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Individual recognition of juvenile salmonids using melanophore patterns

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This article describes two small scale experiments designed to test whether variation in melanophore patterns could be used to identify individual juvenile salmonids that are too small for conventional marking methods. Three independent observers were able to identify 30 individual 0+ Atlantic salmon (*Salmo salar*) parr from photographs taken over an 8-week period with 100% accuracy, on the basis of patterns of melanophores in the region of the eye and jaw. Counts of the number of melanophores in two particularly variable regions of the head of 14 newly-emerged brown trout (*Salmo trutta*) from photographs of the same individuals taken on different occasions were strongly correlated. Eighty-four per cent of the fry were correctly identified on the basis of spot number alone, without any reference to their patterns. Thus a combination of the number and position of head melanophores can be used for recognition of relatively large samples of very young salmonids. Problems with, and potential applications of this method are discussed.

Key words: identification; melanophores; salmonids.

INTRODUCTION

There are a number of more-or-less sophisticated techniques for permanent marking of individual fish once they reach a reasonable size, including Carlin tags, tattoo marking (Hart & Pitcher, 1969; Pitcher & Kennedy, 1977), freeze branding (Laird *et al.*, 1975), and, more recently, passive integrated transponder tags (Brannas *et al.*, 1993). For small (<70 mm) fish, the range of methods for identification of individuals is much more limited, with some form of tattooing being the most satisfactory option (Herbinger *et al.*, 1990). When it comes to first-feeding salmonids, none of these techniques is suitable, since few marking positions are available, mark-induced mortalities can be high, and dyes other than Alcian blue have shown poor retention (Kelly, 1967; Starkie, 1975). This makes it hard to keep track of individuals, either in the field or in the laboratory, and consequently greatly constrains what can be learned about the biology of salmonids at what is a critical time for survival (Elliott, 1989).

During routine trapping and batch-marking of newly-emerged Atlantic salmon (*Salmo salar* L.) and brown trout (*Salmo trutta* L.) alevins in the field (Garcia de Leaniz *et al.*, in prep.), it became apparent that melanophore spot patterns in the head region of these fish were highly variable. Natural

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pigmentation patterns have been used successfully to provide long-term individual recognition in various kinds of animals (for example, swans, Scott, 1978; cheetahs, Caro & Collins, 1986; whales, Katona & Whitehead, 1981), including fish (Persat, 1982; Bachmann, 1984). These studies used areas of pigmentation on relatively large animals. We report here two small-scale experimental tests of whether distinct patterns of the melanophores themselves could be used for individual recognition of much smaller animals, namely juvenile salmonids (Atlantic salmon and brown trout). We also describe a simple technique for quantifying such patterns.

MATERIAL AND METHODS

MEDIUM-TERM (8-WEEKS) RECOGNITION OF 0+ SALMON PARR

On 3 September 1990, 30 Atlantic salmon fry (44–66 mm) were caught by electrofishing in the Girnock Burn, Aberdeenshire, Scotland. They were anaesthetized (MS222), measured, weighed and photographed against a grey background on the left and right side of their head using colour film (Kodak Gold 100 ASA) and a Canon 35 mm camera (Canon T90) fitted with a bellows extension concertina, a 90 mm macro lens and a ring flash to minimize shadows and reflections. Then all fry were marked individually by a combination of fin clipping and Alcian blue spots and placed in a live-box in the Girnock Burn. No mortalities resulted from the handling procedure. The fry were rephotographed 4 and 8 weeks later) so as to show their heads but not their dye marks. The photographs taken on each subsequent occasion were numbered at random with respect to individual identity, shuffled and given separately to three observers who had not previously seen them (CdeL, FAH and NF) for matching on the basis of characteristic constellations of melanophores.

SHORT-TERM (24 H) RECOGNITION IN NEWLY-EMERGED BROWN TROUT

To test whether individual recognition on the basis of melanophore pattern is possible in young salmonids at first feeding, and to develop a simple method for quantifying spot patterns, 14 newly-emerged brown trout alevins (24–25 mm S.L.) were trapped downstream from a redd in the Bruntlan Burn, a tributary of the Girnock Burn, Aberdeenshire on 16 May 1990, anaesthetized, weighed, measured and fin-clipped. They were also photographed on the left and right side of their head as described above. The fish were kept in a live-box until the following day when they were identified on the basis of unique combinations of weights and fin-clips and rephotographed; all the fish survived. To provide a simple quantification of melanophore spot patterns that could be used for screening a larger number of fish, the area just below the eye on each side of the head was divided notionally into two sections using specified parts of the eye as landmarks (Fig. 1). These head sections were chosen because prior inspection of several hundred photographs had shown that these were separated normally by a spot-free zone and had highly variable numbers of visible spots. The two sets of photographs were presented blind to one observer (VM) and the number of pigment spots in each section (A and B, left and right side) was counted.

