



## NOTES

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### WATER TURBULENCE EFFECT ON EGG SURVIVAL AND CHARACTERISTICS OF HATCHED LARVAE OF THE BLACK SEA TURBOT *SCOPHTHALMUS MAEOTICUS* (PALLAS, 1814)

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The impact of different level of turbulence on developing eggs and prelarvae of the Black Sea turbot *Scophthalmus maeoticus* obtained from the pair of wild spawners at the end of natural spawning season has been studied. The incubation was carried out at three different levels of turbulence generated by diffused aeration: without aeration (calm water), low aeration (34 ml per minute) and high aeration (75 ml per minute). In calm water hatching rate (HR) of turbot eggs was 20 % and the hatched prelarvae had the longest standard length (SL = 3.13 mm) and the biggest volume of the yolk sac (VYS = 0.37 mm<sup>3</sup>) as compared to those of low aeration (HR = 18 %; SL = 3.10 mm; VYS = 0.32 mm<sup>3</sup>) and high aeration conditions (HR = 141%; SL = 3.10 mm; VYS = 0.29 mm<sup>3</sup>). One day after hatching the percentage of survived prelarvae from the number of hatched was the highest – 86 % at high aeration, compared with that of low aeration (72 %) and calm water (61 %). Application of turbulence leads to elimination of “poor” quality eggs during incubation, presumably enables hatching of more viable larvae and can reduce mortality of more advanced larvae.

**Keywords:** *Scophthalmus maeoticus*, turbot, turbulence, aeration, fish eggs, fish larvae, artificial reproduction

Valuable commercial fish species and a promising subject for mariculture, Black Sea turbot *Scophthalmus maeoticus* (Scophthalmidae, Pisces) is one of the important components of the Black Sea ecosystem. The Black Sea ichthyoplankton data from the 1950s [9] till 2016–2017 [3, 5] showed a significant number of anomalies and high mortality rate (up to 90 %) of turbot eggs in natural environment. In aquaculture facilities obtaining turbot eggs through artificial stripping of spawners is even less selective than through natural spawning as it is difficult to obtain oocytes just after ovulation. Eggs stripped more than 8 hrs after ovulation have significantly decreased quality and, in case of their fertilization success, result usually in low-quality embryos. Maldevelopment of both Atlantic and Black Sea turbot during embryogenesis was observed in 30 % of stripped and fertilized artificially eggs [1, 7, 8]. Besides, spontaneous chromosomal variability of turbot embryos was observed with cells with aberrant chromosomes varying from 1.5 to 20 % [4].

The efficiency of turbot reproduction depends on numerous biotic and abiotic factors including initial quality of eggs and hydrological conditions under which they develop. The main abiotic factors affecting early life stages survival prior start of exogenous feeding are hydrological conditions including level of turbulence.

In aquaculture facilities hydrodynamic conditions affecting survival and hatching rate of larvae are represented by intensity of water turbulence in incubators [2]. The objective of the work is to study the effect of turbulence level (intensity of aeration in incubators) on turbot eggs and larvae development.

## MATERIAL AND METHODS

The impact of water turbulence on development of turbot eggs and larvae from the artificial dry fertilization of gametes obtained from a couple of wild Black Sea turbot spawners caught by the gill nets during the end of spawning season was monitored in laboratory conditions. Incubation of fertilized eggs (stocked within an hour after fertilization into six 40-liter blue plastic incubators at initial density of  $75 \text{ eggs} \cdot \text{L}^{-1}$ ) and hatched larvae was run under temperature of  $(17 \pm 1)^\circ\text{C}$  under the dim natural light. Incubators were cleaned daily, dead eggs and larvae were removed and calculated, clean water was replaced. Turbulence was maintained through experiment at 3 levels (2 replicates each) using aquarium compressor performing diffuse aeration 15 cm from the bottom of incubator: zero turbulence (no aeration), low (34 ml per minute) and high level of turbulence (75 ml per minute). Oxygen was maintained at  $8.0\text{--}8.2 \text{ mg} \cdot \text{L}^{-1}$ .

