Arch. Pol. Fish. (2011) 19: 259-265
DOI 10.2478/v10086-011-0032-5

RESEARCH ARTICLE

Marking and return method for evaluating the effects of stocking larval vendace, *Coregonus albula* (L.), into Lake Wigry in 2000-2001

Received – 12 April 2011/Accepted – 10 October 2011. Published online: 30 December 2011;

Paweł Poczyczyński, Krzysztof Kozłowski, Jacek Kozłowski, Andrzej Martyniak

Abstract. In 2000 and 2001, larval vendace, *Coregonus albula* (L.), were marked and released into Lake Wigry. The larvae were immersed in alizarin red S. Of the 19.2 million vendace larvae released in 2000, 2 million were marked (10.4% of the overall number of fish released), and in 2001 of the 18.8 million fish released, 7 million were marked (47.8%). In subsequent years, otoliths were excised from caught vendace and the number of them with alizarin marks was determined. It was assumed that all unmarked specimens came equally from natural spawning and stocking assuming that survival is equal in both forms of recruitment. It was confirmed that 82.4% of the vendace caught from the 2000 generation originated from stocking, while this figure was 64.2% of all specimens caught from the 2001 generation. Lake Wigry hosts the most abundant vendace population in Poland, and this stock spawns on a massive scale annually. Even so, the study described herein provides evidence of just how important systematic stocking is to the maintenance of the vendace population in this lake.

Keywords: vendace, otoliths, alizarin red S, marking and return method, marking coefficient, stocking

P. Poczyczyński [✉], K. Kozłowski, J. Kozłowski, A. Martyniak
Department of Fish Biology and Pisciculture
Faculty of Environmental Sciences and Fisheries
University of Warmia and Mazury
Oczapowskiego 5, 10-719 Olsztyn, Poland
Tel. +48 89 523 32 62, e-mail: k.kozlowski@uwm.edu.pl

Introduction

Marking and return studies are one of the best methods for investigating various aspects of the biology of many fish species. They are particularly useful for identifying population dynamics, migration research, and determining stocking effectiveness (Everhart et al. 1975, Mills and Beamish 1980, Babaluk and Cambell 1987, Hilborn et al. 1990, Meunier 1994, Secor and Houde 1995). Techniques for marking fish have improved vastly in the past fifty years. Earlier methods (e.g., clipping fins, freezing, attaching external tags) are increasingly being replaced by more modern methods such as attaching PIT or microtags with binary or alphanumeric codes. However, these methods cannot be used to mark larval fish because of their small sizes. Evaluating the effects of stocking larvae was basically impossible until a method was developed for marking juvenile fish on a mass scale using, among other methods, chemical agents or thermal shock. Marking is the only method that provides reliable data on the success of reinforcing natural reproduction through stocking in a given basin (Kozłowski 2007). Intense research into marking fish using chemicals was the begun in the 1960s. Two distinctively different methods were developed. One of these methods uses strontium chloride. As strontium is a calcium analogue, it is built into the bone structures of fish (Ophell and Judd 1968, Bagenal et al. 1973). Otoliths are analyzed using atomic mass spectrometry (AMS) and atomic absorption spectrometry (AAS). The second, far more popular marking method is to use fluorochromes, which form chelate complexes with calcium ions that are built into skeletal and otolith structures. Markings are visible under ultraviolet light since the calcium-fluorochrome complexes emit fluorescent light (Beverander and Goss 1962, Monaghan 1993).

The abundance of the family Coregonidae in Poland has been dependent on stocking for many years, and until a certain time, the number of lakes in which these fish occurred increased as a result of the implementation of various management strategies. In the 1958-1969 period, the number of coregonid lakes reached 420, which comprised 62% of the total surface area of Polish lakes. Later, disadvantageous environmental changes, decreased stocking intensity, changes in management strategies, and intense fisheries led to smaller Coregonidae populations, and even their extinction in some basins (Bnińska 1998, Wołos 1998a, 1998b, Wołos and Mickiewicz 1998, Mamcarz and Skrzypczak 2002).

