

**THE INFLUENCE OF TEMPERATURE
ON SUCCESSFUL REPRODUCTIONS OF BURBOT,
LOTA LOTA (L.) UNDER HATCHERY CONDITIONS**

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Key words: spawning, thermal manipulation, wild population, hormonal stimulation, burbot
Lota lota.

Abstract

The aim of this study was to define an influence of water temperature on successful reproduction of burbot under the hatchery conditions. Research was conducted during four successive spawning seasons where wild spawners were used. In the first three years of study fish were kept in three different (natural) thermal conditions. In the fourth year it was confirmed that the highest efficacy of synchronization of the spawning could be reached only under controlled thermal regimes. That year one group of spawners was kept at 6°C before spawning and then a sudden decrease of the temperature to 1°C was applied. Restrictively controlled thermal regime during reproduction of burbot in captivity caused the most synchronous spawning of females (2 days-period) in contrast to control group (17 days-period) and even hormonally stimulated (4 days-period). Eggs survival in thermally manipulated group was very high (over 85% in the eyed-egg-stage). This research proved that manipulation of water temperature is the most important technique which should be applied in controlled reproduction of burbot and it is suggested that the temperature is the major factor during final maturation of burbot females. Also, confirmed the fact that incubation of burbot eggs in temperature over 5°C causes its high mortality (100% in the eyed-egg-stage).

**WPLYW TEMPERATURY NA SUKCES ROZRODCZY MIĘTUSA, *LOTA LOTA* (L.)
W WARUNKACH WYLĘGARNICZYCH**

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Słowa kluczowe: rozród, manipulacje termiczne, dzika populacja, stymulacja hormonalna,
miętus *Lota lota*.

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Abstrakt

Celem pracy było określenie wpływu warunków termicznych na sukces rozrodczy miętusa w warunkach wylęgarniczych. Badania prowadzono podczas czterech kolejnych sezonów rozrodczych na dzikiej populacji miętusa. W pierwszych trzech latach badań ryby przetrzymywano w trzech różnych (naturalnych) warunkach termicznych. W czwartym roku potwierdzono w sposób eksperymentalny, że można osiągnąć bardzo wysoką efektywność rozrodczą po zastosowaniu wyłącznie manipulacji termicznych. W tym celu jedną z grup przetrzymywano przed tarłem w wodzie o temperaturze 6°C, a następnie gwałtownie obniżono temperaturę do 1°C. Restrykcyjne manipulacje termiczne w trakcie przetrzymywania tarlaków miętusa wpłynęły na największą synchronizację rozrodu (2 dni) w porównaniu z grupą kontrolną (17 dni) lub nawet stymulowaną hormonalnie (4 dni). Przeżywalność embrionów w „termicznie manipulowanej” grupie była bardzo wysoka (ponad 85% w stadium zaoczkowania). Wyniki wskazują na to, iż najważniejszym elementem procedury rozrodczej miętusa jest manipulacja termiczna, a temperatura jest głównym czynnikiem powodującym finalne dojrzewanie gamet. Potwierdzono także wysoką śmiertelność embrionów (100% w stadium zaoczkowania) w wodzie o temperaturze powyżej 5°C.

Introduction

The burbot, *Lota lota* (L.) is one of the most perspective species in coldwater aquaculture. It is the only freshwater species, which belongs to the order *Gadiformes* (NELSON 1994). Rivers, lakes and brackish waters, which constitute feeding areas for adult specimens, are places where burbot is present (SCOTT, CROSSMAN 1973, PULLIAINEN et al. 1992). Burbot is a predator whose main food are invertebrates and fish (JACOBSON, JÄRVI 1976, PÄÄKÖNEN, MARJOMÄKI 2000). It belongs to a group of reproductive lithopelagophils (KJELLMAN, ELORANTA 2002) and can also be classified as a cold stenothermal species, adapted to life in cold environment (TIITU, VORNANEN 2002). The optimal temperature for burbot feeding is below 12°C (Rass 1983). Spawning season, which is frequently preceded by long migration (PARAGAMIAN 2000, SCHRAM 2000, SLAVIK, BARTOS 2002, PARAGAMIAN et al. 2005) occurs mainly in the middle of the winter, when spawners often reproduce under the ice cover. Semi buoyant eggs are release in the depth of 0.5–1.5 m (SCOTT, CROSSMAN 1973, LEHTONEN 1998, MCPHAIL, PARAGAMIAN 2000), usually in temperature below 4°C (MANN 1996). The first days of incubation occur in similar temperatures as well, reducing mortality rates and developmental defects (KUJAWA et al. 1999b). PULLIAINEN, KORHONEN (1993) reported that some of adult burbot (sometimes even 50%) do not approach breeding every year. Fecundity depends on the female size, which may produce from 100 000 to over 3 000 000 eggs (NIKOLSKIJ 1950, VOSTRADOVSKA 1963, BAILEY 1972). Females of burbot reach maturity at the age of four years, while males usually one year earlier (VOSTRADOVSKA 1963).

