Evaluation of the use of elastomer and paint to mark lowland longjaw galaxias (*Galaxias cobitinis*)

Leanne K. O'Brien and Nicholas R. Dunn

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Abstract

This study evaluated the use of visual implant elastomer, acrylic paint, and fabric paint for use as subcutaneously (under the skin) injected marks for individual or batch identification of the lowland longjaw galaxias (*Galaxias cobitinis*: Galaxiidae; Threatened: Nationally Critical) fishes, in potential habitat use studies. Over the 3 months of the study, survival of marked fish was high, and none of the mortalities that did occur were attributed to the presence of marks. Furthermore, neither the growth nor the relative condition of *G. cobitinis* were significantly affected by any of the trialed products, even when individuals received multiple marks – although fabric paint did have some adverse effects. All elastomer and acrylic paint marks were retained; however, fabric paint did not perform as well and its use is not recommended. Digital image analysis was used to determine mark area at monthly intervals to assess absorption rates. Elastomer marks were more stable than acrylic paint marks. Extrapolation of these data suggest that fabric paint marks may be visible for 7 months, acrylic paint marks for 24 months and elastomer marks for even longer, possibly for the lifetime of *G. cobitinis*. However, elastomer marks were more likely to fragment than the paints, although this did not affect overall visibility. Overall, both elastomer and acrylic paint could be used to achieve long-term marking of *G. cobitinis* without adversely affecting fish. These results are also likely to be applicable to population monitoring studies involving other small galaxiid species.

Keywords: *Galaxias cobitinis*, lowland longjaw galaxias, Galaxiidae, mark, tag, batch mark, visual implant elastomer (VIE), acrylic paint, mark evaluation

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1. Introduction

In population studies, the ability to identify fish through the use of batch or individual marks can greatly increase the scope of ecological questions that can be addressed. Fish are required to be marked in order to estimate population size, as well as individual movements, growth and survival. In preparation for field-based ecological studies, it is important to evaluate the impact and effectiveness of proposed marking materials on the focal organism. First and foremost, it is important that the application of permanent marks does not affect the parameters being investigated. It is also important that the level of mark retention be evaluated. Mark-retention evaluations can have varied results, and species-specific differences in marking effectiveness have previously been demonstrated (e.g. Goldsmith et al. 2003). Marks can be absorbed, expelled or may move from the initial injection area (Woods & Martin-Smith 2004), and the formation of scar tissue, especially if pigmented, can further affect the usefulness of subcutaneous marks (Hill & Grossman 1987). Furthermore, finding suitable locations to inject marks may not be straightforward as the presence of scales and the extent of pigmentation can limit the number of positions of where marks are likely to be visible (Olsen & Vøllestad 2001). Moreover, mark absorption and shedding rates may vary between different parts of the body (Catalano et al. 2001; Goldsmith et al. 2003).

The subcutaneous injection of viscous materials (e.g. elastomer or craft paints), which cure to form solid marks that are visible externally, is increasingly being used to batch-mark very small fish (Willis & Babcock 1998). Moreover, the use of multiple colours and marking positions can allow individual identification. Such methods have been used to mark a wide variety of invertebrates and vertebrates (e.g. Woods & James 2003; Beausoleil et al. 2004; Woods & Martin-Smith 2004), including Galaxiidae (e.g. Dunn 2003; Hansen & Closs 2005; O'Brien 2005). Biocompatible elastomer materials, some of which fluoresce, have been specifically developed for marking and have been found to have higher retention rates than previously used methods (Catalano et al. 2001).

The purpose of this study was to evaluate suitable materials and methods for marking the Nationally Critical (Dunn et al. 2018), lowland longjaw galaxias (*Galaxias cobitinis*: Galaxiidae). This evaluative study was carried out in partial fulfilment of Action 7.4[1] of the non-migratory galaxiid fishes recovery plan (DOC 2004), as a prerequisite for field studies on the dispersal of *G. cobitinis* from drought refugia on the resumption of surface flow in the Kauru River. The results from this study could also be used in the design of programmes to assess and monitor the long-term status and trends in populations of this species.

