USE AND EFFECT OF FREEZE BRANDING ON ROACH (*RUTILUS RUTILUS* L.)

G. EVRARD

Unité de Recherches en Biologie des Organismes, Facultés Universitaires Notre-Dame de la Paix, 61 rue de Bruxelles, 5000 Namur, Belgique.
Courriel : geoffroy.evrard@fundp.ac.be

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ABSTRACT

We tested the effects of freeze branding on growth, survival and mark retention in roach *Rutilus rutilus* L. (82-268 mm in total length). Markings were applied at two positions on the skin: (1) the depressed area above the anal fin, (2) the muscles above the lateral line and between the caudal and dorsal fins. A control group received no mark. The experiment duration was 30 days, and 120 roach were selected and distributed into three lots. Retention rates were high and ranged between 94% for position 2 and 100% for position 1. Survival rate after 1 month was 87% for freeze branded roach at position 2, 90% for position 1 and 92% for unmarked fish. These differences were not statistically significant. A significant decrease of linear growth was observed for individuals marked at position 2 (3.2 mm.month^{-1}), but the implication of the brand itself was unclear. We discuss the short- and long-term properties of freeze branding techniques on roach.

Key-words: Marking technique, Retention, Survival and Growth rates, Cyprinids.

UTILISATION ET EFFETS DU CRYOMARQUAGE SUR LE GARDON (*RUTILUS RUTILUS* L.)

RÉSUMÉ

Nous avons étudié les effets du cryomarquage sur la croissance et la survie du gardon *Rutilus rutilus* L. (82-268 mm longueur totale) ainsi que le taux de rétention. Deux zones de marquage sur le poisson ont été choisies : (1) la zone légèrement renfoncée au-dessus de la nageoire anale, (2) la zone au-dessus de la ligne latérale, entre les nageoires dorsale et caudale. Un groupe contrôle ne fut pas marqué. La durée du test était de 30 jours, et 120 gardons ont été choisis et distribués dans les 3 lots. Les taux de rétention étaient élevés et variaient entre 94 % pour la position 2 et 100 % pour la position 1. Le taux de survie après un mois était de 87 % pour les gardons cryomarqués à la position 2, de 90 % pour ceux marqués à la position 1 et de 92 % pour les poissons non marqués. Les différences n'étaient pas significatives. Une diminution significative de la croissance linéaire apparut chez les individus marqués à la position 2 (3,2 mm.mois^{-1}), mais l'influence du marquage en lui-même n'est pas certifiée. Nous avons discuté des propriétés à court- et long-terme de la technique de cryomarquage appliquée au gardon.

Mots-clés : Technique de marquage, taux de rétention, survie et croissance, Cyprinidae.

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INTRODUCTION

Marking is an essential technique for ichthyologists and managers of halieutic resources. Marking programs can provide a wealth of information (migration, growth, density, etc.) about either individual fish or entire populations, depending on the selection of marks (LUCAS *et al*., 1998). The most familiar application is undoubtedly the mark-recapture estimate of abundance (NIELSEN, 1992). Mark-recapture studies of freshwater fishes (cyprinids and salmonids) use a variety of marking or tagging techniques. All have advantages and drawbacks (HERBINGER, NEWKIRK and LANES, 1990; PRIGNON and MICHA, 1996; LUCAS and BARAS, 2000), but external marks are the easiest to apply and probably the most widely used marks for short-term and geographically restricted projects (MCFARLANE, WYDOSKI and PRICE, 1990).

External tags may cause some undesirable effects, like the dorsal Carlin tag which decreases survival rate in sea trout *Salmo trutta trutta* (FOURNEL, EUZENAT and FAGARD, 1990) and like external transmitters which probably cause mortality in common carp *Cyprinus carpio* (ØKLAND *et al*., 2003). Internal marks also cause problems: coded microwire tags, widely used on a large variety of fishes (BEUKERS, JONES and BUCKLEY, 1995), rarely induce serious effects on growth and survival, but problems have been reported with species-specific differences in tag retention (KLAR and PARKER, 1986; BUMGUARDNER, COLURA and MATLOCK, 1992) and alteration of the behaviour (HABICHT *et al*., 1988) associated with tagging. Several studies have demonstrated that marking/tagging to wild and hatchery-reared “salmon” has a negative effect on survival (HANSEN, 1988; MOFFETT, CROZIER and KENNEDY, 1997), in part attributable to the tag itself, but also reflecting the combined impacts of capture, handling and anaesthetisation (HANSEN and JONSSON, 1988).