At present, the importance of burbot is mostly ecological. As one of the top predators in ecosystem, they often compete with other top predators such as

pike or large salmonids (KIRILLOV 1988). Presently the burbot is threatened in almost all range of his distribution. Hence, the large interest in their restitution and production of fry in aquaculture in order to prevent this species extinction (BABIĄK et al. 1998, HARDY et al. 2008). Due to this situation many studies have been initiated on different aspects of biotechnology and burbot reproduction (KOUŘIL et al. 1985, KUCHARCZYK et al. 1998b, LAHNSTEINER et al. 2004) and fry rearing (KUJAWA et al. 1999c, WOLNICKI et al. 2001, 2002, PÄÄKKÖNEN et al. 2003, SHIRI HARZEVILI et al. 2003, 2004, BINNER et al. 2008, ŻARSKI et al. 2009a).

Reproductive processes in fish and development during early ontogeny stages are controlled and regulated by environmental factors like the photoperiod and temperature of water (LAM 1983, STACEY 1984, KUCHARCZYK et al. 1997b, 1998c, KUJAWA et al. 1997). However, for many teleosts the photoperiod is a dominant and the most important factor in the reproductive cycle (BROMAGE et al. 2001), while the temperature is playing the major role in final gamete maturation, ovulation and spawning (ANGUIS, CAÑAVATE 2005). The influence of temperature on egg quality and synchronization of ovulation, which strictly corroborates with day-length, has also been observed (BYE 1984, DAVIES, BROMAGE 2002). In artificial reproduction of many freshwater fish species, the hormonal induction of final gametes maturation is needed (KUCHARCZYK et al. 1997a,c,d, 2008, SZCZERBOWSKI et al. 2009). Only in limited number of species. i.e. Eurasian perch (KUCHARCZYK et al. 1996, 1998a) or some domesticated (i.e. KREJSZEFF et al. 2009) or aquarium fishes (i.e. KUCHARCZYK et al. 2010) it is possible to prepare spontaneous spawning in captivity.

The aim of this study was to investigate the influence of water temperature on successful spawning of burbot in captivity.

Materials and Methods

Spawners of burbot were captured every year (on four consecutive years) in the Szczeciński Bay (Northern-West Poland). Each season over one hundred breeders were collected and transported to the hatchery. Fish were held in captivity from the beginning of November. Females' weight varied from 0.8 kg to over 3.0 kg and males from 0.5 kg to 1.0 kg. Females for experiment were selected according to following criteria: the belly of females had to be fully distended and bulging, soft and resilient to touch.

The experiment was carried out at the "Czarci Jar" (near Olsztynek, Northern-East Poland) hatchery and the laboratory of Department of Lake and River Fisheries, Warmia and Mazury University in Olsztyn. In the hatchery fish were kept without feeding in 1 m³ tanks (KUJAWA et al. 1999a),

with maximum load of fish up to 30 kg m⁻³. Males and females were kept together. During all years of experiment fish were kept at the same conditions: tanks volume, fish density, water flow, photoperiod (natural), dissolved oxygen level (between 75 and 80%). The only variable was temperature. During the first three years the fish were kept in natural temperature (not modified; water was taken from Drwęca River), whereas in the last year of the study fish from one group were kept in thermostatically controlled temperature.

