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# THE DEVELOPMENT OF THE SWIM BLADDER OF PIKEPERCH *SANDER LUCIOPERCA* (L.) REARED IN INTENSIVE CULTURE

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ABSTRACT. The aim of this study was to describe the development of the swim bladder of pikeperch *Sander lucioperca* (L.) under conditions of intensive culture and to determine when the pneumatic duct regresses. The development of the swim bladder was observed over a 30-day period of larvae culture in a recirculation system. The swim bladder and the pneumatic duct were clearly visible in histological cross sections of 4-day-old larvae. On day 6 after hatching significant changes occurred in the microscopic structure of the swim bladder, e.g. the gas gland and oval primordia appeared. The pneumatic duct gradually atrophied in the larvae with inflated swim bladders between days 11 and 13, while it remained open until day 24-26 after hatching in fish with noninflated swim bladders. The swim bladders of the latter group were irregular in shape and hyperplasia of the epithelium and connective tissues and the presence of macrophags were noted. Pikeperch larvae with noninflated swim bladders also suffered from lordosis and slower growth rates.

Key words: PIKEPERCH, (*SANDER LUCIOPERCA)*, SWIM BLADDER, INTENSIVE CULTURE

## INTRODUCTION

Pikeperch *Sander lucioperca* (L.) is a rapidly growing species whose meat is highly valued for its taste. As it is valuable to both commercial fisheries and anglers (Wołos et al. 1998, Bnińska and Wołos 2001), the demand for its fry is high. Pikeperch have been the subject of numerous studies aimed at either developing the principles of hormonally stimulated reproduction (Schlumberger and Proteau 1996, Steffens et al. 1996, Zakęś and Demska-Zakęś 1999, Demska-Zakęś and Zakęś 2002) or the cultivation of larval and juvenile stages under controlled conditions (see the literature review in Hilge and Steffens 1996). Although the cultivation of pikeperch juvenile stages under controlled conditions using artificial feed is well established (Zakęś and Demska-Zakęś 1996, Zakęś 1997 and 1999, Zakęś et al. 2001), the effectiveness of the larval culture of this species remains unsatisfactory (Ruuhijärvi et al. 1991, Schlumberger and Proteau 1991, Hilge and Steffens 1996, Szkudlarek and Zakęś, unpublished data). Similar results were obtained over a decade ago in larval cultivation of the walleye *Stizostedion vitreum* Mitchill (Colesante et al. 1986, Barrows et al.

1988, Kindschi and MacConell 1989). The authors of these papers indicated that the process of swim bladder inflation has a significant impact on the effectiveness of the culture. As other percoids, this species is physoclistous, and at a certain moment in its ontogenesis the pneumatic duct atrophied facilitating swim bladder inflation with air (Marty et al. 1995, Rieger and Summerfelt 1998). Understanding the biology of *S. vitreum* and applying the appropriate technical solutions resulted in a significant increase in the percentage of fish with inflated swim bladders, thus improving the effectiveness of the culture (Summerfelt 1996).

Studies focused on the biology of larval stages and ontogenesis, especially (in physoclistous fish) during the period when inflation of the swim bladder is possible, should be conducted in intensive culture and at optimal environmental conditions. It is known that larval development and behavior are not only affected by environmental conditions such as temperature or light intensity, but also by the capacity, shape and color of basins used in larval culture (Moore et al. 1994).

The aim of the research presented in this paper was to describe the development of the swim bladder and determine when the pneumatic duct atrophied in pikeperch larvae in intensive culture.

## MATERIAL AND METHODS

The experimental material involved pikeperch larvae obtained during hormonally stimulated and controlled reproduction conducted at the Dgał Experimental Fisheries Center of the Inland Fisheries Institute in Olsztyn. The larvae were cultivated in tanks with a volume of 0.2 $m^3$ that were part of a closed water circulation system. Water flow was maintained at 1 - 2 l $min^{-1}$. The physical and chemical water parameters during culture for inflow and outflow water, respectively, were: temperature – 19.74 (± 0.15) and 19.58 (± 0.14)°C; total ammonia nitrogen – (TAN = $NH_4^+$-N + $NH_3$-N) 0.08 (± 0.02) and 0.21 (± 0.01) mg TAN $l^{-1}$; nitrites - 0.09 (± 0.13) and 7.67 (± 0.23) μγ N–$NO_2$ $l^{-1}$; oxygen concentration – 7.58 (± 0.25) and 6.67 (± 0.38) mg $O_2$ $l^{-1}$; pH – 7.69 (± 0.13) and 7.67 (± 0.23). The larvae were fed ad libitum with mixed feed (*Artemia* sp.) and artificial feed (FK 0G Felleskjøpet Havbruk, Norway) with a chemical composition of protein - 57%, fat - 13%, carbohydrate - 10%.