Lake Wigry, with a surface area of 2186.7 ha, is the tenth largest lake in Poland. It is of a mixed trough–morena origin, with a highly varied shoreline and bottom topography. There are 18 islands in the lake with a total surface area of 68.4 ha. The maximum depth is 73 m, which makes Lake Wigry the fifth deepest lake in Poland. The population of vendace, *Coregonus albula* (L.), in Lake Wigry is probably the most abundant in Poland. Its biomass is estimated at 222 kg ha^{-1} (Świerzowski 1999). Currently, annual catches range from 10 to 20 tons. The aim of the present study was to evaluate the effects of stocking Lake Wigry in 2000 and 2001 with marked larvae using the stocking-return method. In order to perform calculations, the marking coefficient (MC), which is described in the Materials and methods section, was applied.

Materials and Methods

The stocking material originated from vendace spawners caught in Lake Wigry. The eggs were incubated at a hatchery (Wigierski National Park, Poland). The first marking of larvae vendace was done on March 10, 2000. Alizarin red S, was used to mark two-day-old larvae. The fish were immersed in an alizarin solution (Tsukamoto 1988, Nagięć et al. 1995). The water supply was shut off to a tank with a volume of 800 dm^3 in which there were 2 million larval vendace; then 170 g of alizarin was dissolved in the water (concentration 200 ppm). The water temperature was 5.3°C. During marking, which lasted for 4 hours, the amount of dissolved oxygen in the water was monitored systematically. After 2 hours, the oxygen concentration decreased to 43.8%, which is why the water in the tank was supplemented to a volume of about 1000 dm^3. After four hours of immersion, water flow was restored in the tank. No increased mortality was noted during marking. It can take up to 14 days for alizarin to chelate with calcium ions. To verify the effectiveness of the marking and determine its quality, 100 vendace larvae were reared for two weeks in an aquarium. Then otoliths were excised from 36 fish, mounted with Entellan Neu, and viewed under a Nikon OPTIPHOT 2 in UV-B at a magnification of 800x. The markings were legible on all of the otoliths viewed. A second marking was performed in the same hatchery on March 19 and 20, 2001 when 9 million vendace larvae were marked. Because of the large number of larvae and the oxygen deficit in the water that occurred in the previous year, this time marking was performed in two rounds and in a greater number of tanks. On the first day, marking was conducted in two tanks, one with a volume of 650 dm^3 and the second with a volume of 450 dm^3. The water temperature was 5.9°C. Again, alizarin S was used at a concentration of 225 ppm. Marking was completed after three hours. The oxygen content during the procedure did not decrease below 9.0 mg dm^{-3}, which was about 74% saturation. On the second day, marking was performed in one tank with a volume of 650 dm^3 and in three with a volume of 450 dm^3. The water temperature was 6.3°C. Immersion time in the alizarin solution at a concentration of 200 ppm was three hours. The concentration of oxygen in the water remained above 71%. After the conclusion of marking, 100 vendace larvae from each tank were held for rearing. After 16 days the otoliths were excised and examined using the same procedures applied to the fish in 2000. Brightly shiny marks were confirmed in all 124 of the otoliths examined. In 2000, Lake Wigry was stocked with 19.2 million vendace larvae, of which 10.4% had been marked. In the following year, 18.8 million larval vendace were released, of which 47.8% were marked.

The material analyzed came from commercial and experimental catches conducted in 2002-2005 (Table 1).

Table 1

Number of otoliths obtained from the 2000 and 2001 generations of vendace by age group

	Age group					
Generation	1+	2+	3+	4+	5+	Total
2000	17	299	61	8	13	398
2001	58	346	30	14	-	448

The otoliths examined came from fish of the 2000 and 2001 generations. The age of the fish was determined based on scale readings. The otoliths chosen for examination were mounted on glass slides with Entellan Neu. After a week they were, polished with fine-grade 1000 and 1200 waterproof sandpaper. One-sided polishing was applied to the otoliths of young fish aged 1+ to 3+. The otoliths of older fish were polished on both sides. During polishing, the otoliths were examined under a light microscope at a magnification of 400x. Polishing was stopped once the center of the otolith, where the mark had been recorded, was exposed. Processing the material required precision and great care since the otoliths are fragile and are easily damaged. Additionally, the otolith center is convex, and excessive polishing can cause it to collapse. During polishing, 112 otoliths were damaged, including all of those from fish aged 5+, 15 from fish aged 4+, 12 from fish aged 3+, and 72 from fish aged 2+. Of the remaining 734 otoliths, 76 were illegible, thus 658 otoliths were used to analyze the alizarin marks in legible otolith centers. The prepared materials were examined under a Nikon OPTIPHOT 2 microscope in UV-B at a magnification of 200-800x.

