ЮБИЛЕЙНА НАУЧНА КОНФЕРЕНЦИЯ ПО ЕКОЛОГИЯ (СБОРНИК С ДОКЛАДИ)
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EXPERIMENTAL APPLICATION OF VISIBLE ELASTOMER IMPLANTS FOR TAGGING OF PUMPKINSEEDS (*Lepomis gibbosus* L.)

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Abstract. Experimental laboratory tagging of 120 pumpkinseed sunfish, *Lepomis gibbosus* L. with subcutaneous injection of a fluorescent elastomer was done. Visible Implant Elastomers (VIE, Northwest Marine Technology, Shaw Island Washington, WA, USA) was used. Tag position was under anterior part of the dorsal fin. Three colours fluorescent elastomers were investigated: yellow, red and orange. Pumpkinseeds mortality rate, length and weight growth were evaluated for a period of 112 days. Tag retention and visibility on the end of the experiment was assessed. Results show that VIE is an effective tagging method and had no negative effects on pumpkinseed growth and surviving.

Key words: Tagging, Visible Implant Elastomer*, Lepomis gibbosus,* growth, tag retention

INTRODUCTION

Pumpkinseed*, Lepomis gibbosus* L. is a freshwater fish originated from North America (SKOTT & GROSSMAN, 1973). It was introduced in Europe as a potential sport and garden fish about a hundred years ago (KÜNSTLER, 1908; HOLČIC, 1991; WELCOMME, 1992). In Bulgarian inland waters comes via the Danube River. It is widespread due to its extreme adaptability in novel environments. *L. gibbosus* inhabits the shallow water bodies and slow running rivers with soft bottoms and submerged vegetation. The native North American pumpkinseeds have reached a maximum length about 40 cm. Non-indigenous European population have slower growth rate and smaller maximum sizes (COPP & FOX, 2007). Its food consists mainly from benthos, especially Chironomidae (NIKOLOVA *et al.*, 2008). Pumpkinseed is a multispawning fish with male parental care (BALON, 1975). Because particular life-history traits, pumpkinseed is defined as a potential invasive species. Monitoring of spreading and assessment of population size in the new habitats are extremely important. In order to obtain such biological information, it is necessary to identify individual or groups of fish. Common techniques in fish investigation involve the use of external or internal marks. Such tags should have no effect on mortality, behaviour, growth and reproduction of the marked species. Also, tags should be easily recognized and should be retained for the long time (BERGMAN *et al*., 1992). One tagging method that shows considerable potential is a visible implant fluorescent polymer (Northwest Marine Technology, Shaw Island Washington, WA, USA). This tag is injected subcutaneously using a small needle in a variety of body positions where form a permanent, easy recognizable and non-toxic mark. This system can be used for individual or batch marking (BRENNAN *et al*., 2001). Visible implant elastomers (VIE) are applicable in laboratory and field conditions. VIE has been used to tag many fish species (FREDERICK J.L. 1997; BAILEY *et al*., 1998; CATALANO *et al*., 2001; OLSEN & VOLLESTAD, 2001; JENSEN *et al.,* 2008), amphibians and crayfishes (VASCONCELOS & CALHOUN, 2004; MAZLUM, 2005).

The aim of the present study was to assess the potential impact of VIE tagging on the growth and surviving of pumpkinseed, *L. gibbosus* in laboratory experiment. As well, was tested the rate of tag loss and their visibility over 110-day period.

MATERIAL AND METHODS

Experimental design

A total of 137 pumpkinseed sunfish, *L. gibbosus* were used for the experiment. 114 fish were tagged and 23 were used as a control.

Fish were collected from quarry lakes near by Sofia city (West Bulgaria) by traps and angling. After 10-day acclimatization period, fish were anesthetized with 0.01 % oil of cloves and than marked. After tagging fish were distributed into three 110-L aquariums. A constant water temperature (12-18 ^{o}C) and oxygen level (7.0-8.0 mgL^{-1}) were maintained. Pumpkinseed was reared for 112 days with granulated forage for Salmonids.