The samples for histological examination were collected throughout the 30-day-long culture (fish age 0-29 days after hatching). Initially, samples were collected daily (0-17 days after hatching), and then every other day (18-29 days after

hatching). A total of 24 fish samples were collected, with an average of 10 specimens in each. The fish were caught and placed in containers with the anesthetic PROPISCIN (Kazuń and Siwicki 2001). Total body length (Lt ± 0.1 mm) and body weight (BW – 0.01 mg) were measured, and then the specimens were preserved in Bouin's liquid (Zawistowski 1986). These samples were dehydrated in ethanol solutions (from 75 to 100%), over-exposed in xylene and submerged in paraffin blocks. The samples were then cut (longitudinal and transverse cross sections) using a rotating microtome into 5 µm thick sections and then stained using either hematoxylin and eosin (HE) or the Mallory method (Zawistowski 1986, modified by Demska-Zakęś). The histological preparations were analyzed using a light microscope with a focus on the shape, size, location and microscopic structure of the swim bladder and the pneumatic duct. MultiScanBase v. 8.08 software (Computer Scanning System Ltd., Warsaw, Poland) was used during the studies. The growth rate data were analyzed using one-factor variance analysis (ANOVA). The differences were regarded as statistically significant at $P < 0.05$.

## RESULTS

The average, total length of pikeperch larvae on the day of hatching was 4.28 (± 0.28) mm (Fig. 1). The transparent larvae had oval yolk sacs of a significant size. They contained a large globule of oil in the anterior part and a protein yolk in the posterior part. The mouth was located in the ventral part of the head and was occluded. Swim bladder primordia were not visible under the microscope. By the fourth day after hatching the yolk sac was significantly resorbed, but the relatively large oil globule remained. The mouth was located at its final position and was open, and the larvae began to consume its first exogenous food. The longitudinal and transverse larval cross sections from above the digestive tract indicated the presence of a swim bladder. It was shaped like an oblong sack and was connected with the digestive tract (near the esophagus) through the pneumatic ducts (Photo 1). The duct was open in the caudal part of the swim bladder. The outer wall of the swim bladder was made of a fibrous shell, while the inner wall was comprised of a cylindrical, single-layer epithelium (in the cranial section) or it was cuboidal (hexagonal) in shape (the rest of the margin). The lumen of the pneumatic ducts was limited by the cuboidal epithelium, although the internal epithelium cells in both the bladder and the ductus pneumaticus were not very well marked.

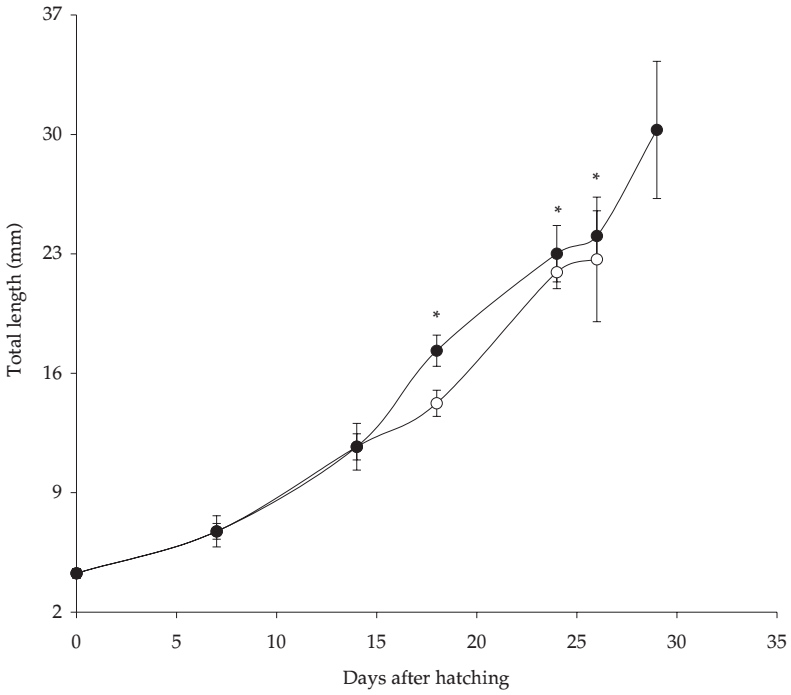


Fig. 1. Growth rate of pikeperch larvae with inflated (filled marks) and noninflated (empty marks) swim bladders (average ± SD) (* - statistically significant differences ($P < 0.05$)).