Water temperature was measured twice daily. Females were checked every three days before the first spawning, and twice daily after the first spawning. During the first three years of study, all females spontaneously matured without any hormonal stimulation. As a spawned females had been identified specimens without signs, mentioned above, and vest of eggs (which were not released in the tank) were certified after soft pressure of abdomen covers. For experimental observations 56, 49 and 52 females were selected in the first, second and third year of study respectively. In the last (fourth) year of study, females were randomly divided into three groups (20 specimens in each group), where:

- group 1 (control group) – fish were kept in natural conditions (average temperature about 1°C);

- group 2 (hormonally treated group) – fish were kept in natural water conditions but later were hormonally induced to spawn, using Ovopel (Unic-Trade, Hungary) at the dose of one pellet kg⁻¹. One Ovopel pellet containing 18–20 µg of highly active analogue of GnRH and 8–10 mg of metoclopramide – a dopamine antagonist (HORVATH et al. 1997);

- group 3 (temperature manipulated group) – fish were kept two weeks at 6°C and later water temperature was rapidly (during one day) lowered to 1°C.

Males were mature since they were brought to the hatchery and it was possible to squeeze semen from males during the entire period of captivity. During experiment some spawns characteristics, such as: number of ovulated females, ovulation time, survival of eyed-egg stage embryos, were noted. Because during the first three years of study the fish spawned spontaneously in tanks, it was impossible to recognize females fecundity. Similar situation was observed in the control group during the last year. In these groups spawned females were moved from the tanks. To determine embryos survival eggs were removed from tanks (water with eggs was siphoned through fine mesh) and incubated in Weiss jars. In the 4th year, in groups 2 and 3 when the females were matured and ready to spawn (eggs were certified by gentle pressure of abdomen), the eggs were stripped manually into plastic containers, mixed with the semen from 3–5 males (randomly chosen) and fertilized by adding water according to method described by KUCHARCZYK et al. (1998b). Eggs collected each day were incubated separately in Weiss jars. In the first three years of study, eggs were incubated in open-water-system where natural

water temperature was maintained. In the 4th year of investigation eggs were incubated in Weiss jars in recirculating system, equipped with cooling device, which allowed keeping the temperature of incubation below 4°C, till eyed-egg-stage. The eggs survival was examined after three days of incubation and at the eyed-egg stage. All these manipulations have been carried out after slight anaesthesia of fish (0.5-0.7 cm³ dm⁻³) with 2-phenoxyethanol (Sigma-Aldrich, Germany).

Statistical differences between groups (embryos survival to the eyed-egg-stage) in the fourth year of study were analyzed using one-way analysis of variance (ANOVA) and Tukey's *post-hoc* test at significance level below 5% ($P < 0.05$).

Results

In the first year of the experiment the temperature of water was steady and on a quite high level till the middle of January. At night from the 9th to 10th of January water temperature dropped below 5°C (Figure 1). Sudden thermal break down stimulated spawners' rapid final gametes maturation. As a result all of the females matured properly and the gametes were intensively collected in short intervals. The quality of eggs was satisfactory and their survival remained on high level up to eyed-egg-stage (Figure 2 and Figure 3).

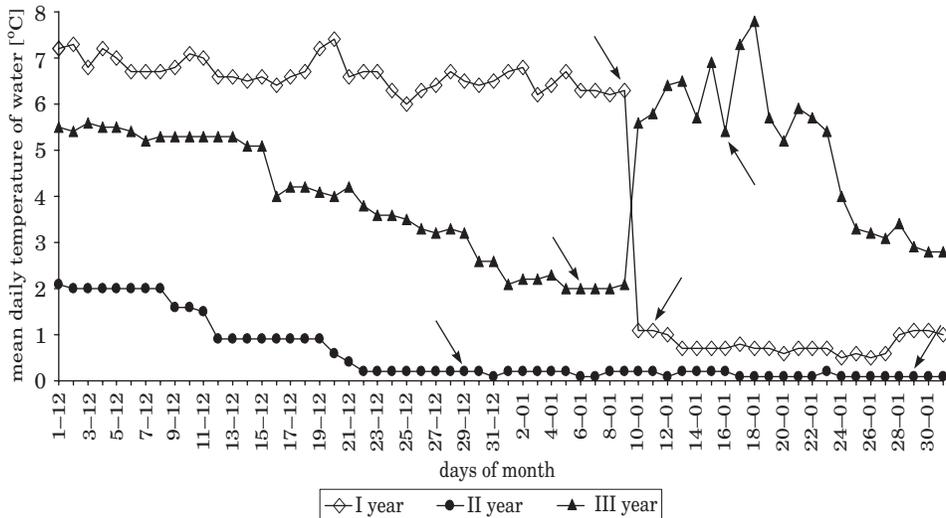


Fig 1. Fluctuations of water temperature during consecutive three years of study (arrows are showing time of the begin and the end of the burbot spawning)