Estimating the effects of stocking during which only a portion of the material released was marked required introducing the marking coefficient parameter (P), which expresses the ratio of fish marked to all vendace produced through artificial breeding and released into a given lake. In 2000 this parameter was 9.61 (100%:10.4%), while in 2001 it was 2.09 (100%:47.8%).

Assuming that mortality in marked and unmarked hatch is equal (Czerkies 1998), it was possible to calculate the percentage of catches that originated from stocking. To do this, the marking coefficient was multiplied by the share of marked fish in the catches.

Results

Of the total number of fish stocked into Lake Wigry in 2000, otoliths from 312 specimens were read, while in 2001, the otoliths from 346 vendace specimens were read (Table 2).

Table 2

Number of otoliths analyzed microscopically from vendace from the 2000 and 2001 generations by age groups

	Age group			
Generation	1+	2+	3+	Total
2000	17	243	52	312
2001	58	276	12	346

The best quality marks were noted in fish aged 1+. In older fish, while the marks on the otoliths were less visible, they were still distinct enough to exclude identification error. Among the 312 otoliths examined from the 2000 generation, 27 (8.6%) had distinct alizarin marks, with the most (22) noted in the 2+ age group. This comprised 9% of the otoliths analyzed in this age group. Among the fish aged 1+ and 2+ examined, marked otoliths were noted in 2 and 3 fish or in 1.8 and 5.8%, respectively (Table 2). Of the 346 otoliths examined from the 2001 generation, 109 alizarin marks were confirmed (31.5%). Similarly, the most marks were noted in fish aged 2+ (85 individuals, which was 37.9% of the whole sample). The number of marked otoliths from fish aged 1+ and 3+ was 22 and 2, which was 30.8 and 16.7%,

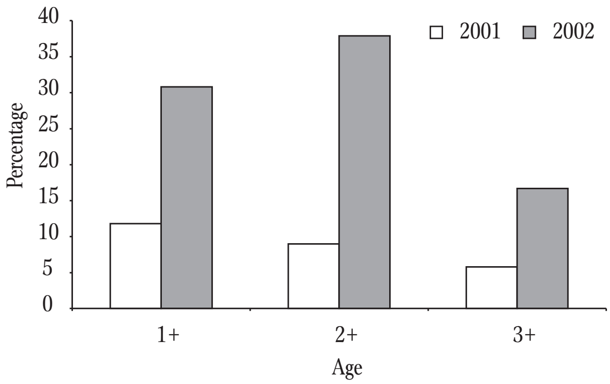

Figure 1. Share (%) marked larval vendace in the entire sample and by age group.

respectively, of the analyzed individuals in these age groups (Fig. 1; Table 2).

The proportions between the share of marked larvae released into the lake and the share of marked otoliths in the material collected were similar. In the generation from 2000, the share of marked larvae was 10.4%, and the share of marked otoliths was 8.6%. Based on the proportion of marked fish to the number of fish caught, 82.4% of the vendace caught came from stocking. In the 2001 generation, marked larvae comprised 47.8%, and the share of marked otoliths was 31.5%. The share of vendace from the 2001 generation was 64.2%.

Discussion

Stocking Polish lakes with coregonids has been the focus of many studies of the effectiveness of this measure as well as the general principles for the management of these species (Falkowski 1998). In Poland, the most common type of vendace stocking material is freshly-hatched larvae. The share of vendace summer fry in stocking is slight and, according to Wołos (1998a) it comprises barely 0.4% of all the stocking material released into 123 basins that he analyzed. Lake Wigry has been and continues to be stocked exclusively with larvae. The average number of larvae released into this lake in the 1977-2004 period was 15.8 million larvae annually (7241 larvae ha^{-1}; Kozłowski 2007), which is nearly twice the average amount of stocking material released other Polish lakes at 3860 larvae per ha^{-1} (Wołos 1998b). Larval vendace were released into Lake Wigry systematically, and the stocking frequency coefficient was 1, which indicates annual stocking. According to data published by Leopold (1998a), coefficient values above 0.7 were noted in only 2.6% of the lakes analyzed in Poland.