Individual weight (W, g) and length (TL, cm) of the fish were measured. To assess the impact of tagging on the growth, the lengths and weights of marked and control fish were compared with t- test at the beginning and end of experiment. The rate of mortality was measured as the cumulative percentage of dead fish per group. Tag retention rate was also considered as the cumulative percentage of fish that lost whole tags at the end of experiment.

Tagging process

Three colored (yellow, orange, red) fluorescent elastomer implants was used. The polymer consisted from two components, which were mixed just before work in proportion 1:1. The elastomer mark was subcutaneously injected with a 1mm hypodermic needle, in the left side under the anterior part of dorsal fin (Fig. 1). The average length of the marker was 5 mm.

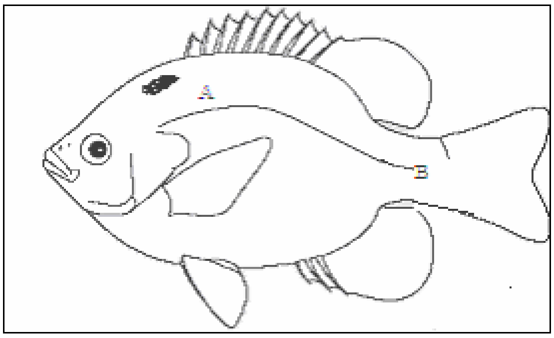

Fig. 1. *Tag location of tag on the pumpkinseed sunfish*, *Lepomis gibbosus.*

RESULTS AND DISCUSSION

Our study has found that tag visibility didn't change during all experimental period. No deterioration in tag quality or fragmentation can occur. Tag can be detected by naked eye, but tag visibility was increased significantly when blue LED light was used (JONES, 2002). Tag detection was very easy with using UV light passed through fish body (Fig. 2). It was found that orange and red fluorescent colours, tested in this study were more detectable, because of the blue-greenish colored pumpkinseeds body. HALE & GRAY (1998) also found that red was the most easily detectable tag colour in rainbow trout. The main reasons for the decreasing of the marks visibility was expansion of the tissue around the marker (OLSEN & VOLLESTAD, 2001) or tag fragmentation caused by muscular growth (MORGAN & PAVELY, 1996). Otherwise, in natural environment the brightly colors can increase predation on tagged fish (CATALANO *et al*., 2001). For VIE tags, body location significantly influenced mark retention and visibility (BRENNAN *et al*., 2005). The application of tags under dorsal fin allowed easily visual observation. This tag position allowed us marking very small fish – up to 0.2 g. VIE tags were successfully used in very small fishes and even fish larvae (FREDERICK, 1997; BAILEY *et al*., 1998; OLSEN & VOLLESTAD, 2001; JENSEN *et al.,* 2008).

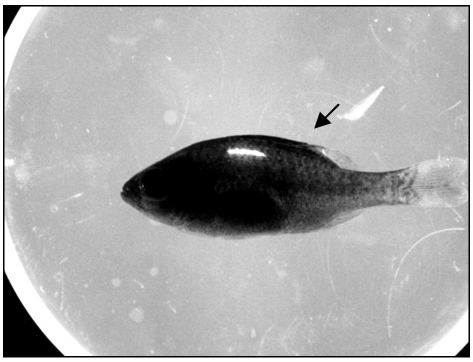

Fig. 2. *Visibility of fluorescent elastomer implant under UV – light.*

Percentage of the tag loss at the end of experimental period was 10.5 (Table 1). This result was relatively high compare to some other studies that used the same tagging system (HALE & GRAY, 1998; WILLIS & BABCOCK, 1998; GRIFFITHS, 2002). DEWEY & ZIGLER (1996) reported 99% tag retention after six months period of field and laboratory tests with adult fish *Lepomis macrochirus*. However, tag loss was higher in small sized fish from the same study. The highest loss rate reported was 27% over 2 years, with immediate losses of 3-7% over 24h (BAILEY *et al*., 1998). One of the crucial factors for tag loss is a lack of experience in tag application. Incorrect tag injection and body moving, causing uncured elastomer to be ejected trough the application hole (BUCKLY *et al*., 1994).