Whatever the mark selected, the main assumptions must be dealt with in order to conduct a successful study (NIELSEN, 1992; LUCAS and BARAS, 2000), and the most important one is that it doesn’t affect growth, mortality, and behaviour.

To carry out an estimate of density and biomass of roach in a part of the lowland river Meuse, we have focused our choice on freeze branding, first, for the following reasons: it is an easy and quick procedure for marking a large number of animals; it has little apparent effect on the fish (BRYANT *et al*., 1990); it is suitable for all sizes-classes; it is adapted to a short-term study and has a relatively moderate cost (BOURGEOIS, O’CONNEL and SCOTT, 1987; NIELSEN, 1992).

Freeze branding is widely used for marking small salmonids (FAY and PARDUE, 1985; FOURNEL *et al*., 1990; KNIGHT, 1990), channel catfish *Ictalurus punctatus* (BROCK and FARRELL, 1977), and walleyes *Stizostedion vitreum* (LAJEONE and BERGERHOUSE, 1991), but it has not been widely tested and used on cyprinids like roach, our target species.

A suitable mark does not alter the fish physiology, behaviour and survival, and will be easily identified. Meeting these assumptions is particularly challenging for studies and, in this way, testing freeze branding on a sample of fish will assess marking-induced mortality and retention rates and prove the effectiveness of this technique for short-time studies on cyprinids.

In this study, we tested the effects of freeze branding on survival, growth and mark retention in roach. The purpose was also to identify an optimum position for brand positioning. The findings of the present experiment were prerequisites for the design of a field mark-recapture study to determine the dynamic parameters of roach population in the river Meuse in the summer of 2002 (EVRARD and MICHA, 2003).

MATERIAL AND METHODS

General procedures

The freeze branding apparatus (Figure 1) used in this study consisted of a 10-L reservoir of liquid nitrogen (N_2), enclosed in an isolated wood box for easier transport and security, and a metal hose (15 cm long, 35 mm ∅), with a tap, extending through a lateral aperture on a wall of that box. Two detachable branding irons, which are engraved with distinct symbols (5 to 10 mm long), can be fastened to the system like an iron pin. Two or three minutes after the tap is opened, one flant of the fish is held against the iron for approximately 4 s, for marking. Brands become visible on the fish within an average of 2-3 days after marking.