RESULTS

MEDIUM-TERM (8-WEEK) RECOGNITION OF 0+ SALMON PARR

The degree of expansion and contraction of particular melanophore spots often varied in photographs of the same fish taken on different occasions, but their relative positions remained unchanged. However, the three observers were

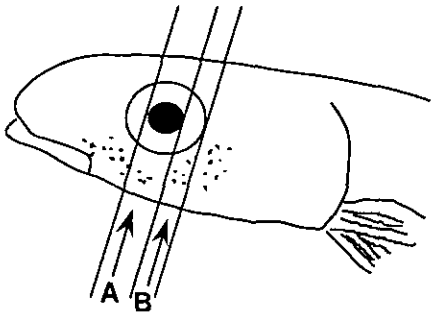
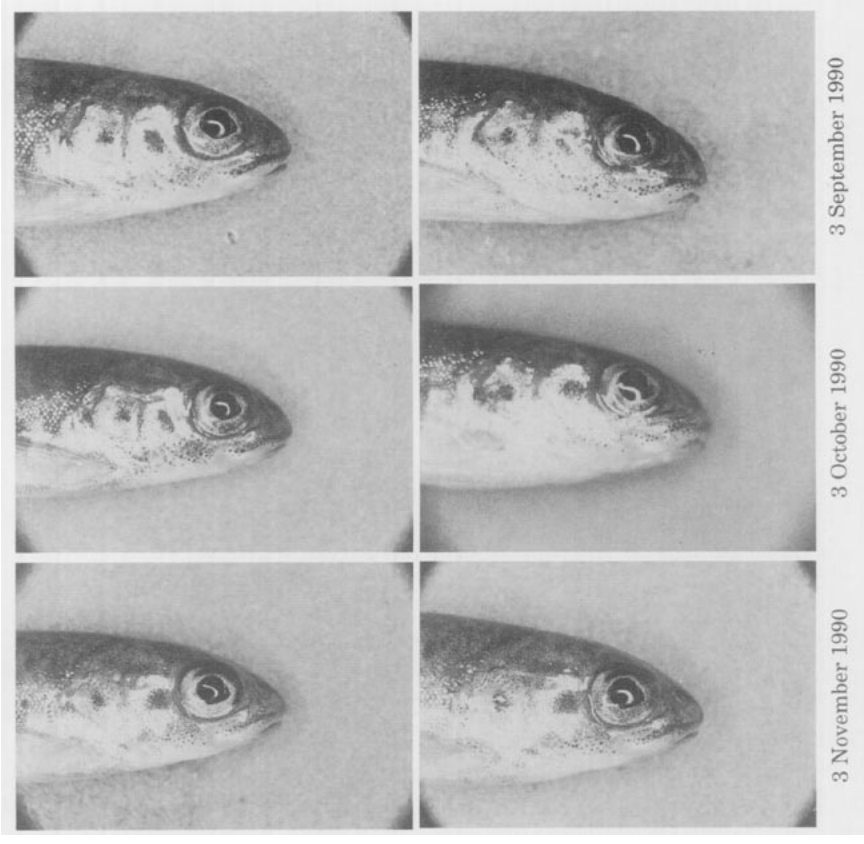


FIG. 1. Diagram of the head of a salmonid fry, indicating the two regions used for quantifying spot patterns.



Fish No. 15 Fish No. 16

FIG. 2. Side view photographs of the head of two Atlantic salmon parr taken on three occasions at 4-weekly intervals.

able to match up all the photographs with those taken 4 and 8 weeks later for all 30 fish, taking 1–5 min to identify each. Figure 2 shows some examples of the kinds of features used in this exercise.