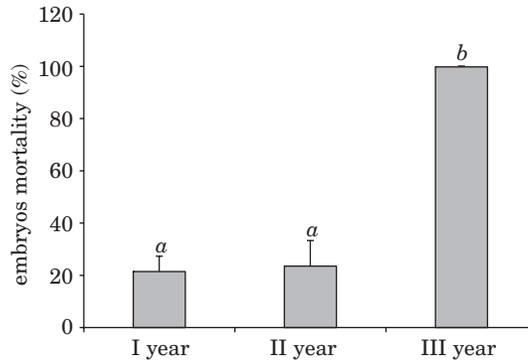


Fig. 2. Mortality of embryos in the 4th day of incubation (data marked with the same letter did not differ statistically)

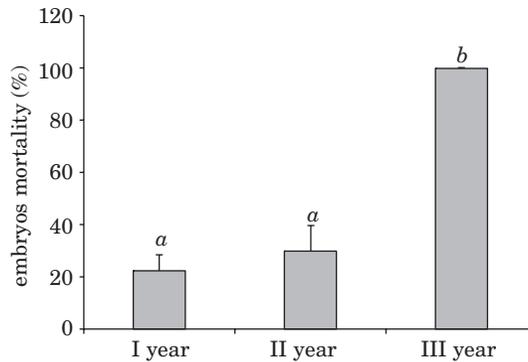


Fig. 3. Mortality of embryos in the eyed-egg-stage (data marked with the same letter did not differ statistically)

The temperatures in the 2nd year were completely different. The spawning season was cold which influenced negatively the synchronization of maturation of spawners (Figure 1). The temperature of water was near 0°C at the end of December. After more than a week of steady temperature conditions only some females matured. Gametes were collected during long period of time, when the temperature was steady and oscillated around 0°C. However, the quality of eggs (from all females) was, again, satisfactory. Survival on the 3rd day of incubation was high (Figure 2). Over 70% of embryos survived to the eyed-egg stage (Figure 3). Spawning in 2nd year of study started as the earliest and ended as the latest one.

At the beginning of December in the 3rd year of study, the temperature of water oscillated around 5°C. Then it decreased during 5 weeks to 2°C and after that, in the next few days, females matured. After 4 days from the 1st spawning, the temperature of water increased suddenly to the same level as for

beginning of December (Figure 1). It caused a shorter period of spawning. Despite the good quality of gametes, survival of embryos was unexpectedly low. After three days of incubation mortality was about 99% (Figure 2) and all incubated eggs did not survived to the eyed-egg stage (Figure 3).

Natural temperature of water during the 4th year of study was similar to this of the 2nd year of experimentation (Figure 4). The data obtained in the last year of study are presented in Table 1. Fish stimulated hormonally or thermally, spawned successfully. It was in contrast to results obtained from the control group. There were no differences in embryos survival between all groups, but remarkable differences were noted in duration of spawning. Females from the control group spawned during 17 days, whereas spawning of treated groups (groups 2 and 3) lasted only few days (Figure 5).