Galaxias cobitinis is a small (< 90 mm Total Length (TL)) fish, which restricts the methods that can be used to permanently mark individuals. However, as a galaxiid, *G. cobitinis* lacks scales (Cuvier 1817), and strongly defined pigmentation (McDowall & Waters 2002), suggesting subcutaneous marking is a suitable method for identifying individuals of this species.

In the present study, we evaluated the use of visual implant elastomer (VIE) against two readily available craft paints, one of which has previously been used by the authors to mark non-migratory *Galaxias* (Dunn 2003) and *Neochanna* (O'Brien 2005) species, for use as batch- or unique-marks in studies involving *G. cobitinis*. The survival, growth, and relative condition of captive *G. cobitinis* were monitored over a 3-month period to identify possible detrimental effects of these materials. Mark performance was assessed at several locations on the body, and digital image analysis was used to determine mark area at monthly intervals, to accurately assess absorption rates and estimate long-term mark retention.

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[1] 'Action 7.4: By June 2004, trial the use of visual implant elastomer tagging (or other suitable methods) with lowland longjaw galaxias. If successful, use tagging to determine (by June 2006) lowland longjaw galaxias dispersal from drought refuges following the resumption of surface flow in the Kauru River' (DOC 2004).

2. Methods

2.1 Mark types

Three materials were chosen for comparison: Chromacryl® Students' water resistant acrylic paint (cool yellow – this coloured had previously been found to work well by Dunn (2003); Chroma Australia Pty Ltd, Sydney, Australia;), visual implant elastomer (red and green – colours provided by the manufactuer in the test kit; Northwest Marine Technology, Shaw Island, Washington State, USA), and dimensional fabric paint (violet gloss – different from the aforementioned colours; Semco Crafts Pty Ltd, Taren Point, Australia). These materials were chosen based on their common usage for marking fish, all were labelled as non-toxic, and, in the case of the fabric paint, the flexible properties of the cured product (as stated on the label) with all being flexible on curing in air. The two paints did not require any specialised preparation, whereas the elastomer was mixed according to the manufacturer's instructions for preparing small quantities (these came with the product and are also available on the manufacturer's website: https://www.nmt.us/wp-content/uploads/2017/11/VIE-Project-Manual-Nov-2017-1.pdf. One elastomer trial colour (2 cc) was sufficient for at least 56 marks; however, there was product wastage involved in the mixing process and, once prepared, elastomer needs to be used within 1–2 hours (Northwest Marine Technology instructions: https://www.nmt.us/wp-content/uploads/2017/11/VIE-Project-Manual-Nov-2017-1.pdf) — although this can be extended through storage on ice (Goldsmith et al. 2003).

2.2 Experimental design

Galaxias cobitinis were held in twelve 120-L lidded storage bins with a trickle-through flow system that were situated outdoors. These tanks contained a variety of substratum and flow conditions (see Dunn & O'Brien (2018) for further details).

Two marking studies were conducted simultaneously. The first study assessed the retention and visibility of the different materials for use as batch marks. *Galaxias cobitinis* were stocked in eight individual tanks, at a density of six fish per tank. In each tank, three *G. cobitinis* were injected once, with one of the three mark materials, giving a total of eight replicates for each material. Batch-marking occurred in the fleshy caudal peduncle region (the posterior of the body supporting the tail) in muscle tissue away from vital organs. Acrylic paint marks placed in the caudal peduncle region have previously proven successful for *Galaxias paucispondylus* and *G. vulgaris* (Dunn 2003), and *Neochanna burrowsius* (O'Brien 2005), and this position is recommended for marking small fish by the Department of Conservation (DOC 2003). Controls, which were the remaining three *G. cobitinis* in each tank, were not marked but could be individually differentiated by differences in their initial length.

The second study investigated the feasibility of using multiple elastomer marks to individually identify *G. cobitinis*. Fish were held in four individual tanks, at a density of 6, 10, 12 or 20 *G. cobitinis* per tank, as part of a simultaneous study investigating optimal densities for holding *G. cobitinis*, as described by Dunn & O'Brien (2018). Each *G. cobitinis*, (excluding the 3 control fish in each tank described in the first study) was marked with a unique combination of up to two colours in two of four locations (see Appendix 1). Marks were placed on the dorsal surface of *G. cobitinis* in the trunk region, either anteriorly (immediately behind the head) or posteriorly (midway between the head and dorsal fin), in muscle tissue away from vital organs (Fig. 1). Moreover, marking in this position also allowed *G. cobitinis* to be identified without handling, if required.

