Method Article

Colour marking of small fish with a marking stand for Dermojet®

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A B S T R A C T

We designed a marking stand for the Dermojet®, which substantially improves fish marking via needleless subcutaneous injection of dye. The marking stand allows to increase the nozzle-to-fish distance, adjust this position and to keep the jet injector fixed during operation as well as dye refilling. A laser pointer enables a precise and small-scale aiming. Using this marking stand we marked the caudal fin of small fish with Alcian blue for a flume experiment. In total we marked 204 gudgeon (Gobio gobio) and spirlin (Alburnoides bipunctatus) of 9–14 cm length with up to two dots per fish. Weighing, measuring and marking one sedated fish took 30 to 60 s. Immediate marking success was 100%. Fish were kept indoors in tanks for 7–12 days post-marking and the colour mark remained visible for the complete study period. During our flume experiment the colour marks at the caudal fin were detectable on all fish regardless of swimming position. With this easy and fast method fish can be marked gently, reliably and efficiently.

- Application of a high-pressure jet injector for needleless and accurate colour marking of fish.
- Manual for marking the caudal fin of small fish with Alcian blue.

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### Method details

#### Introduction

During the past decades fish in field and laboratory studies were marked with external tags, fin clips or dye to distinguish individuals or groups. One common method is subcutaneous injection of dye [1] because of easy application and identification, low costs and none or minor effects on fish. To enable identification of small fish via visual observation in a flume experiment, we choose colour marks on the caudal fin by needleless injection.

The jet injector named Panjet® (itself developed for dental use on humans) was frequently used by fish biologists [2–5]. To ensure an intra-/subcutaneous dye injection on fish and prevent damage on internal tissue, modifications on the Panjet® were mentioned [2,4] and different recommendations for the dye application were given [2,6]. However, the Panjet® is no longer available. An alternative needleless injector is the Dermojet®. It is similar in size and handling, but exhibits a very high non-adjustable injection pressure making an accurate use on fish fins difficult. Marking of fins by dye injection is mentioned in various studies [2,6] and a different marking code on fins allows the identification of individual fish [7]. The distance between injector nozzle and fish has a significant impact on the dye penetration depth and the mark quality. Moreover, because of the thin tissue of fins, the distance between fish and injector plays an important role for fin integrity, especially for small individuals. For the Panjet®, the recommended distance varies from 2 to 30 mm [2,4–6] and needs to be increased for smaller fish as well as for the Dermojet® that has an even higher injection pressure.

Since we wanted to mark comparatively small fish for a flume experiment, we had to develop a gentle and precise way of working with the Dermojet®. According to a preliminary test on dead fish (small cyprinids) marking the caudal fin required a distance between Dermojet® nozzle and fish fin of 310 mm. Therefore, precise marks cannot be accomplished by holding the jet injector by hand. To solve this problem, we developed a marking stand for the Dermojet®. It allows to increase the nozzle-to-fish distance, adjust this position and to keep the jet injector fixed during operation as well as dye refilling. A laser pointer enables a precise and small-scale aiming.

#### Method

**Equipment description and preparation**

**Description Dermojet® stand**

The baseplate of the Dermojet® stand (Fig. 1) is made of solid aluminium. The linear actuator is fixed on this baseplate using a connector clamps base. A specially manufactured connection bar links the Dermojet® to the linear actuator, while a nozzle in the middle of this bar holds the laser pointer. The connector clamps flange, which is continuously adjustable in height, allows to bring the connection bar with the Dermojet® and laser pointer into position by turning the handwheel. The Dermojet® is fixed by two flange clamps, which are modified in such a way that you can see the fill level in the dye reservoir, cock the Dermojet® and unscrew the Dermojet® dye reservoir without having to remove the entire Dermojet® from the stand (see also photo in the top right corner of Fig 1). The laser pointer is held by a clamping joint with wing screw, which allows to align the laser beam in any direction. The entire construction is solid and very stable so that the fine adjustments
Marking preparation and procedure

Dermojet® preparation. Before using the Dermojet®-Polymedical, we advise the reader to carefully read and follow the manufacturer’s instructions. Our descriptions should be understood as complementary information and not as stand-alone instruction for use of the Dermojet®.

Sterilise the Dermojet® upon arrival and after every usage as described by the manufacturer.

Preparation of sedation. Fish are sedated with 2-phenoxyethanol solution (500 – 600 mg l⁻¹) in a narcosis tub. Therefore 12 l water from the holding tank is filled in an opaque box (400 × 300 × 250 mm) and aerated. Add 2-phenoxyethanol (Carl Roth GmbH & Co.KG, Ref. 4348.2) directly into the water stir briefly and wait at least 30 min with running aeration to support dissolving. Control that the solution is properly solved before sedating the first fish.

Preparation of Alcian blue solution. Mix 20 ml Alcian blue dye solution (equals ~ 175 - 200 dye points per Dermojet® releases) at the rate of 50 mg Alcian blue powder (Carl Roth GmbH & Co.KG, C.I. 74240) and 0.045 ml Sterilium (BODE Chemie GmbH, Germany Ref. 981069) per 1 ml aqua dest. One needs to ensure that all equipment is clean and the solution is properly solved.