Survival rate of embryos was estimated by daily calculation of the percentage of survived embryos from initial stocked number (%) up to hatching of the larvae. Survival and development of larvae were estimated at the time of hatching and 24 hours later. Percentage of survived larvae (%), body standard length (SL, mm), diameter of oil drop (OD, mm) and volume of yolk sac (VYS,  $\text{mm}^3$ ) were registered; measurements were performed under binocular microscope MBC-10 at magnification  $8 \times 4$ .

Statistical data analysis was carried out using STATISTICA 10. Data sampling was checked for normality using the Kolmogorov – Smirnov test. The coefficients of variation of larvae body standard length (CVSL, %), of diameter of oil drop (CVOD, %), and of the yolk sac volume (CVVYS, %) were calculated. To compare the data, analysis of variance ANOVA was applied. Differences were considered statistically significant at  $p \leq 0.05$ .

## RESULTS

Incubation in calm water resulted in hatching rate (HR) 20 % (Fig. 1) of the larvae having larger standard length ( $\text{SL} = (3.13 \pm 0.08) \text{ mm}$ ) and volume of the yolk sac ( $\text{VYS} = 0.37 \text{ mm}^3$ ) (Fig. 2) in comparison with those of low aeration (HR = 18 %;  $\text{SL} = (3.10 \pm 0.05) \text{ mm}$ ;  $\text{VYS} = 0.32 \text{ mm}^3$ ) and high aeration conditions (HR = 14 %;  $\text{SL} = (3.10 \pm 0.07) \text{ mm}$ ;  $\text{VYS} = 0.29 \text{ mm}^3$ ).

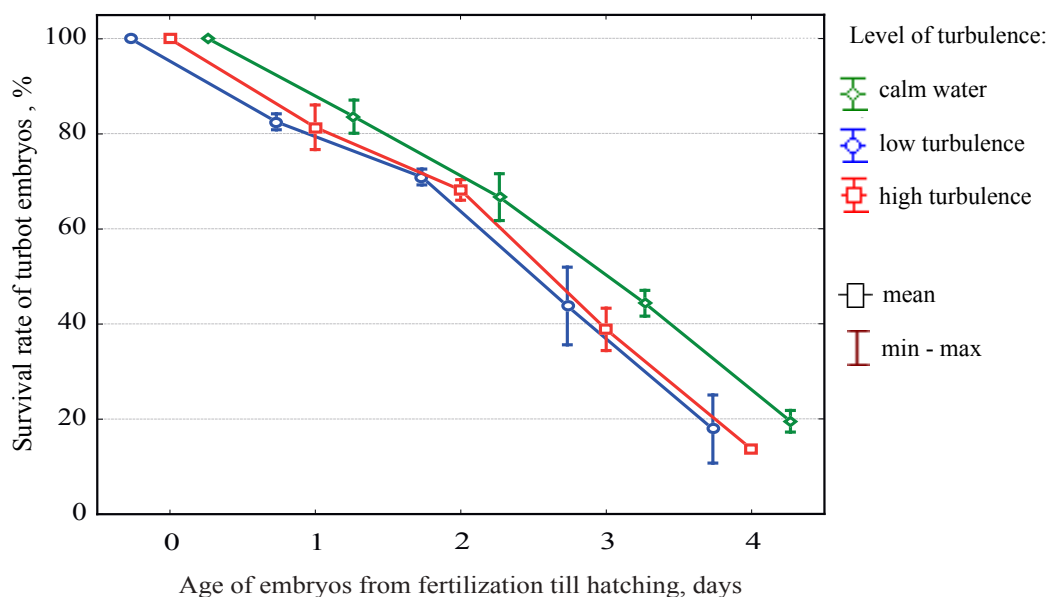
The highest coefficient of variation of standard body length  $\text{CVSL} = 2.5\%$  and the least scattering of the coefficients of variation of diameter of oil drop  $\text{CVOD} = 2.8\%$  and of yolk sac volume  $\text{CVVYS} = 0.29\%$  were observed for hatched larvae incubated without turbulence (Table 1). Absorption of yolk sac and oil drop in this case was slower and more effective; consequently, the larvae of different body length (SL) and condition were able to hatch.