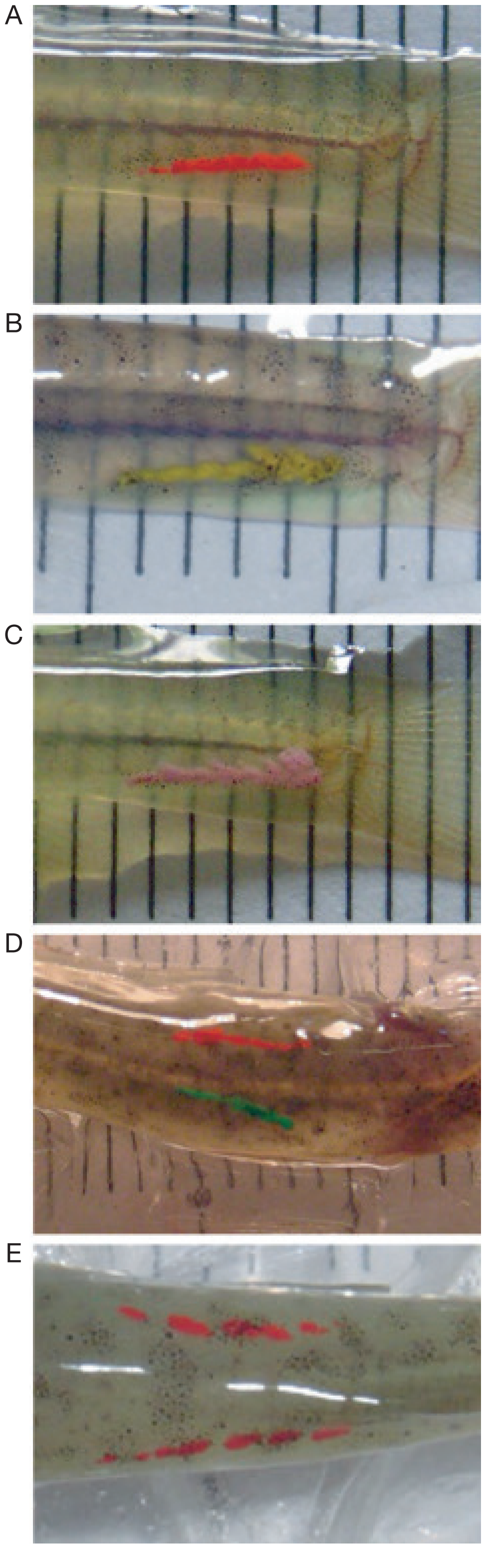

Figure 1. Representative examples of the different mark types administered to *Galaxias cobitinis:* A. batch mark, red elastomer; B. batch mark, yellow acrylic paint; C. batch mark, purple fabric paint; D. individual mark, anterior, red and green elastomer; and E. individual mark, posterior, red elastomer. Scale bars are in millimetres.

2.3 Fish handling and marking procedure

Marking was performed once on 26 February 2005. *Galaxias cobitinis* were anaesthetised before being placed on a sturdy paper towel that had been soaked in Aqua Plus (Rolf C. Hagen (U.S.A.) Corp.), to reduce stress and skin damage during handling. When marking *G. cobitinis* on the dorsal surface, individuals were placed on a piece of plastic bubble wrap packaging to maintain them in an upright stance. Marking materials were injected using a fine-bore hypodermic needle (1.0 ml, 29G × ½" Terumo U-100 Insulin; Terumo Medical Corporation, Elkton, USA). The average length of the marks was 4.1 ± 0.1 mm (mean ± 1 SE). Following injection, the paper towel was used to wipe excess material from the entry point to ensure that the mark was completely internally placed – this allows complete healing of the injection site, reducing the potential for mark expulsion.