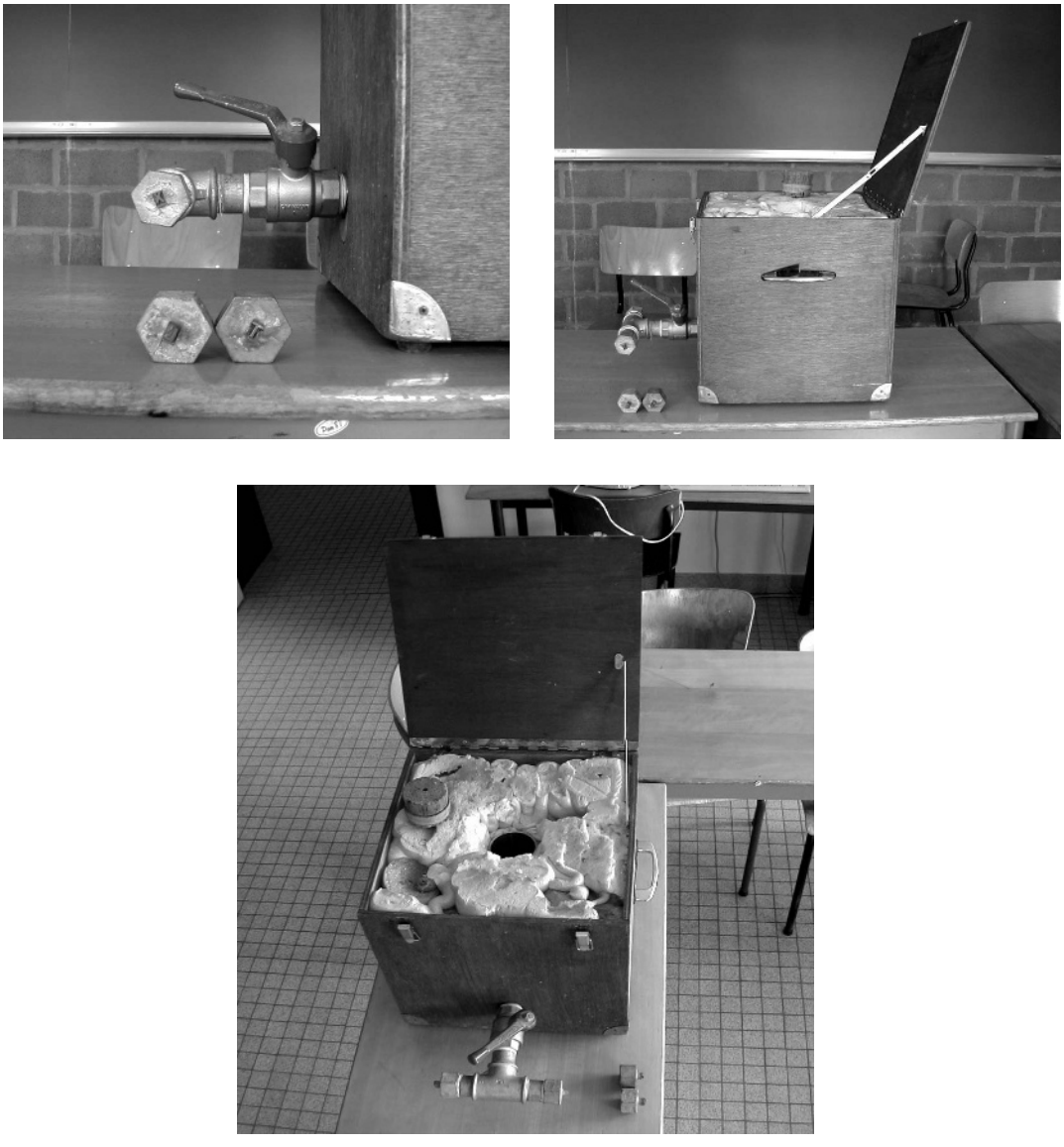

Figure 1
Appareillage de cryomarquage utilisé lors de l'étude (différentes vues).

Figure 1
Different views of the freeze branding apparatus used in this study.

Experiments

Freeze branding and control in a closed-natural environment

The fish used in this study were caught by electrofishing in the river Meuse, Belgium, near the village of Dave (50°25'03"N, 4°53'08"E), one day before the start of the experiments. After marking the fish, roach were placed in two large bow-nets (150 × 75 × 75 cm, 10 mm mesh) in the backwater of Tailfer connected to the river Meuse (50°24'07"N, 4°52'57"E) for future control. Fish were not anaesthetised prior to branding, like recommended by Nielsen (NIELSEN, 1992).

This closed-natural environment allowed us an easy control of fish and an evaluation of short-term incidence on survival and growth in marked roach in habitat conditions similar to those existing in the river.

120 roach were divided into three lots, two for evaluating the best mark placement and one for control:

– Lot 1: 40 individuals (85-240 mm TL) were freeze branded at position 1;

– Lot 2: 40 individuals (90-251 mm TL) were freeze branded at position 2;

– Lot 3: 40 individuals (82-268 mm TL) received no marks and were used as a control lot.