By the end of the first 24 hours after hatching the percentage of survived larvae from the number of hatched was the highest (86 %). The percentage of survived larvae resulted from low aeration conditions was 72 %. The lowest survival was obtained for the larvae hatched in calm water – 61 %.

**Table 1.** Variability of larvae characteristics at hatching under different level of turbulence

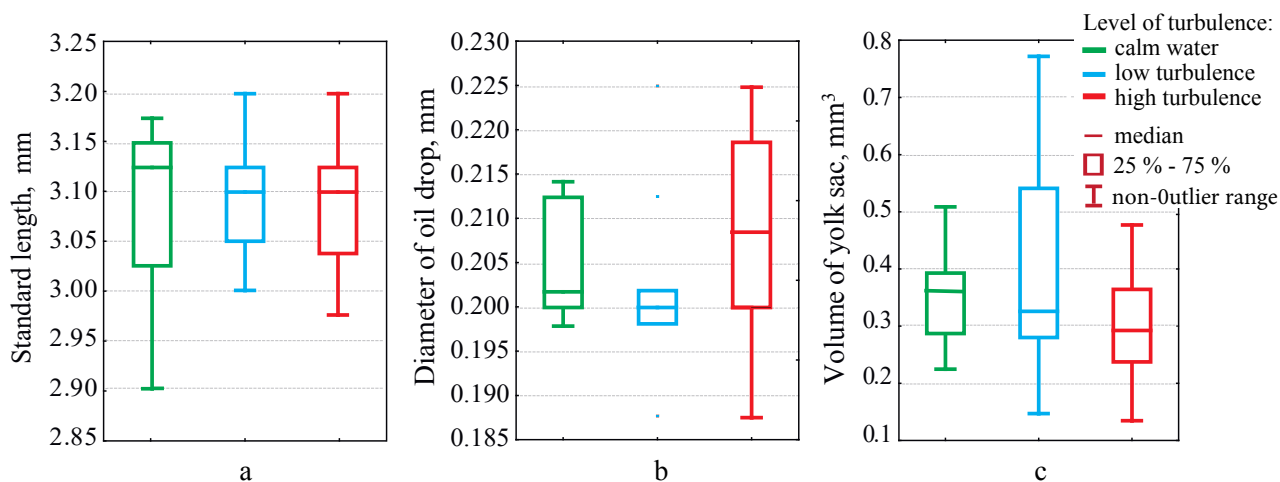
**Таблица 1.** Вариабельность размерных характеристик личинок на выклеве при различной интенсивности продувки

| Level of turbulence | Rate of hatching | SL, mm | CVSL, % | OD, mm | CVOD, % | VYS, $\text{mm}^3$ | CVVYS, % |
|---------------------|------------------|--------|---------|--------|---------|--------------------|----------|
| Calm water          | 20 %             | 3.13   | 2.5     | 0.203  | 2.8     | 0.37               | 0.29     |
| Low turbulence      | 18 %             | 3.10   | 1.7     | 0.202  | 5.2     | 0.32               | 0.42     |
| High turbulence     | 14 %             | 3.10   | 2.3     | 0.208  | 5.5     | 0.29               | 0.37     |



**Fig. 1.** Survival rate of turbot embryos (%) during incubation from fertilization till hatching in calm water (green line), low (blue line) and high (red line) level of turbulence