During the experimental period, the accumulated mortality for tagged group reached 11.4%. Mortality in control group was significantly higher (17.4%)(P>0.05). The observed mortality was probably not due to the VIE tag. Most probable reasons were high density of reared fish and food competition. A high mortality rate associated with tagging procedures was been found in a study of reef fish, with rates up to first 24 hours (ASTROGA *et al*., 2005). Almost all other studies using VIE have found that mortality caused by tagging was zero or not significantly different from that of control fish in such species as bluegills (*Lepomis macrochirus*) and snapper (*Pagrus auratus*) (DEWEY & ZIGLER, 1996; WILLIS & BABCOCK, 1998; OLSEN & VOLLESTAD, 2001).

Table 1. *Tag loss and mortality rates of the experimental and control groups.*

Groups	n	Tag loss rate (%)	Dead fish	Mortality rate (%)
Experimental	114	10.52	13	11.4
Control	23	-	4	17.39

On Fig. 3 was presented data of weight measuring of control and experimental group. At the beginning of the study, the mean individual weight of experimental animals (5.85 ± 0.5 g) did not differ significantly from that of control group (5.15 ± 0.8 g)(P>0.05). At the end of 112-day period the mean increase in weight for both groups was about 1 g. There was no significant difference in tagged and untagged individuals (P>0.05).

The similar results were obtained from body length measurements (Fig. 4). No statistical differences were found between experimental and control group according their mean length in the beginning and end of study (P>0.05). Other studies with fishes and crustaceans found no influence of these tag types on growth performance of animals (DEWEY & ZIGLER, 1996, HUGHES *et al*., 2000; OLSEN & VOLLESTAD, 2001; ASTROGA *et al*., 2005). Only in some cases was observed decreased growth rate in tagged group (WOODS & MARTIN-SMITH, 2004).

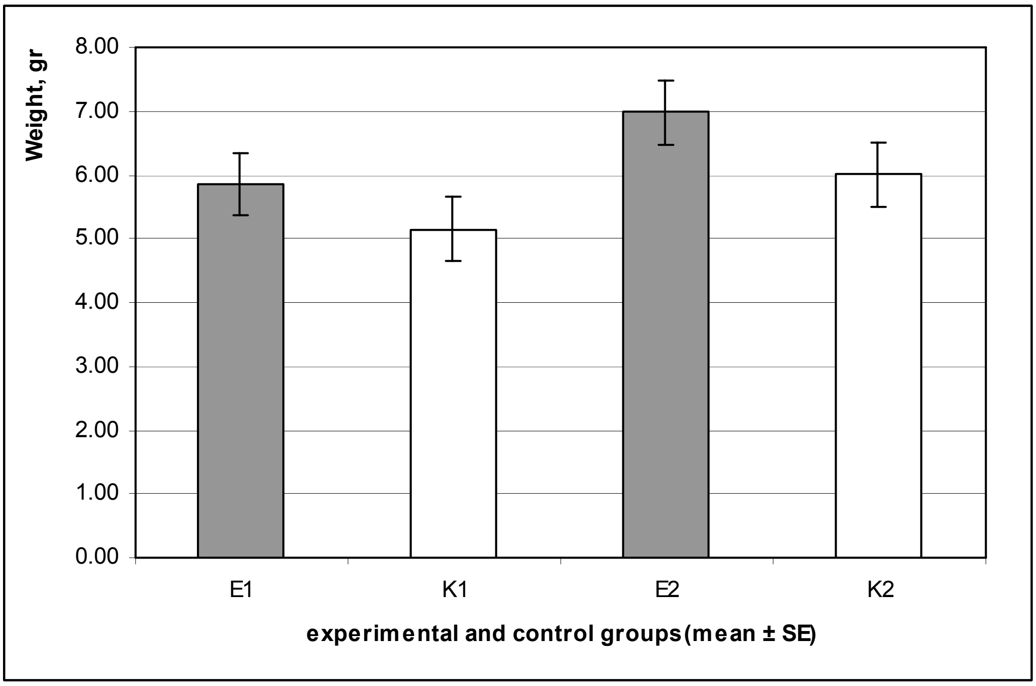

Fig. 3. *Mean weight (W, g) of the experimental and control fish at the beginning and end of study. Marks: E1- experimental group before tagging; E2-experimental group after tagging; K1-control group at the beginning of study; K2-control group at the end of study.*