We chose only two different positions for marks on skin. Position 1 was the little depressed area of muscles above the anal fin. Position 2 was located above the lateral line and between caudal and dorsal fins. These positions are commonly used for scale removal, for age determination, because the scales' cover is not or little eroded at these places (BAGENAL, 1974; SUMMERFELT and HALL, 1987; STEINMETZ, MÜLLER and GRIEDER-KÜHN, 1991). After 1 month, we estimated survival rates, growth rates and mark retention rates. Retention rates and survival rates data were treated by Pearson χ^2 test whereas statistical comparison between growth rates of each lot were made with the help of Student's t test (DAGNELIE, 1992).

Moreover, during marking sessions of roach carried out for the field mark-recapture study (EVRARD and MICHA, 2003), the marking speed was estimated. It was, in fact, calculated as the average number of fish per hour of operations by one person.

RESULTS

Freeze branding and control in a closed-natural environment

Retention rate

The L-shaped freeze brand blackened tissues and deformed scales. It was recognisable on more than 94% of treated fish after 30 days (for position 2). The retention rate reached 100% for fish marked at position 1 (Table I). Retention rates were similar to those reported by other authors. LAJEONE and BERGERHOUSE (1991) reported 95% of branded walleyes retained recognisable marks after 5 months in a rearing pond. There is no significant difference in retention rate between the 2 positions ($\chi^2 = 2.00$; a = 0.05; 1 df).

Survival rate

The presence of a mark and its position had little effect on survival (Table I). Survival rate after 1 month was higher than 87% for roach marked at position 2, 90% for position 1 and 92% for unmarked fish. No significant difference appeared either between marked and unmarked fish ($\chi^2 = 0.55$; a = 0.05; 2 df) or between the 2 positions ($\chi^2 = 0.12$; a = 0.05; 1 df).

Table I

Efficiency of freeze branding and effects on survival and growth of roach in closed-natural environment. (N: number of roach; LT: average total length and length interval; P: mean weight; Ns: total number of survivors; Nsq: total number of survivors which retained the mark; SR: survival rate; RR: retention rate; GR: average growth rate).

Tableau I

Efficacité du cryomarquage et impacts sur la survie et la croissance du gardon dans un environnement naturel clos. (N : nombre de gardons ; LT : longueur totale moyenne et intervalle de longueur ; P : poids moyen ; Ns : Nombre total de survivants ; Nsq : Nombre total de survivants ayant conservé le marquage ; SR : taux de survie ; RR : taux de rétention ; GR : taux moyen de croissance).

Freeze branding (27 march 03)					Control (26 april 03)							
Lot	Mark	N	LT (mm)	P (g)	Ns	Nsq	LT (mm)	P (g)	SR (%)	RR (%)	GR (mm/month)	GR (g/month)
1	L-shaped position 1	40	144 (85-240)	47	36	36	148	50	90.0	100	4.4	4.5
2	L-shaped position 2	40	150 (90-251)	49	35	33	154	53	87.5	94.3	3.2	3.0
3	No mark	40	139 (82-268)	49.5	37		144	54	92.5	/	4.0	4.0

Growth rate

Absolute growth rates observed during the experiment varied from 3.2 mm month^{-1} for fish marked at position 2, to 4.4 mm.month^{-1} for position 1 (Table I), and was 4.0 mm. month^{-1} in unmarked fishes. In terms of biomass, growth reached between 3 g.month^{-1} and 4.5 g.month^{-1} for roach marked respectively at position 2 and position 1. A statistical analysis made on linear growth data for the three groups (difference between TL at time 30 days and at time 0) showed significant effects, both between roach marked at position 2 and control fish ($t = 2.79$; $p = 0.995$; 70 df), and between the fish marked at position 1 and 2 ($t = 5.63$; $p = 0.995$; 69 df). There was no significant difference in growth rates between unmarked roach and those branded at position 1 ($t = 1.70$; $p = 0.95$; 71 df). Consequently, we decided to mark the roach at position 1 for our mark-recapture study.

Marking speed

With a little time of practice, a trained person can mark an average of 430 fish per hour. In our case, it took approximately 6 h for a team of 4 people to mark the 10,300 roach caught for the mark-recapture study. These figures are in agreement with those for freeze branding and PIT tagging of Atlantic salmon smolts used in the "Saumon 2000" project (BOURGEOIS, O'CONNEL and SCOTT, 1987; PRIGNON and MICHA, 1996).