Both unmarked and marked *G. cobitinis* were measured to the nearest 0.5 mm TL and weighed to the nearest 0.1 g using an electronic balance (Ohaus® Scout Pro, Ohaus Corporation, Parsippany, New Jersey, United States of America). The initial mean length of all unmarked and marked *G. cobitinis* was 50.1 ± 0.97 (range 41.5–57.0) and 52.1 ± 0.3 (46–59) mm TL, respectively, and initial mean weight of all unmarked and marked *G. cobitinis* was 0.575 ± 0.035 (range 0.3–0.8) and 0.606 ± 0.014 (range 0.3–1.0) respectively. Batch-marked *G. cobitinis* were then placed on a millimetre-graduated background and a still image of the mark was captured using a frame-mounted digital camera (Pentax Optio 33WR) in macro mode. Once handled, *G. cobitinis* were then placed in a temporary holding setup, consisting of 12 aerated 25-L bins, where they were treated for bacterial and fungal infections, provided with stress-reducing preparations to aid recovery, see Dunn & O'Brien (2018), and monitored for 24 hr, before being transferred to the outdoor tank facility. This anaesthetising, measuring, weighing, and photographing procedure was re-conducted for all individuals at subsequent monthly intervals (March, April, and May).

2.4 Image analysis

Computer analysis allowed the efficient and accurate determination of mark size. Images of marks were saved and subsequently manipulated in jpeg format. The outline of each mark was selected using the 'select colour range' function in the imaging software Adobe Photoshop 6.0 (Adobe Systems Incorporated 2000). Within this function, the 'eye dropper' tool was used to select the mark colour and the 'fuzziness' adjusted to ensure that the exact mark outline was selected. Once selected, the mark colour was changed to white by deleting it from the image, to enhance the contrast of the mark against the pigmentation of the *G. cobitinis*. Manipulated images were then imported into the software Image Lab 2.2.4.0 (MCM Design 2001). The scale of each image was individually calibrated using the graduated background on which the *G. cobitinis* were photographed. The mark area was selected using the 'trace' function and the area of each mark (mm^2) was calculated by the software based on the number of selected pixels.

2.5 Statistical analysis

Analysis of variance (ANOVA) was used to compare between growth in length and relative condition of *G. cobitinis* receiving different mark types, multiple marks, and unmarked (control) fish, based on initial (February) and final (May) data. Relative condition (*Kn*) was calculated for *G. cobitinis* using the allometric equation of the form: $Kn = W/a{\cdot}L^b$, where W is an individual's weight (g), L its total length (mm), and a the y-intercept and b the slope of a fitted power equation (Le Cren 1951; Anderson & Gutreuter 1983). Data were only included for *G. cobitinis* held at densities of six fish per tank that had been fed similar rations. If mortality occurred, captive *G. cobitinis* were added to the tanks to maintain the assigned densities; however, these fish were not marked, and their growth and relative condition were not included in the analyses.

To assess mark absorption, the mean monthly loss of mark area was calculated between successive measurement periods. Similarly the proportion of mark remaining since February was calculated. Multivariate analysis of variance (MANOVA) was then used to examine differences in absorption between mark types. All analyses were conducted in Statistica 6.0 (Statsoft Inc. 2001).

3. Results

3.1 Effects of marks on *G. cobitinis*

Over the 3-month period of the study, all of the 48 *G. cobitinis* that were individually marked with two elastomer marks on their dorsal surface survived. By contrast, there was 79% survival for both the batch-marked and unmarked *G. cobitinis*. Half of all mortalities (n = 5) occurred when *G. cobitinis* entered submersed pumps through 3-mm openings in the outer casing (see Dunn & O'Brien 2018); an outbreak of the parasite *Ichthyophthirius multifiliis* (white spot, ich) led to four mortalities; and one fish was accidentally fatally injured during tank cleaning. Incidences of misadventure and parasitic infection were equally prevalent in marked and unmarked *G. cobitinis*, and there was no difference in mortality between fish marked with any of the three different mark materials or unmarked control fish.