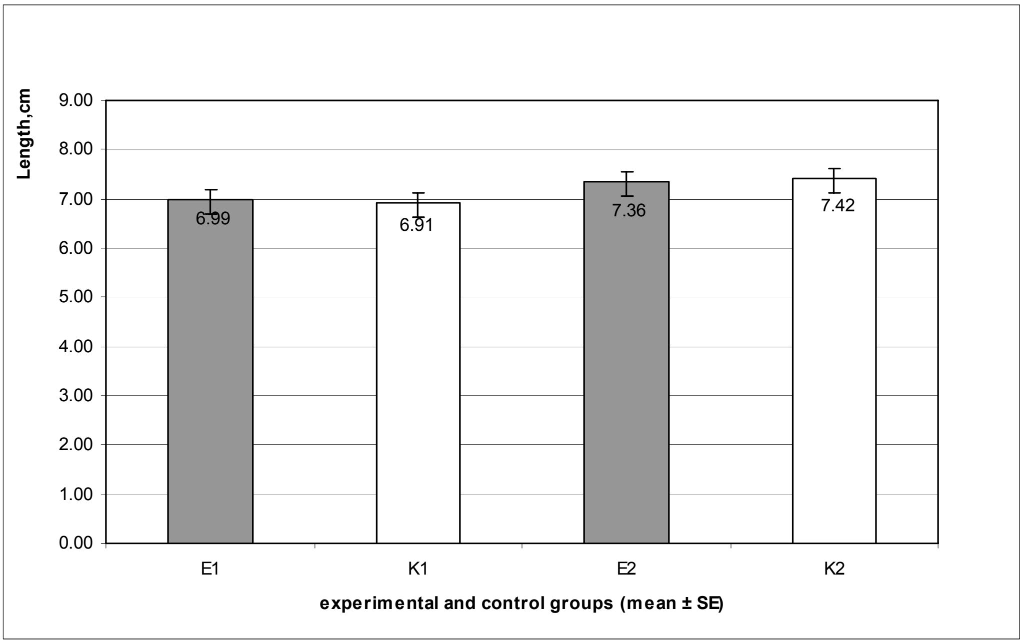

Fig. 4. *Mean length (L, cm) of the experimental and control fish at the beginning and end of study. Marks: E1- experimental group before tagging; E2-experimental group after tagging; K1-control group at the beginning of study; K2-control group at the end of study.*

CONCLUSIONS

Overall we found that visible implant elastomer were useful tool for tagging of pumpkinseeds, *Lepomis gibbosus* with small size. Tag colour influenced tag visibility and red and orange elastomer tags were recommended. No significant effect of tagging on growth and mortality rates was observed. Tag retention is relatively high and probably depends on experience of the person applying the tags.

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ЕКСПЕРИМЕНТАЛНО ПРИЛОЖЕНИЕ НА ЕЛАСТОМЕРНИ ИМПЛАНТИ ЗА МАРКИРАНЕ НА РИБИ ОТ ВИДА *Lepomis gibbosus* L.

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(Резюме)

Осъществено е маркиране на 120 броя риби от инвазивния вид слънчева рибка *Lepomis gibbosus* L. чрез субкутанно инжектиране на елестомерни импланти (Visible Implant Elastomers, Northwest Marine Technology, Shaw Island Washington,WA, USA). Маркирането е осъществено в две позиции и с три цвята флуоресциращи еластомери. Проследено е в 90 дневен експериментален период влиянието на еластомерите върху оцеляемостта на рибите, тяхното тегловно и линейно нарастване. Оценени са промените в състоянието и видимостта на маркировката в края на експеримента. Дискутират се възможностите за определяне на числеността, биомасата на рибите в полеви условия при маркиране с еластомерните импланти.