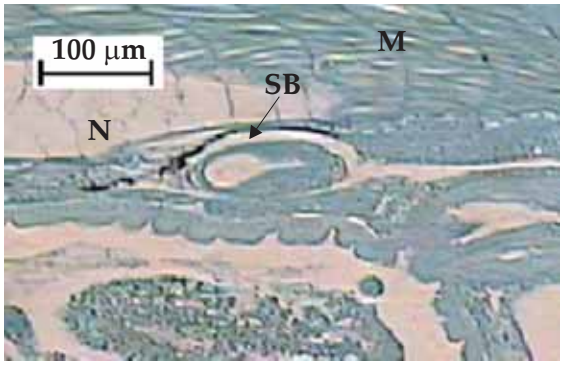


Photo 1. Longitudinal cross-section of pikeperch larvae on day 4 after hatching: SB – swim bladder, N – notochord, M – muscles.

On the sixth day after the hatch both the yolk and oil droplet were almost entirely resorbed. At this stage, the swim bladder was an oval sphere (Photo 2A). The size of its lumen increased substantially and changes in its histological structure were visible. A multi-layered gland epithelium (cuboidal) protruded towards the opening (gas gland primordium) and was observed in the cranioventral part of the swim bladder. A multi-layered epithelium was also observed in the caudodorsal part; however, it