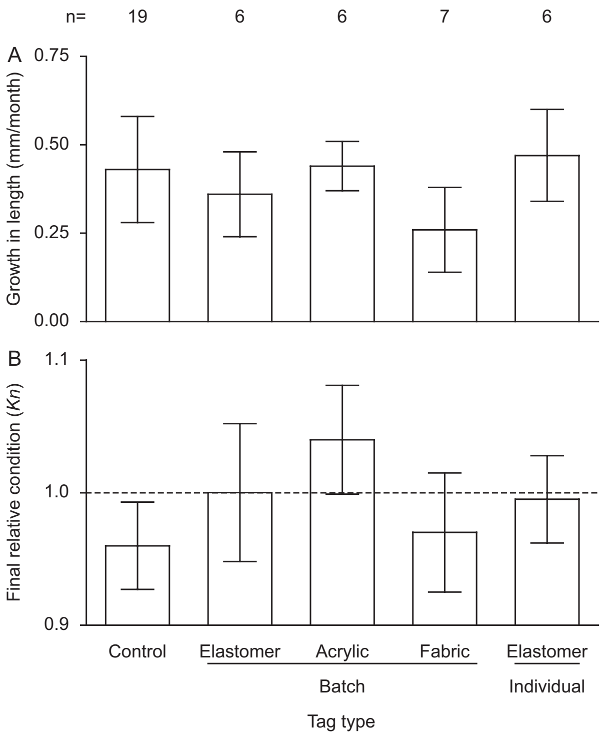

Figure 2. Difference between *Galaxias cobitinis*: A. mean growth in length (mm/month ± 1 SE); and B. final relative condition (*Kn* ± 1 SE) between control, batch (elastomer, acrylic and fabric) and individually marked (elastomer) mark types.

There was no significant difference in the growth in length and or change in relative condition of marked versus unmarked *G. cobitinis* or between the different mark types (growth: F = 1.1, d.f. = 4, 40, P = 0.4, Fig. 2A; relative condition: F = 2.0, d.f. = 4, 40, P = 0.1, Fig. 2B). However, *G. cobitinis* with fabric paint marks did have lower growth in length and lower relative condition, which, while not statistically different from the controls, is still of concern. Moreover, immediately after injection, internal bleeding was observed around some fabric paint marks, which remained visible for several weeks after marking but dissipated over the 3 months, suggesting that *G. cobitinis* were adversely reacting to this paint material. Similar bleeding was not observed in association with elastomer or acrylic paint marks.

3.2 Mark performance

There was no difference in the retention of marks placed in the caudal peduncle area compared with those placed in the dorsal muscle blocks (see Fig. 1). After 3 months, individually marked *G. cobitinis* were still readily identifiable by their unique marks, and all batch marks had been retained, with the exception of one fabric paint mark. Several fabric paint marks could only be discerned after careful examination of the caudle peduncle, however. The area of fabric paint marks was initially greater than that of the other mark types, despite the same method of application (Fig. 3). This was due to diffusion of the fabric paint into the surrounding tissue and between muscle blocks of the fish. This tendency to disperse may have also caused fabric paint marks to be absorbed more readily as, after 3 months, although the area of fabric paint marks was comparable to other mark types, these marks were more diffuse and thus difficult to distinguish.

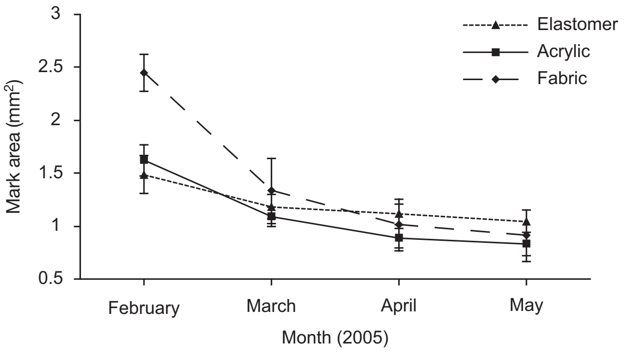

Figure 3. Change in area (mm^2; mean ± 1 SE) of each batch mark type administered to *Galaxias cobitinis* over the 3 months of investigation.