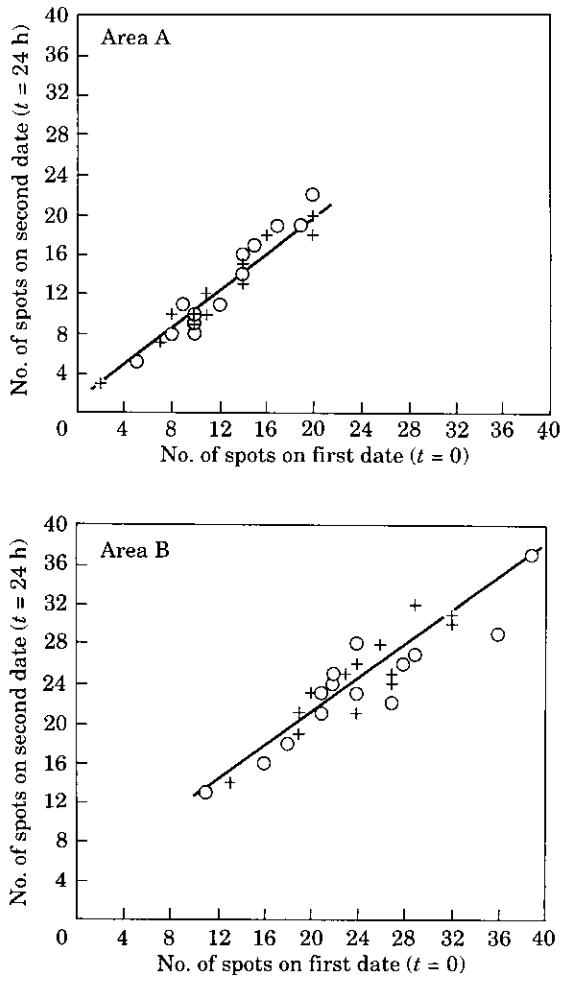


FIG. 3. The relationship between spot counts in regions A and B on the left (○) and right (+) sides of the head of brown trout alevins photographed on two separate occasions.

SHORT-TERM (24 H) RECOGNITION OF FIRST-FEEDING TROUT

Figure 3 compares the number of spots in areas A and B on the left and right sides of the head in photographs of the same fish taken on two different occasions. Agreement is very good: for region A 93% of the variance in spot count in the second set of pictures (A_2) could be predicted from the spot count in the first (A_1). Results from regression analysis were: $A_2 = -0{\cdot}31 + 1{\cdot}03\ A_1$ [r^2(adj)=0·925; P<0·001]. The equivalent coefficient of determination for area B is 83% [B_2=5·40+0·774 B_1; r^2(adj)=0·825, P<0·001]. The two regression coefficients were significantly different (ANCOVAR, $F_{1,50}$=6·78, P<0·05); for some reason, the melanophores in region B seem to be more labile, or else more difficult to identify in a photograph. Using just the spot counts in the head regions, 12/14 fish (84%) were identified correctly. The remaining two fish could be identified on the basis of idiosyncratic constellations of melanophores, as described above for salmon.

DISCUSSION

Thus juvenile brown trout and Atlantic salmon have individually distinct patterns of melanophores in the head region that are visible soon after emergence and that (in salmon at least) persist for at least 8 weeks. These can be used by human observers to recognize reliably fish that are too small for individual marking by any other technique. By using a simple counting method to identify most fish, or at least to reduce substantially the pool of candidates, the technique can be relatively simple for inexperienced workers to apply. In addition, the technique involves limited extra handling, takes little more time than routine measuring and weighing under anaesthesia, makes use of widely available equipment and can be applied in the field (Garcia de Leaniz *et al.*, in prep.). Application of the technique is relatively cheap (approximately 27p per fish for film and processing), and capital costs are fairly modest (£1200).

Disadvantages of the technique are: that melanophores appear to be poorly developed in yolk-sac fry, which limits the accuracy of the technique at this stage (from first feeding onwards this ceases to be a problem); that head melanophore patterns are often obscured by guanine deposits among hatchery-reared fry, making individual recognition very difficult in intensive husbandry conditions; that the method is time consuming and rather hard on the eyes [although computerized image analysis, used by Strachan *et al.* (1990) for analysing markings in mackerel, might help to solve this problem]; and that it requires magnification, so that some form of macro-photography is essential. This latter point means that head melanophore patterns cannot be used easily for individual recognition during behavioural studies, though pilot trials in an artificial stream using a video camera fitted with a $\times 20$ magnification lens have yielded promising results.

These studies have shown that melanophore patterns can be used with a degree of confidence to identify individual Atlantic salmon and brown trout at an age when existing techniques are unsuitable. The degree of stability and the time over which the technique can be applied remains to be documented fully, although it has been used successfully in a programme of serial trapping of Atlantic salmon dispersing from a natural redd to identify the same individuals on several occasions between April and July (Garcia de Leaniz *et al.*, in prep.). At smolting, when salmonids begin to lose head melanophore patterns and parr marks, fish will normally be large enough for individual marking by other, more conventional techniques.

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