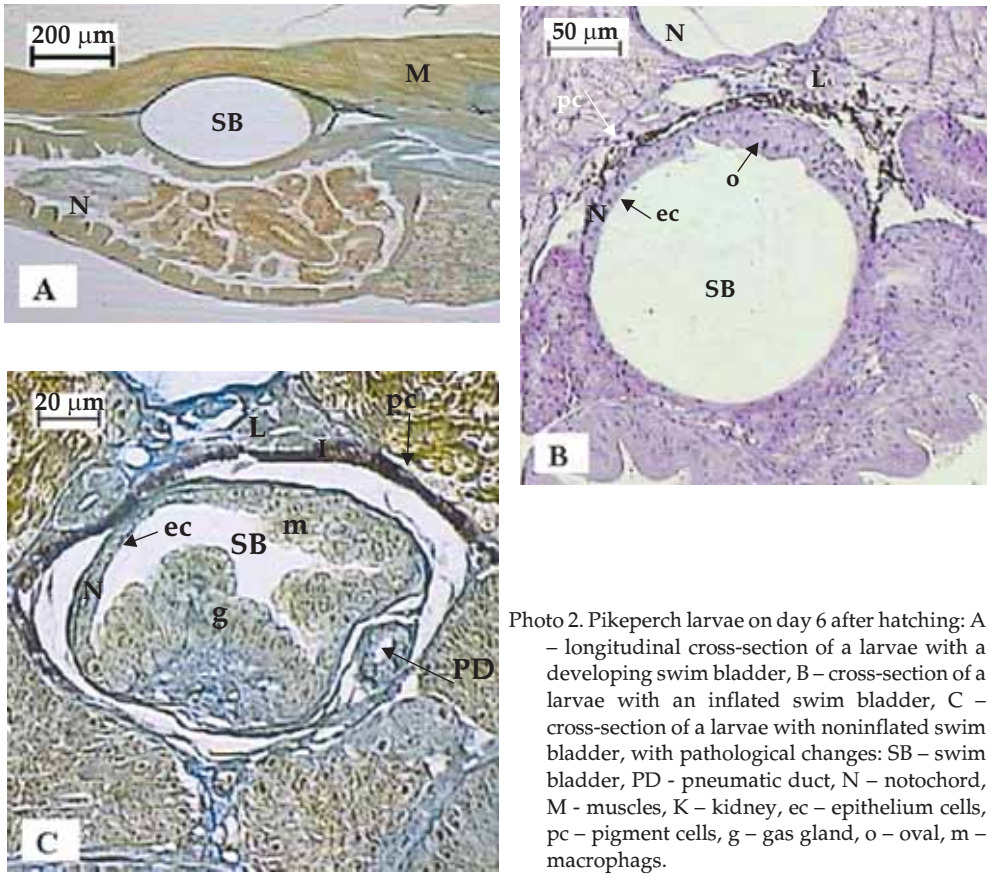


Photo 2. Pikeperch larvae on day 6 after hatching: A – longitudinal cross-section of a larvae with a developing swim bladder, B – cross-section of a larvae with an inflated swim bladder, C – cross-section of a larvae with noninflated swim bladder, with pathological changes: SB – swim bladder, PD - pneumatic duct, N – notochord, M - muscles, K – kidney, ec – epithelium cells, pc – pigment cells, g – gas gland, o – oval, m – macrophags.

was squamous and was the primordium of the so-called oval (Photo 2B). The wall in the remaining part of the swim bladder was layered with single-layered hexagonal epithelium. The epithelium was surrounded by connective tissue, which included smooth muscle tissue. Pigment cells were observed around the outer bladder walls, especially in the dorsal part. The pneumatic duct was open and clearly visible. The noninflated swim bladder had a similar structure, although its lumen was smaller and had collapsed due to the hypertrophy of the epithelium of the gas gland and oval (Photo 2C). Macrophags were found inside the bladder. During subsequent days, pathological changes intensified, while the volume of the inflated swim bladders increased and the thickness of their walls decreased. A few capillaries were noted under the epithelium of the gas gland. The pneumatics duct was open. The atrophy of the pneumatic duct was observed beginning on day 11 after hatching in larvae with

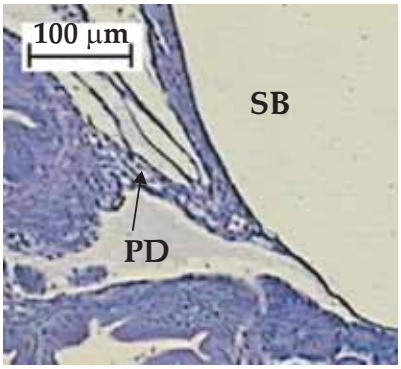


Photo 3. Longitudinal cross-section of a 13-day-old pikeperch specimen: SB – swim bladder, PD - pneumatic duct.

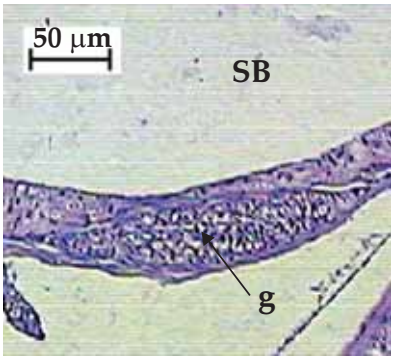


Photo 5. Cross-section through anterior, ventral part of the swim bladder of a 18-day-old pikeperch specimen: SB – swim bladder, g – gas gland.


Photo 4. Pikeperch (day 18 after hatching) with noninflated swim bladder and deformed spine.

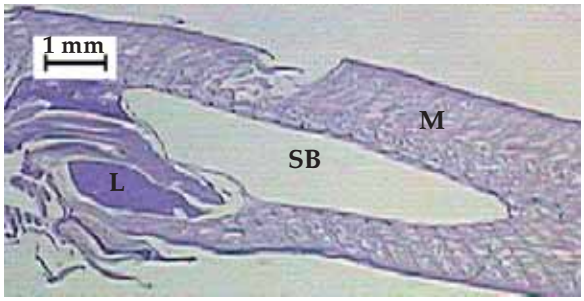


Photo 6. Longitudinal cross-section of a 21-day-old pikeperch specimen with an inflated swim bladder: SB – swim bladder, M – muscles, L – liver.