The rate at which marks are absorbed will affect the length of time that they are visible. In the present study, mark absorption predominantly occurred in the first month following implantation. Subsequently, both fabric and acrylic paint marks continued to be absorbed, while the mean area of elastomer marks was comparatively stable. MANOVA results indicated that both the mean monthly loss in mark area and the proportion of mark area remaining after 3 months combined, were significantly different between mark types (F = 4.6, d.f. = 4, 30, P = 0.005), with fabric paint marks having greater absorption than elastomer marks (Fig. 4A).

Data from the 3-month study were extrapolated using exponential decay curves to evaluate the period of time over which each mark type was likely to remain visible (Fig. 4B). Fabric paint marks were difficult to distinguish at a size of c. 0.2 mm^2, which equated to 20% of the initial area, and so this area was considered to be the lower limit of an effectively visible mark. Extrapolation indicated that fabric paint marks, on average, were unlikely to be visible 7 months after initial marking, while acrylic paint marks may be visible for 24 months after marking and elastomer marks may be visible for even longer, as their absorption rate appeared to stabilise (Fig. 4B).

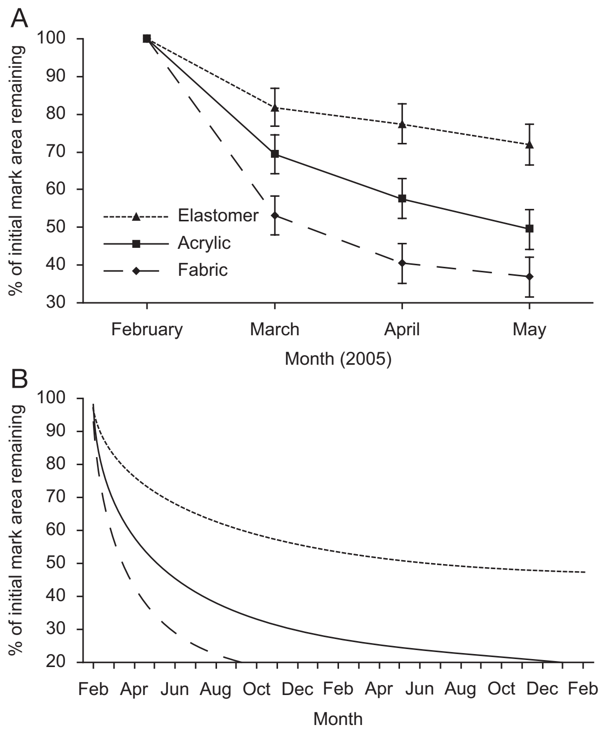

Figure 4. Rate of absorption (percentage of initial mark area remaining) of each mark type administered to *Galaxias cobitinis*: A. mean ± 1 SE over the 3 months of investigation; and B. extrapolated to evaluate the period of time over which each mark type was likely to remain visible.

Mark fragmentation – that is the loss of small sections within the area of the mark and distinct from overall absorption of the mark – was observed in some cases (Fig. 5). Examination of digital images captured in May found

