Availability and abundance of food affects fertility and consequently, the quality and quantity of gametes produced (Bieniarz, Epler 1991). The metabolism of fish and their nutritional needs increase with a rise in temperature. The author's observations indicate that ceasing feeding stops maturation of gonads in females and leads to resorption of ovaries. Maturation of males seems to be less susceptible to food deficiencies. Frozen mussels, fish meat and offal are useful and used as food (Kolat 2006). An avera-

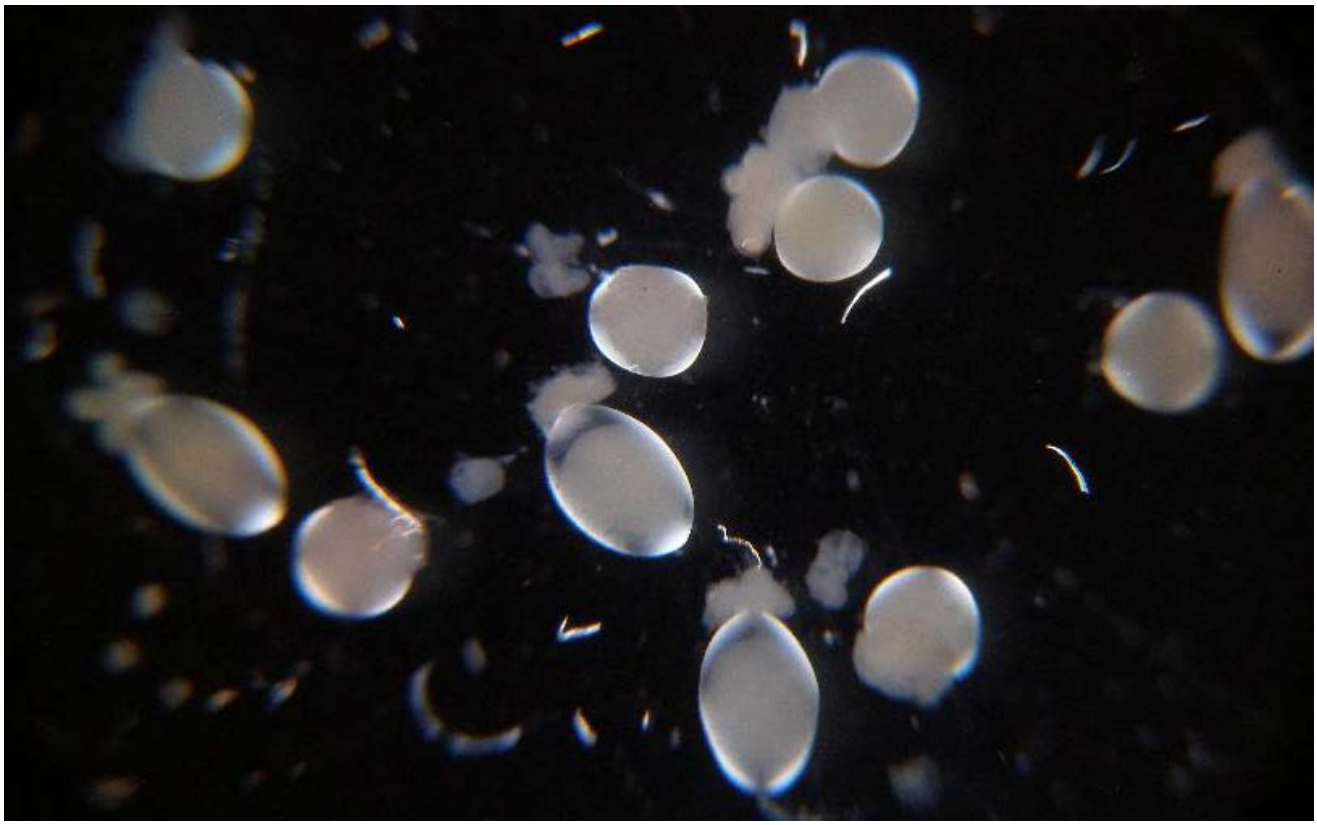

Fig 6. Fertilized and unfertilized spawn an hour after fertilization.

ge daily food portion for a round goby is estimated at 2 grams (Sapota 2005).

In the pre-spawning period, males demonstrate evident territoriality and after they occupy the nest, they remain in its vicinity and chase other fish away. There was one dominant male in each aquarium, this could result from the differences in the maturation rate and individuals' sizes. The climax of reproduction is usually preceded by specific behavior of individuals of both sexes (Bieniarz, Epler 1991). The male round goby that remained in the nest was constantly removing movable elements from the nest and "cleaning" the ceiling with the abdominal part of his body. This might be associated with the functioning of the cement gland, the purpose of which is to increase the efficiency of attachment of eggs by females. Spawning occurred after a few-day period of an evident increase in females' activity and was characterized by an evident synchronization between them in each aquarium. Dominance and spawning by one male and females' ovulation synchronization may be regulated by the activity of pheromones which play an important role in reproductive behavior (Bieniarz, Epler 1991, Corkum 1998).