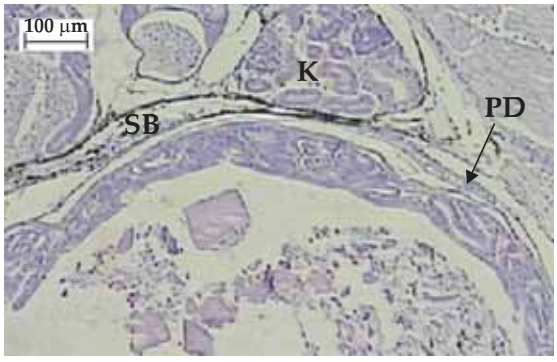


Photo 7. Cross-section through a pikeperch specimen with noninflated swim bladder (day 24 after hatching): SB – swim bladder, PD - pneumatic duct, K – kidney.

inflated swim bladders. Two days later the duct was occluded (Photo 3).

Statistically significant differences ($P < 0.05$) were observed in larval growth rates on day 18 after hatching (Fig. 1). Fish with noninflated swim bladders were 14.24 mm long, on average, and their skeletons were deformed (Photo 4). The swim bladder, connected by an open pneumatic duct with the

esophagus, was irregular in shape and filled with hypertrophic cells and macrophags. Larvae with inflated swim bladders (average body length – 17.33 mm) did not exhibit symptoms of lordosis. The swim bladder lumen was wide and surrounded by a squamous epithelium. Gas gland cells covered a large area of the cranioventral wall, and at the base of them were numerous capillaries of the rete mirabile (Photo 5). A highly vascular oval was observed in the caudodorsal part of the swim bladder. Over the subsequent days, the swim bladder grew and occupied an increasingly large proportion of body volume (Photo 6). On the other hand, progressing pathological changes were observed in the throats of the fish with noninflated swim bladders (Photo 7). The gradual occlusion of the pneumatic duct was observed from day 24 after hatching. The histological cross sections of larval bodies that were collected on day 27 after hatching showed no trace of the pneumatic duct.

## DISCUSSION

The studies revealed that among pikeperch specimens cultivated in water at a temperature of 19.6°C, the swim bladder starts to appear within day three or four after hatching and in the following days this organ develops and then inflates with air. The development of the swim bladder in walleye *S. vitreum* was observed to be similar. Larvae of this species cultivated at a temperature of 21°C start to inflate their swim bladders on day 5 after hatching (Barrows et al. 1988), while at lower temperatures of 15.2-19.0°C this occurs on day 6 after hatching (Marty et al. 1995). During this period, significant changes occur in pikeperch - the yolk sac is resorbed, the mouth opens and larvae start to consume their first exogenous food. The process of swim bladder inflation, which occurs simultaneously with the resorbence of the yolk sac, the consumption of exogenous food and active swimming, has also been observed in other fish species. Ostaszewska et al. (1997) confirmed that the swim bladders in vimba *Vimba vimba* L. larvae begin to inflate when 71-76% of the yolk sac is resorbed. Salmonids do not inflate their swim bladders and they stay at the bottom of basins until the yolk sac is fully resorbed (Tait 1960). Tait confirmed that the beginning of inflation depends on water temperature at which cultivation takes place. Since salmonids are phytostomous and have an open pneumatic duct, they can inflate their swim bladders for as long as three months after hatching.

The period of swim bladder inflation in physoclistous fish is limited by changes which occur in the digestive tract and the regression of the pneumatic duct (Doroshev

et al. 1981). The current study revealed that among larvae of *S. lucioperca* with inflated swim bladders, the pneumatic duct begins to atrophy gradually on day 11 after hatching and by day 15 it is completely closed. Thus, swim bladder inflation in this species can occur on day 5 after hatching and can last until days 11 or 12. *S. vitreum* inflate their swim bladders during a similar period of time. Marty et al. (1995) confirmed that the 13-day-old larvae of this species loose the ability to inflate the swim bladder. Thus, any manipulation of environmental conditions or the application of various technical solutions aimed at increasing the percentage of larvae with inflated swim bladders are pointless after day 12 of cultivation (Marty et al. 1995). The relatively short time periods during which physoclistous fish can or must inflate their swim bladders has also been confirmed by Doroshev and Cornacchia (1979), Doroshev et al. (1981), Barrows et al. (1988) and Battaglene and Talbot (1990). It is worth mentioning that the pneumatic duct in larvae with noninflated swim bladders remains open for a much longer period. The current study confirmed that the pneumatic duct was visible until days 24-26 after hatching in *S. lucioperca* larvae with noninflated swim bladders, and in *S. vitreum* until the end of the experiment (day 19 after hatching) (Marty et al. 1995). The long period during which the pneumatic duct, despite its physiological uselessness, is present in specimens with noninflated swim bladders has not yet been explained.