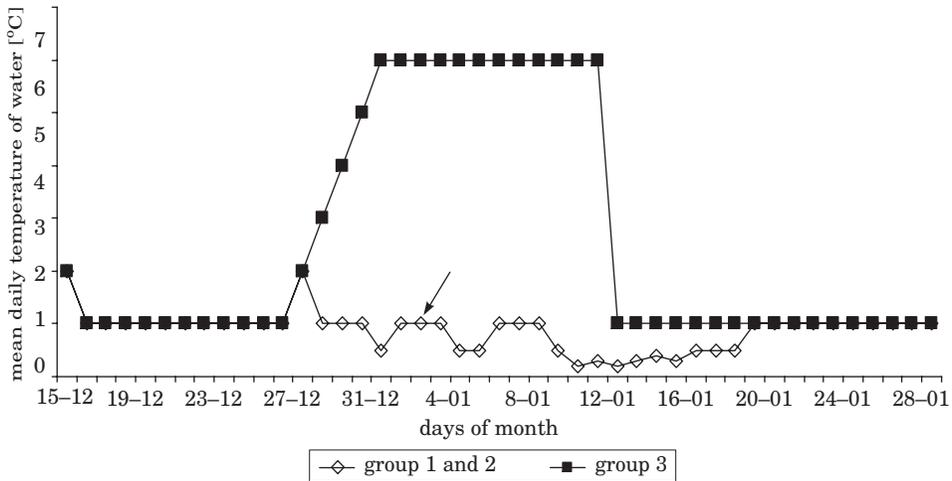


Fig. 4. Daily fluctuations of water temperature during the 4th year of study (arrow indicates day of hormonal stimulation of females from group 2)

Table 1
Results of reproduction of burbot in the 4th year of study

Specification	Natural conditions (group 1)	Hormonal stimulated (group 2)	“Warm” water/low temperature impact (group 3)
Number of females	20	20	20
Percentage of ovulated females	65%	95%	95%
Spawning time (days)	17	4	2
Survival of embryos at 3 rd day of incubation	95.0 ± 2.2 ^a	94.0 ± 3.1 ^a	95.2 ± 2.1 ^a
Survival of embryos to the eyed-egg stage	86.1 ± 2.3 ^a	85.2 ± 3.5 ^a	85.6 ± 3.2 ^a

* Data in rows marked with the same letter did not differ statistically

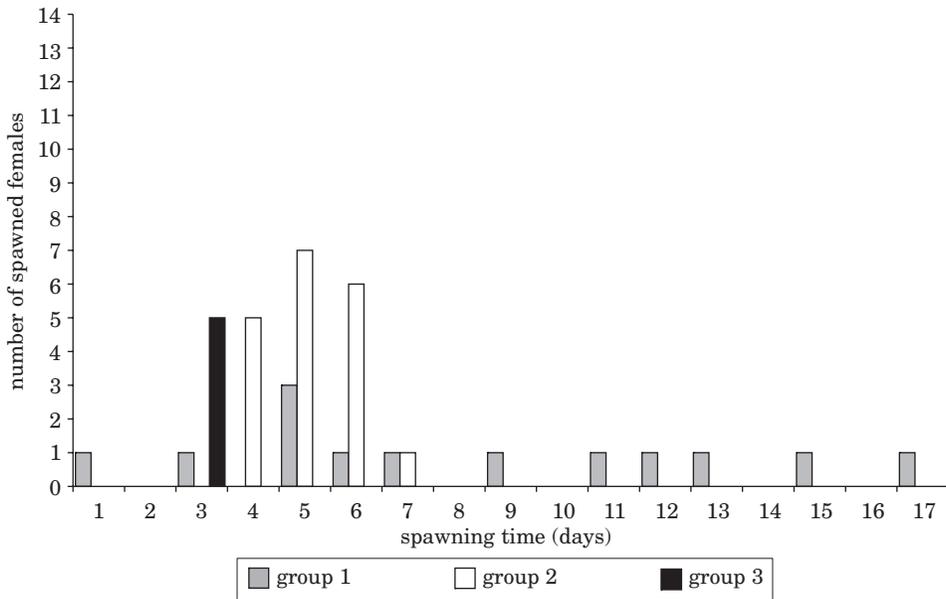


Fig. 5. Number of spawned females during 4th year of study

Discussion

In order to execute the artificial spawning of burbot the spawners need to be captured from their natural habitat. One of the major reasons for such operation is extreme mortality of brood-fish in ponds (KLESZCZ et al. 2001). Additionally data dealing with domestication of burbot is not available. KUCHARCZYK et al. (2004) reported that less than 50% of fish ate trout pellets under controlled conditions but fish survival in captivity was excellent. A change in burbot diet into living or frozen fish caused an increased percentage of fish which take food to about 70%. Nevertheless, there is an existing high risk of introducing of pathogens with “outside-fish”.