The statistical analysis of vendace stocking and catches in Lake Wigry did not indicate significant dependencies between these variables (Kozłowski 2007). In this study, the analyses of correlations between stocking and catches one and two years following stocking did not exhibit any significant statistical correlations between these variables. No statistically significant dependencies in the analysis of correlations between stocking, taking into consideration the two and three year moving variables, and catches were noted. While the lack of statistically significant dependencies between stocking and catches of vendace have been confirmed in many lakes, this does not provide evidence that stocking is ineffective. The lack of correlations can be explained by the great variability in the amounts of stocking material released, breaks in stocking, the application of various kinds of stocking materials, the success of natural spawning, and, as is the case in Lake Wigry, low levels of exploitation (Kozłowski 2007). According to a study by Leopold (1998b) conducted on 60 vendace lakes in Poland, the mean number of larvae released that corresponded to 1 kg of commercial vendace ranged from 720 to 2,138 individuals depending on the basin. During this study period, Lake Wigry, with 1,273 larvae released per kg of vendace caught, was classified as a lake with medium stocking effectiveness.

Applying fluorescent markers using the immersion technique is a relatively simple procedure that is possible to perform in virtually any hatchery. Its advantages include the low cost and the possibility of marking large numbers of fish in early developmental stages. While the marking procedure is performed without any great difficulty, reading fish otoliths requires sacrificing the fish and specialist equipment such as a fluorescent microscope. Preparing the otoliths is also a labor-intensive task. Additionally, otoliths are often damaged while being polished, which excludes them from further analysis. While preparing 423 otoliths for analysis, Czerkies (1997, 1998) discarded 233 as damaged ones with illegible centers; this comprised more than 50% of the material that had been collected. The number of damaged otoliths in the current study was significantly lower; because the likelihood that the otolith would be damaged was high, the second otolith was always collected from the same fish.

In experiments with marking fish later released into open basins, an important issue is the permanence of the markings. The results of the current study indicate that alizarin marks remain visible in fish otoliths for many years. Tsukamoto (1995) observed in *Pagrus major* (Temminck and Schlegel) marks five years after marking, while in grayling, *Thymallus thymallus* (L.), marks were confirmed to be at least two years old, and brown trout, *Salmo trutta* L., retained otolith marks for three years (Nagięć et al. 1995). In the case of vendace from lake Wigry, otolith marks were noted in fish aged 3+, but it is plausible that the marks remain visible for at least four years.

Studies conducted to date on fish markings indicate that as time passes the marks in a certain number of fish fade. According to Muth and Bestegen (1991) this phenomenon occurs in the youngest fish and is linked to ultraviolet solar radiation. However, Eckmann et al. (1996), in a study of larval vendace marked with alizarin did not report the subsequent loss of marks, and in a control group of vendace held for a year, all of the fish retained markings.

The technique developed in Poland for marking larvae with fluorochromes can be applied to many different fish species: whitefish; vendace; grayling; pike, *Esox lucius*, L.; rainbow trout, *Onchorhynchus mykiss* (Walbaum); peled, *Coregonus peled* (Gmelin); pikeperch, *Sander lucioperca* (L.); common bream, *Abramis brama* (L.); Wels catfish, *Silurus glanis* L. Studies of the effects of stocking have been performed with the first four species listed (Goryczko et al. 1994, 1998, Nagięć et al. 1995, 1998). Czerkies (1998) conducted an experiment in which Lake Garbaś Duży was stocked with two types of vendace stocking material: hatch (300,000 individuals) and summer fry (15,000 individuals). The larvae were marked with alizarin, while the summer fry were marked with tetracycline. Of the 170 fish caught, the author confirmed alizarin marks in 4.5% of the individuals, while no marks were noted in the other individuals, which was explained by the strong autofluorescent, amphoric otolith centers. However, these did fish exhibit daily increment patterns on their otoliths that are typical of stocking and which indicated that they originated from summer fry stocking.

Stocking larval vendace marked with alizarin red S into Lake Werbellin (Germany) showed how effective natural spawning is there (Eckmann et al. 1998). Among the 1072 individuals examined, only 1.3% of the fish had marked otoliths. In the studies in Lake Wigry indicated quite the opposite situation. The share of marked fish was higher at 8.6% in the 2000 generation and 31.5% in the 2001 generation, which, when recalculated, indicated a high share of fish from stocking in the catches at 82.4% and 64.2%.