Males actively care for eggs deposited in the nest , providing continuous circulation of water and eating unfertilized and dead eggs. Males do not show signs of active feeding and feed only when the food in the direct vicinity of the nest. In a natural environment, such a situation may lead to the fish's death due to exhaustion after the period of guarding the eggs (Sapota 2005).

During the reproductive season, the round goby from the Gulf of Gdańsk, shows a twofold intensification of spawning which is related to the nature of this fish's batch spawning (Tomczak 2001). The observed 3–4–week gap period between subsequent ovulations in females is similar to the one indicated by Kulikowa (1985) who specified it as a period of 15–28 days, depending on the temperature. Due to the consistent presence of two fractions of oocytes in various maturity stages in the ovaries of fish caught in the spring and summer (Tomczak 2001; author's observations), it is possible to obtain probably more than two spawnings during the season with omission of the cooling phase. During spawning of the round goby under controlled conditions with the use of fish caught in April from water with a temperature of around 8–10°C, maturation synchronization of individuals of both sexes was not achieved. Dysfunctions frequently occur in spawners of many species obtained from the natural environment. This contributes to the lack or low efficiency of spawning. In such a case, it is recommended to use hormonal injections leading to synchronization of ovulation with spermiation (Zakęś, Demska-Zakęś 2005); therefore, hormonal stimulation should be considered during further attempts of round goby reproduction under controlled conditions. Observations carried out during this experiment indicate that the male does not participate in the process of sticking eggs to the nest which is suggested by some authors who claim that this is the primary function of the cement gland discharge (Charlebois et al. 1997). The female independently stacks eggs in tightly aligned rows and stick onto the surface with a characteristic "frill" on the vegetal pole.

Artificial reproduction with the use of fertilization ex situ is the basic tool of aquaculture due to the increase in fertilization efficiency, the ability to adjust fertilization and spawn incubation conditions, as well as the selection of spawners. The ability to select the time and place of spawning is a factor facilitating the works (Babiak, Głogowski 1996). Artificial spawning of the round goby with the "dry" method does not cause many problems. Females caught in the spring matured and were ready for spawning within a short period of time between 7 to 11 days, influenced only by thermal stimulation. The only difficulty was losing eggs after the inspection of oocytes' maturity, likely to be caused by the stress of fish during their handling. This operation could not be carried out with the use of any method other than applying pressure to the abdominal integuments. The use of anesthesia in an an aesthetic solution could be the method which would eliminate stress in the fish but not all species of fish tolerate it well and it causes their breathlessness (Kucharczyk et al. 1997). In the case of females, obtaining genital products with the use of the intravital method encounters no problems; in the case of males however, it was difficult. Small quantities of sperm were obtained in this way which may be related to the batch spawning character of the round goby where the male is ready to spawn throughout the reproduction period. On the other hand, during the very act of spawning, a small amount of sperm is used (author's observations). Use of the extravital method has a serious disadvantage of causing loss of spawners. The fact that it is independent of the male spermiation time is this method's advantage. This enables the researcher to carry out artificial spawning throughout the early spring period, when the females have gonads at the 4th stage of the Nikolski scale and the males exhibit various gonad maturity stages (Tomczak 2001).

Features of fertilized eggs of the round goby (not very adhesive, falling onto the seabed) predisposes it to incubation in Californian type apparatuses. Water circulation around the eggs which lays motionless on the bottom of the apparatus is typical for these devices (Bieniarz, Epler 1991). Such a situation is analogical to the incubation process in the case of natural spawning which does not take place in the Weiss apparatus suggested by Kolat (2006) where the vertical stream of water causes continuous movement of the spawn. Mesh nets used in the experiment turned out to be the optimal solution for a small amount of spawn, allowing monitoring of the spawn's condition and care procedures on an ongoing basis.

The spawn incubation period ranged from 17 to 22 days, depending on the temperature (around 350–360 degree days). These results clearly differ from the data of Kolat (2006) who specified the period between fertilization and hatching as 10 days, at a temperature of 20°C. Such large differences are very difficult to explain. It is likely to be caused by the eggs incubation method. This is supported by a small difference in the resorption of the yolk-sac in round gobies hatched from Weiss jars (Kolat 2006) and incubated on a mesh nest. In Weiss jars which are widely used in aquaculture, the water flows vertically upwards which enforces constant spawn movement (Steffens 1986). The spawn incubated on mesh nets remains motionless and is constantly washed by water, similarly to California-type devices. Mechanical stimulation may initiate earlier hatching which is supported by records of larvae hatched early from the eggs that had fallen out of incubators. Another reason for earlier larva hatching in fish may be low oxygen contents in water, due to its deficit at the final stage of embryo development (Steffens 1986).

The general body structure of hatched fish was consistent with the description in literature (Moskalova 1996). The fish had fully developed fins and showed beginnings of pigmentation. On this basis they can be defined as post-larvae (Sapota 2005). After hatching, young round gobies were also able to feed independently and were readily accepting trout starter food which they were provided.

CONCLUSION

The adopted methodology of natural and artificial spawning can be successfully applied to round goby Neogobius melanostomus reproduction under controlled conditions. During the reproductive season, the round males spawn at least twice. The interval between the first and second spawning is 3–4 weeks. The male does not participate in the process of sticking the eggs to the nest. Round gobies hatched from eggs have fully developed fins and are capable to feed.

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