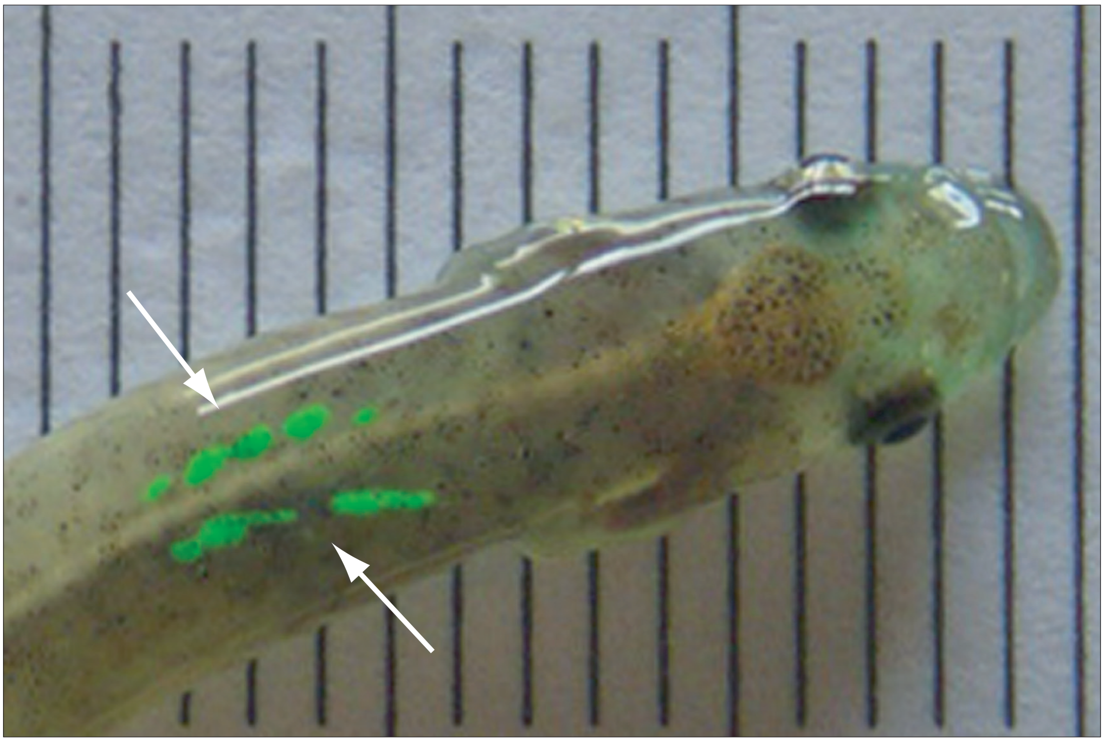

Figure 5. Representative example of fragmentation (arrowed) of green elastomer individual marks on the anterior of the trunk of a *Galaxias cobitinis*. Scale bar is in millimetres.

that elastomer marks were fragmented in 83% of cases, whereas fragmentation only occurred in 67% of purple fabric paint marks and 50% of yellow acrylic paint marks. Thus, although the calculated area of elastomer marks stabilised, patterns in absorption were more likely to cause these marks to appear fragmented, like a dashed line. However, this fragmentation did not affect overall visibility, as the marks were sufficiently long to ensure some retention.

4. Discussion

This study demonstrated that *G. cobitinis* can be effectively marked with batch or individual marks. Overall, the survival of marked fish was high and no mortality could be directly attributed to the presence of marks. The growth and relative condition of *G. cobitinis* were also not adversely affected by the injection of any of the three mark materials tested, even when multiple marks were applied—although these parameters were slightly reduced in fish bearing fabric paint marks and fish also appeared to react to this material. This supports previous research that showed that elastomer marks did not affect the growth of age-0 brown trout (*Salmo trutta*: Salmonidae; Olsen & Vøllestad 2001), common bully (*Gobiomorphus cotidianus*: Gobiidae; Goldsmith et al. 2003) or perch (*Perca fluviatilis*: Percidae; Goldsmith et al. 2003), and that there was no difference in the growth of fish marked with a range of acrylic paints and control fish (Hill & Grossman 1987). However, other studies have found differences in growth rate between fish marked with different materials. For example, in a similar study that directly compared elastomer and acrylic paint marks, Malone et al. (1999) found that the growth rates of small (< c. 35 mm TL) *Coryphopterus glaucofraenum* (Gobiidae) with acrylic paint marks were slightly lower than those with elastomer marks. This suggests that elastomer marks may be more suitable than acrylic paint if a study required the marking of *G. cobitinis* that are smaller than those used in the present study (i.e. < 52.5 mm TL).

Galaxias cobitinis is a scaleless fish, which has largely transparent skin with low levels of pigmentation (McDowall & Waters 2002). These characteristics resulted in high visibility of marks in all locations investigated over the 3 months of our study. The most visible marks were those placed on the dorsal surface, which also enabled *G. cobitinis* individuals to be identified in tanks without the need for handling. This result contrasts with previous studies that have noted that skin pigmentation can obscure marks (e.g. Olsen & Vøllestad 2001; Goldsmith et al. 2003). The high visibility of marks in *G. cobitinis* ensures that even small individuals can be adequately marked, even with multiple marks, while avoiding sensitive areas, such as close to internal organs, the head region, and near main arterial and venous systems.