The results of the study described above clearly confirm the view that the occurrence of vendace in Polish lakes is mainly because of stocking programs. Even the exceptionally abundant population of this species in Lake Wigry, despite its effective natural reproduction, is supported largely by stocking programs. It is plausible that if stocking were discontinued, vendace would disappear from this lake as it did in the early 1900s.

References

Babaluk J.A., Cambell J.S. 1987 – Preliminary results of tetracycline labelling for validating annual growth increments in opercula of walleyes – N. Am. J. Fish. Manage. 7: 138-141.

Bagenal T.B., Mackereth F.J.H., Heron J. 1973 – The distinction between brown trout and sea trout by the strontium content of their scales – J. Fish Biol. 5: 555-557.

Bevelander G., Goss R.J. 1962 – Influence of tetracycline on calcification in normal and regenerating teleost scales – Nature (London) 193: 1098-1099.

Bnińska M. 1998 – Coregonid management and environmental quality – In: Coregonid management. Conditions and effectiveness (Eds) A. Wołos, M. Bnińska, Wyd. IRS, Olsztyn: 29-43 (in Polish).

Czerkies P. 1997 – Comparing effects of stocking Lake Garbaś Duży with vendace hatch and fry – In: Proc. II Conference for Lake Fisheries. Significance and management, Wyd. IRS, Olsztyn: 105-109 (in Polish).

Czerkies P. 1998 – Comparing effects of stocking Lake Garbaś Duży with vendace hatch and fry – In: Coregonid management. Conditions and effectiveness (Eds) A. Wołos, M. Bnińska, Wyd. IRS, Olsztyn: 81-87 (in Polish).

Eckmann R., Czerkies P., Helms C., Kleibs K. 1998 – Evaluating the effectiveness of stocking vendace (*Coregonus albula* (L.)) eleutheroembryos by alizarin marking of otoliths – Arch. Hydrobiol., Spec. Issues Advanc. Limnol. 50: 457-463.

Everhart W.H., Eipper A.W., Youngs W.D. 1975 – Fish marking – In: Principles of Fishery Science (Eds) W.H., Everhart, W.D. Young, Cornell University Press, Ithaca, New York: 141-216.

Falkowski S. 1998 – Effectiveness of whitefish (*Coregonus lavaretus* L.) management in lakes with the highest yields of this fish in Poland – Arch. Pol. Fish. 6: 361-379.

Goryczko K., Kowalewski M., Czerkies P., Witkowski A. 1994 – Evaluation of the effectiveness of stocking grayling hatch in streams inhabited by natural populations of this species – Komun. Ryb. 5: 12-13 (in Polish).

Goryczko K., Nagięć M., Witkowski A., Murawska E. 1998 – Stocking Pomeranian rivers with grayling, *Thymallus thymallus* (L.), summer fry based on the example of the Radunia – Rocz. Nauk. PZW 11: 81-85 (in Polish).

Hilborn R., Walters C.J., Jester D.B. 1990 – Value of fish marking in fisheries management – Am. Fish. Soc. Symp. 7: 5-8.

Kozłowski K. 2007 – Characteristics of vendace (*Coregonus albula* L.) populations from Lake Wigry and selected issues in managing this species – Department of Fish Biology and Culture, UWM Olsztyn, PhD Thesis (in Polish).

Leopold M. 1998a – Link between the effectiveness of vendace management and stocking frequency – In: Coregonid management. Conditions and effectiveness (Eds) A. Wołos, M. Bnińska, Wyd. IRS, Olsztyn: 23-28 (in Polish).

Leopold M. 1998b – Vendace management effectiveness and morphometric characteristics of lakes – In: Coregonid management. Conditions and effectiveness (Eds) A. Wołos, M. Bnińska, Wyd. IRS, Olsztyn: 17-22 (in Polish).

Mamcarz A., Skrzypczak A. 2002 – Coregonids in the Great Masurian Lakes – In: Biology and Management of Coregonid Fishes (Eds) T. Tood, G. Fleisher – Arch. Hydrobiol., Spec. Issues Advanc. Limnol. 57: 157-169.

Meunier F.J. 1994 – Données sur la croissance de l'anguille (*Anguilla anguilla* L.) dans le cours moyen du Rhin, region Alsacienne – Bull. Fr. Pęche Piscic. 335: 133-144.