It is known that the swim bladder does not inflate in a significant percentage of physoclistous fish larvae in intense culture. Marty et al. (1995) contends that this phenomenon affects individuals which, at the moment the surface membrane of the mouth breaks and there is an intake of air (the initial phase of swim bladder inflation, Rieger and Summerfelt 1998), ingest organic pollution and accompanying developing microorganisms, from the water surface, which is a potential source of infection. Infection is accompanied by numerous macrophags in the swim bladder; this is confirmed by the present study and the observations of Marty et al. (1995), who noted a large amount of bacteria and remnants of organic material in 13-day-old *S. vitreum* larvae. This same phenomenon was seen to a lesser degree in fish with inflated swim bladders. Thus, it can be contended that the degree of pollution in the surface waters impacts the swim bladder inflation process. Confirmation of this conclusion is provided to some extent by the studies of Kindschi and Barrows (1993), Barrows et al. (1993) and Moore et al. (1994) in which the number of fish incapable of inflating their swim bladders is lessened when surface waters are sprinkled. This technique of water delivery to cultivation basins prevents the accumulation of pollution on surface membranes. This has also been confirmed in *S. lucioperca* (Szkudlarek, unpublished data).

The phenomenon of noninflated swim bladders results not only in larval mortality on a mass scale, but also causes spine deformations (lordosis), lower growth rates and increased cannibalism. The amount of energy larvae with noninflated swim bladders expend on metabolism is much greater than that of larvae with inflated bladders. Their energy needs are surely higher due to the necessity of continuous, active movement through the water. The lack of a properly functioning swim bladder limits their movement which may impose difficulties with the intake of food and decrease their growth rate (Chatain 1986, current study). Limited mobility also renders them easier prey, which may increase cannibalism (Summerfelt 1996). Lordosis was observed among larvae with uninflated swim bladders. This thesis is supported by the results of the authors' studies and those obtained by Kindschi and Barrows (1993), Kitajima et al. (1994) and Summerfelt (1996). Spine deformation is often caused by deficiencies of nucleoproteins and vitamins; however, pathologic changes in larvae with uninflated bladders are different in character (Summerfelt 1996).

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## STRESZCZENIE

ROZWÓJ PĘCHERZA PŁAWNEGO SANDACZA, *SANDER LUCIOPERCA* (L.) W WARUNKACH INTENSYWNEGO PODCHOWU

Celem niniejszej pracy było prześledzenie rozwoju pęcherza pławnego sandacza w warunkach intensywnego podchowu i określenie momentu zaniku przewodu powietrznego. Rozwój pęcherza pławnego obserwowano w trakcie trzydziestodniowego podchowu larw w obiegu recyrkulacyjnym. W tym czasie średnia długość larw wzrosła od 4,28 do około 30,27 mm (rys. 1). Pęcherz pławny wraz z przewodem powietrznym był dobrze widoczny na przekrojach histologicznych przez ciało 4-dniowych larw (fot. 1). W szóstym dniu po wykluciu obserwowano duże zmiany w budowie pęcherza pławnego; pojawiły się zawiązki gruczołu gazowego i owala (fot. 2A i B). W kolejnych dniach, u prawidłowo rozwijających się larw, objętość pęcherza pławnego systematycznie się zwiększała (fot. 6). Powiększyła się również powierzchnia gruczołu gazowego i owala oraz nastąpił rozwój sieci dziwnej (fot. 5). Od 11 do 13 dnia po wykluciu obserwowano stopniowe zarastanie przewodu powietrznego (fot. 3). U ryb z nienapełnionym gazem pęcherzem pławnym przewód powietrzny utrzymywał się dłużej, do 24-26 dnia po wykluciu. Takie pęcherze pławne miały nieregularny kształt i wykazywały zmiany patologiczne. Pierwsze ich symptomy obserwowano już u 6-dniowych larw. W nienapełnionych gazem pęcherzach pławnych stwierdzono obecność makrofagów oraz nadmierny rozrost nabłonka gruczołu gazowego i owala (fot. 2C). Zmiany te pogłębiały się aż do końca trwania eksperymentu (fot. 7). Ponadto larwy sandacza z nienapełnionym pęcherzem pławnym rosły wolniej ($P < 0,05$, rys. 1) i miały zdeformowany szkielet (lordoza) (fot. 4).

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