The level of elastomer mark retention observed in our study (100% over 90 days) compares well with results quoted for other studies – 93% over 14 days for snapper (*Pagrus auratus*: Sparidae; Willis & Babcock 1998); 100% over 77 days for *S. trutta* (Olsen & Vøllestad 2001); 72% over 125 days for *G. cotidianus* (Goldsmith et al. 2003); and 100% over 125 days for *P. fluviatilis* (Goldsmith et al. 2003). However, mark retention over a set time period may not necessarily indicate the stability or absorption of marks over a longer time period – and this problem is compounded when evaluating the longevity of different mark materials if there are high levels of retention over a short period. In this study, digital image analysis was used to determine monthly absorption rates as an indicator of mark stability. This method enabled the stability of elastomer and acrylic paint to be differentiated, with elastomer marks having the lowest absorption of the injected materials. Further, the extrapolation of monthly data indicated that while the long-term retention likelihood of fabric paint marks was only 7 months, acrylic paint marks were likely to be retained for 2 years and elastomer marks for even longer. These findings are supported by field observations of acrylic paint marks injected into galaxiids being visible after 1 year (Dunn 2003) and 3 years (L. O'Brien, unpubl. data). Since *G. cobitinis* is considered a short-lived species, possibly living only 1–2 years (McDowall & Waters 2002), it is likely that elastomer and acrylic paint marks would last the lifetime of most *G. cobitinis* individuals.

Although elastomer marks performed well in all other regards, they were the most likely to fragment into a series of distinct segments. Fragmentation of elastomer marks has been documented in other evaluation studies (Woods & James 2003; Woods & Martin-Smith 2004), with a retention study using spiny lobsters (*Jasus edwardsii*: Palinuridae) showing that fragmentation was greatest in marks inserted across muscle fibres compared with those orientated with these fibres (Woods & James 2003). This suggests that fragmentation is caused by localised absorption due to greater blood flow between, compared to within muscle blocks. In the case of *G. cobitinis*, it is likely to be difficult to inject a mark dorso-ventrally within muscle blocks due to the small size of the fish. Thus, the occurrence of fragmentation emphasises the importance of injecting a long mark (c. 4 mm long in a c. 50 mm TL *G. cobitinis*) that transverses several muscle blocks.

In conclusion, our investigations found that the long-term marking of *G. cobitinis* can be successfully achieved using either elastomer or acrylic paint. The fabric paint trialled here is not considered suitable for marking fish because, although it did not significantly affect their growth, relative condition or survival, its use did lead to slight differences in these parameters and potential adverse reactions, and it also did not perform as well as the other materials tested. Elastomer marks had the greatest visibility and long-term retention. However, waterproof acrylic paint was a cost-effective alternative to elastomer, and may be more useful in field situations as it can be used for longer periods before it hardens and requires no preparation. Thus, the final choice of material will be dependent on the purpose and duration of a particular study, and fiscal constraints (see Table 1 for a summary of the three materials). If elastomer is used, we would recommend taking acrylic paint into the field as a 'backup' in case the elastomer does not mix properly or cures before the required number of fish have been marked. The findings of this study are also likely to be applicable to population studies of other galaxiid species in which marking is required.

Table 1. Comparison of the three mark materials trialled.

	MARK MATERIAL		
	ELASTOMER	ACRYLIC PAINT	FABRIC PAINT
Cost (NZ$)	>100*	<5	<5
Preparation	Involved	None	None
Mark visibility	High	High	Low
Adverse effects	None	None	Possible
Durability	High	Moderate	Low
Applicability	Best for studies greater than 2 years in duration	Best for studies less than 2 years in duration	Use not recommended

* At the time of the study (2005), the elastomer trial pack cost US$ 40 plus US$ 43 freight.

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Appendix 1

Schematic diagram showing the individual marking locations for *Galaxias cobitinis*

Twenty *G. cobitinis* were individually marked using two colours of elastomer (R = red and G = green) in two of four locations on the dorsal surface of the trunk. For example, mark number 1 would see two red marks placed anteriorly on the trunk (immediately behind the head), while mark number 5 has two red marks placed posteriorly (midway between the head and the dorsal fin) on the trunk.