DISCUSSION

It is generally admitted that any manipulation can affect survival of fish and, above all, the type of marks used in the experimental conditions (HANSEN and JONSSON, 1988; NIELSEN, 1992). Clearly, the handling and/or anaesthetisation process associated with tagging of any type can itself have a significant effect on subsequent survival, and this

is likely to be more pronounced in wild fish which are particularly susceptible to loss of scales (HANSEN, 1988). Our findings have confirmed that freeze branding can be a useful method for marking roach, and potentially other cyprinids. Survival and mark retention rates in our experiments, perhaps more particularly for individuals branded on position 1 (above the anal fin), were high and comparable to those reported for other species, like walleye of similar and larger sizes (LAJEONE and BERGERHOUSE, 1991). Freeze brands were detected on 99% of marked Razorback sucker (*Xyrauchen texanus*) and Colorado squawfish (*Ptychocheilus lucius*) after 15 months (HAINES, SEVERSON. and MODDE , 1998). Longer retention times and efficiency of marking render the use of freeze branding a convenient alternative to tagging with passive integrated transponders (HAINES, SEVERSON and MODDE, 1998). Effectiveness and good retention of freeze brands have been also demonstrated in crustaceans, like the coconut crab *Birgus latro* L. (FLETCHER, FIELDER and BROWN, 1989). We observed no significant difference in the survival between unmarked controls and freeze-branded individuals in our experiment, so it seems unlikely that this method of marking affects short-term mortality rates. Of course, an approach of mortality and mark retention in smaller, younger fish (> 80 mm) would complete our study.

On a wide range of sizes, freeze branding appears to be efficient, with a 100% retention rate after 1 month. The survival of roach does not seem to be affected by marking, but an effect on growth was visible for fish marked at position 2. A decrease of linear growth may have several causes. Experimental conditions, manipulation, and type of mark can affect growth and mortality (HANSEN and JONSSON, 1988; NIELSEN, 1992). The observed effect on growth suggests that exogenous factors, such as water quality and a shortage of food, may come into play (CROZIER and KENNEDY, 2002). In fact, we cannot be certain that marking was the only factor responsible for this growth slowing down. On the long-term, branding marks would probably be retained for 5 months and more, as in salmonids (REFSTIE and AULSTAD, 1975; DUMAS, 1977). As the results of a mark-recapture study carried out in the summer of 2002 on the river Meuse suggested (EVRARD and MICHA, 2003), field recaptures of branded roach confirmed a retention period of more than 4 months, and probably limited effects on long-term mortality. This study demonstrated that freeze branding could easily be use as an alternative to other marking techniques, with the advantages of being easier, quicker and, especially, cheaper. For some species, like razorback sucker and Colorado squawfish, freeze brands were more easily recognised than elastomer marks, an equally rapid and cheap marking technique (HAINES, SEVERSON and MODDE, 1998).

The ideal marking technique does not exist. Most of all the characteristics of the ideal mark or tag (see LUCAS and BARAS, 2000 p. 295) are met for our short-term study, on roach stock estimate in the river Meuse (EVRARD and MICHA, 2003). A weakness is that some problems associated with brand identification become increasingly likely as time passes, reducing the effectiveness of external marks for long-term studies (KNIGHT, 1990). Possible confusion with naturally occurring injuries, and risks for the operator are drawbacks that must be taken into account (LUCAS and BARAS, 2000). Similar results, and perhaps better ones, could be acquired using other marking techniques. Panjet alcian blue tattooing could be tested on roach, based on the fact that this procedure has been shown to have no significant long-term effect on salmonid (>85 mm) condition, feeding or behaviour (TWOMEY and GILLER, 1990; BRIDCUTT and GILLER, 1993). Clipping pelvic fins would also be a rapid and cheap manner for fish stock identification. Moreover, DIDIER and MICHA (1996) observed no influence of this kind of marking on short-term mortality in roach.

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