The essential rule in keeping burbot spawners in captivity prior to breeding is to ensure an adequate low temperature regime, in which fish not only survive but also digest food. This corroborates with the quantity of gastric acids secreted by burbot organism. At temperature of 1°C the quantity of secreted gastric acids is much larger than at 10°C (GOMAZKOV 1961). However, at the same time, the temperature should be maintained on a quite high level, which prevents spontaneous breeding in basins. In the first year of this study the temperature of 6°C appeared to be adequate to keep the thermal regime as mentioned above. The initiation of spawning was protracted, in opposite to all years of study and the

duration of spawning time was in most cases shortened. Females were ready just after the thermal breakdown, which affected artificial spawning very positively. This phenomenon creates perspectives for thermal control of ovulation time quite precisely. The synchronization of breeding obtained in that year was very similar to synchronization after hormonal stimulation under controlled conditions, as executed by KUCHARCZYK et al. (2004).

On the other hand, a slow downgrade of temperature in the second year of experimentation did not negatively influenced the reproduction of burbot. Despite the quite prolonged period of spawning, the final effect was pretty satisfactory – characterized with 70% survival of embryos to the eyed-egg stage. A similar result of artificial spawning was reported by KUCHARCZYK et al. (1998b) and KLESZCZ et al. (2001). At such low temperatures fish behaved very calm, facilitating the process of reproduction. But a very long period of such process was very exhausting for fish. Each day small portions of eggs were collected and therefore it was necessary to use lots of incubation devices. With addition, during the spawning season the temperature of water below 5°C was needed for a long period of time, which may cause difficulties in many fish farms. It might also affect the time of larvae hatching. In present study, each portion of eggs was hatched at a different moment causing differentiation in growth (and finally in food competition and cannibalism) (KUJAWA et al. 2002). It requires also larger number of rearing tanks.

Analysis of the 3rd year of study led to the conclusion that the slow but quite large downgrade of temperature in consecutive five weeks, was the reason of females' maturation. Spawning began at 2°C temperature of water and its was very promising, henceforward good quality eggs were collected. The effect of positive and significant gradation of temperature during the process of final maturation confirmed that even at rise of temperature, to the level at which normal development of embryos is impossible, after the 4th day of spawning, the females were still ovulating. The extreme mortality (over 99%) of embryos was caused by the high temperature of incubation. Mass mortality of incubated embryos occurred after the sudden increase of temperature to above 5°C. Other authors (STEINER et al. 1996, KUJAWA et al. 1999b) reported that this level of temperature is lethal for burbot embryos. It should be quoted that any trial to incubate eggs in such thermal regimes resulted in almost immediate mortality.

In many freshwater fish, synchronization of ovulation under controlled conditions is possible, mainly with hormonal treatment (KUCHARCZYK et al. 1996, 1998a, 2005, 2008, ZAKEŚ, SZKUDLAREK 1998, KOUŘIL et al. 2007, KREJSZEFF et al. 2008, 2009, SZCZERBOWSKI et al. 2009, ŻARSKI et al. 2009b). It is particularly essential in artificial reproduction of wild spawners (KUCHARCZYK et al. 1997a, KUCHARCZYK et al. 1998a, HONG, ZHANG 2003, HEYRATI et al.

2007). It improves the work during the spawning season and permits more effective utilization of hatchery facilities. Data from the last year of this study showed that it is possible to obtain much better synchronization of burbot spawning in captivity after application of hormonal stimulation (KUCHARCZYK et al. 1998b, KUCHARCZYK et al. 2004) as well as after thermal stimulation. Moreover, the influence of temperature on burbot females final maturation seems to be the major factor.

Probably, maintenance of breeders in water temperature of 6°C and thereafter sudden decrease to 1–2°C should be the reason of high synchronization of breeding and satisfactory effects on spawning. Unfortunately, such temperature manipulations are not always possible for application in commercial fish farms. The results obtained in the 3rd spawning season of burbot show that similar environmental and thermal conditions are the reason of failure in recruitment success of this species in particular year. Thus, the dramatic progress of environment pollution and climatic changes are very important factors, which require intensive researches in burbot aquaculture. Especially when endemic populations of this species and their habitats are vulnerable by human activities (PARAGAMIAN et al. 2008).

Translated by AUTHORS

Accepted for print 18.11.2009

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