Mills K.H., Beamish R.J. 1980 – Comparison of fin - ray and scale age determinations for lake whitefish (*Coregonus clupeaformis*) and their implications to estimates of growth and annual survival – Can. J. Fish. Aquat. Sci. 37: 534-544.

Monaghan J.P. 1993 – Comparison of calcein and tetracycline as chemical markers in summer flounder – Trans. Am. Fish. Soc. 122: 298-301.

Muth R.T., Bestgen K.R. 1991 – Effects of sunlight on tetracycline – Trans. Am. Fish. Soc. 113: 370-373.

Nagięć M., Czerkies P., Goryczko K., Witkowski A., Murawska E. 1995 – Mass - marking of grayling, *Thymalus thymalus* (L.), larvae by fluorochrome tagging of otholiths – Fish. Manage. Ecol. 2: 185-195.

Ophell I.L., Judd J.M. 1968 – Marking fish with stable strontium – J. Fish. Res. Bd. Can. 25: 1333-1337.

Secor D.H., Houde E.D. 1995 – Larval mark - release experiments: potential for research on dynamics and recruitment in fish stocks – In: Recent developments in fish otolith research (Eds) D.H. Secor, J.M. Dean and S.E. Campana, Univ. South Carolina Press: 423-444.

Świerzowski A. 1999 – Locating and estimating stocks of pelagic fish using acoustic catch methods in selected lakes in Wigry National Park – In: Functioning and protection of aquatic ecosystems in protected areas (Eds) B. Zdanowski, M. Kamiński, A. Martyniak, Wyd. IRS, Olsztyn: 535-555 (in Polish).

Tsukamoto K. 1988 – Otolith tagging of ayu embryo with fluorescent substances – Nippon Suisan Gakk. 54: 1289--1295.

Tsukamoto K. 1995 – Use of otolith - tagging in a stock enhanecement program for masu salmon (*Oncorhynchus masou*) in the Kaji River, Japan – In: Recent developments in fish otolith reserch (Eds) D.H. Secor, J.M. Dean, S.E. Campana, Univ. South Carolina Press: 403-422.

Turkowski K. 2002 – Economic conditions and the effectiveness of stocking lakes with coregonid fishes – Rozprawy i Monografie, 68. Wyd. UWM Olsztyn, 86 p. (in Polish).

Wołos 1998a – General characteristics of coregonid management in 132 Polish lakes – Arch. Pol. Fish. 6: 265-278.

Wołos A. 1998b – Coregonid management and species succession – In: Coregonid management. Conditions and effectiveness (Eds) A. Wołos, M. Bnińska, Wyd. IRS, Olsztyn: 24-27 (in Polish).

Wołos A., Mickiewicz M. 1998 – Characteristics of lakes and management of vendace and whitefish – In: Coregonid management. Conditions and effectiveness (Eds) A. Wołos, M. Bnińska, Wyd. IRS, Olsztyn: 9-15.

Streszczenie

Zastosowanie metody znakowanie – zwroty do oceny efektów zarybień jeziora Wigry larwami sielawy, *Coregonus albula* (L.) w latach 2000-2001

W roku 2000 i 2001 przeprowadzono znakowanie w alizarynie S larw sielawy, *Coregonus albula* (L.), przeznaczonych do zarybienia jeziora Wigry. Spośród 19,2 mln larw sielawy w 2000 roku poznakowano 2 mln (10,4% całkowitej ilości wpuszczonych ryb) a w roku następnym z 18,8 mln ryb, poznakowano 7 mln (47,8%). W kolejnych latach od wszystkich odłowionych sielaw pobierano otolity i ustalano ile z nich posiadało znaczek alizarynowy. Założono, że wszystkie osobniki niepoznakowane pochodzą, w równym stopniu, tak z naturalnego tarła, jak i z zarybień, przyjmując podobną przeżywalność ryb z obu źródeł rekrutacji. Stwierdzono, że w odłowach sielaw z pokolenia 2000 ryby z zarybień stanowiły 82,4%, a z pokolenia 2001 64,2% wszystkich odłowionych osobników. Jezioro Wigry jest zbiornikiem o największej liczebnie populacji sielawy w Polsce, w którym co roku odbywa się jej masowe tarło. Jednak wyniki opisywanych badań świadczą o niezbędności systematycznych zarybień dla utrzymania populacji sielawy w jeziorze.