**Рис. 1.** Выживаемость эмбрионов калкана от момента оплодотворения икры до выклева личинок, инкубируемых без продувки (зелёная линия), при слабой продувке (синяя линия), при сильной продувке (красная линия)



**Fig. 2.** Standard length SL, mm (a), diameter of oil drop OD, mm (b), volume of yolk sac VYS, mm<sup>3</sup> (c), of larvae at the time of hatching incubated in calm water (green line), low (blue line) and high (red line) level of turbulence

**Рис. 2.** Стандартная длина SL, мм (а), диаметр жировой капли OD, мм (б), и объём желточного мешка VYS, мм<sup>3</sup> (с), личинок на выклеве при инкубации без продувки (зелёная линия), при слабой продувке (синяя линия), при сильной продувке (красная линия)

## DISCUSSION

The low hatching rate of turbot eggs obtained in our experiment is a result of multiple reasons, especially, that wild turbot spawners caught by gill nets at the end of natural spawning season were stressed, and, more likely, the eggs stripped from female were delayed more than 12 hrs after ovulation. Nonetheless, our results support previously reported data on the survival of more viable turbot larvae under turbulent conditions [7]. Calm water or very low water turbulence increased survival through incubation and hatching of turbot with minor developmental defects, and resulted in increased number of maldeveloped hatched larvae.

Increasing aeration (up to 10–30 ml per minute) decreased the number of hatched larvae from initial number of eggs through elimination of nonviable embryos at different stages of development. Experimental study of water turbulence impact (diffuse aeration) on the southern flounder, *Paralichthys lethostigma*, also showed that high rate of aeration during incubation favours further survival of newly-hatched larvae of better quality [6], that can be explained by the fact that a certain level of turbulence contributes to selection of more viable embryos.

**Conclusion.** The carried out trials suggest that though diffuse aeration of 30–75 ml per minute affected general survival rate of turbot from the start of incubation till hatching, elimination of embryos with developmental defects in turbulent conditions may turn out to be an effective technique of selection of more viable prelarvae. As a result, survived selected prelarvae can show their robustness during further development.

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**ВЛИЯНИЕ ТУРБУЛЕНТНОСТИ ВОДЫ  
НА ВЫЖИВАЕМОСТЬ ИКРЫ И РАЗВИТИЕ РАННИХ ЛИЧИНОК  
ЧЕРНОМОРСКОГО КАЛКАНА *SCOPHTHALMUS MAEOTICUS* (PALLAS, 1814)**

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Изучено влияние различных режимов турбулентности воды на развитие икры и личинок черноморского калкана *Scophthalmus maeoticus*, полученных от производителей из естественной популяции в конце нерестового сезона. Инкубацию проводили в трёх режимах турбулентности: без продувки, при слабой продувке (34 мл в мин), при сильной продувке (75 мл в мин). При инкубации икры без продувки доля выклева составляла 20 %, личинки на выклеве имели крупные размеры тела ( $SL = 3,13$  мм) и больший по размерам желточный мешок ( $VYS = 0,37$  мм<sup>3</sup>), чем личинки при слабой (доля выклева 18 %;  $SL = 3,10$  мм;  $VYS = 0,32$  мм<sup>3</sup>) и сильной (доля выклева 14 %;  $SL = 3,10$  мм;  $VYS = 0,29$  мм<sup>3</sup>) продувке. К концу первых суток после выклева выживаемость личинок, инкубируемых в режиме сильной продувки, составила 86 %, без продувки — 61 %, при слабой продувке — 72 %. Показано, что наличие продувки вызывает гибель некачественной икры и способствует выклеву наиболее жизнеспособных личинок. Таким образом, элиминация нежизнеспособных личинок на этапе инкубации является эффективным способом снижения смертности личинок в дальнейшем.

**Ключевые слова:** *Scophthalmus maeoticus*, калкан, продувка, барботаж, икра рыб, личинки рыб, искусственное воспроизводство