Filling the Dermojet®. In order to fill the Dermojet®, unscrew the injection head and remove it with the reservoir. Draw up Alcian blue solution into a new 2 ml one-way syringe. Subsequently attach a
syringe filter (pore size 5.0 μm, nominal ø 25 mm, Carl Roth GmbH+Co.KG Ref. SE4M075I99) on the syringe and press the Alcian blue solution slowly through the filter into the reservoir. This should be done as a precaution to avoid the risk of clogging the fine Dermojet® nozzles with particles. Stop 2 mm below the “max-filling” line to avoid spilling and screw the injector head back onto the Dermojet® body.

Calibration. After filling, the Dermojet® has to be emptied of surplus air in the system, which is done by firing several times. Besides, the aiming system has to be calibrated, which one can do simultaneously to save dye. Set the Dermojet® into the marking stand and adjust/check the correct nozzle-to-fish distance (in our case 31 cm). Subsequently, fire the Dermojet® on a blank sheet and adjust the laser pointer carefully to the centre of the resulting dot of ejected dye. This procedure should be repeated two more times. When in use, the position of the Dermojet® and the laser pointer should be controlled regularly and we advise the reader to analyse the accuracy and laser orientation after every refilling.

Marking procedure. The fish are sedated and marked one by one. The first fish is transferred into the narcosis tub. We recommend to cover/close the tub with a dark coloured lid for the first minute to calm the fish and prevent it from jumping out of the tub. When sufficiently well narcotised (shown by loss of body stability respectively irregular gill movement), the fish is placed on a wet tray, its caudal fin on a wet cloth. Weight (g), total length and body height (mm) is noted. Afterwards the tray with the fish is placed on the baseplate below the Dermojet®. Ensure that the caudal fin is sufficiently fanned. The Dermojet® is cocked and the tray is aligned so that the laser point matches the desired marking area on the fin. In order to protect the surrounding fish tissue from dispersed dye, a protective foil cover is placed on the fish tail, except for the marking area. Subsequently, a transparent splashback is placed around the fish to protect the devices and the operator and the Dermojet® is fired. If several marks are required the Dermojet® is cocked again and the fish is repositioned at which a clean foil cover should be used. After the marking the fish is rinsed of surplus dye and transferred to the wake-up tub. Here the immediate marking success is controlled and the fish is observed. When the fish starts to wake up it is transferred into its tank. Afterwards everything is prepared for the next fish (dispose the cloth, wash the tray and the foil cover, place a new wetted cloth on the tray and cock the Dermojet®).

Method validation

Test organisms. Gudgeons (Gobio gobio (L., 1758)) and spirlin (Alburnoides bipunctatus (Bloch, 1782)) were caught by electrofishing in the catchment of the River Rhine, and transported to the Federal Waterways Engineering and Research Institute (BAW) in Karlsruhe, Germany. Fish were stocked to and held in aerated squared glass fibre tanks (2.0 × 1.95 m; 0.5 m water level) with water flow through (abiotic conditions for gudgeon: temperature 16.40 ± 1.23°C, oxygen saturation 100 ± 2.0%; for spirlin: temperature 18.77 ± 1.07°C, oxygen saturation 100 ± 0.92%; mean ± s.d.). One to three days after arrival fish were marked. After recovering for at least 3 days, they participated in the experiment. Across the entire time, all fish were fed daily with a mixture of dried small invertebrates. All care, marking and experimental procedures were conducted as stated and permitted by the responsible authorities (Regierungspräsidium Karlsruhe, Germany, license no. AZ 35-9185.82/A-18/17).

Validation. Of 142 gudgeons and 142 spirlin that were sedated, weight and measured, 109 and 95 fish with 9–14 cm length were marked with a colour dot, respectively. Marking procedure for one sedated fish took 30 to 60 s. After the marking fish were kept for another 7–12 days to conduct a behavioural experiment. In this time period (immediate and longer-term) post marking mortality of gudgeon and spirlin was zero. Overall only one non-marked gudgeon died (despite being skinny the fish showed no signs of any disease or infection).

After carefully and passively rinsing the fishtail of the surplus dye immediately after marking, the mark pattern was clearly visible at the designated spot on the fish tail. Immediate marking success was 100%. For each species out of the 142 sedated fish 108 participated in the behavioural experiment. In the course of the experiment different people noted the mark patterns of the used
Fig. 2. Example of colour mark with the Dermojet®, Spirlin with the blue mark on the lower tip of the caudal fin six days after marking.

fish. The theoretical (noted in the marking process) and observed (noted in the experimental process) mark pattern distribution of the fish matched, indicating that the colour marks were well visible and identifiable for at least 12 days (Fig. 2). Due to the transparency of the caudal fin, colour marks were visible from both sides during the flume experiment regardless of swimming position.

Conclusions

The above introduced marking stand enables the usage of the Dermojet® for fish marking via needleless subcutaneous injection of dye. The Dermojet® is fixed in the marking stand to ensure a consistent nozzle-to-fish distance, generating the proper injection pressure. Due to a laser pointer on the marking stand, precise colour dots can be made on different areas on the small fins, allowing to create different mark patterns. With the presented procedure, marking of the fish can be done gentle, reliable and quite efficient.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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