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## A fast and reliable method for mark-recapture water beetles (Coleoptera: Dytiscidae) and other Arthropoda

**Riassunto:** *Un metodo veloce e affidabile per la marcatura dei coleotteri d'acqua (Coleotteri: Dytiscidae) e altri artropodi*

Un nuovo metodo per marcatura di Coleotteri Dytiscidae è stato sviluppato. Consiste nella marcatura di un'elitra in campo tramite un mini-trapano a batteria, con cui si effettua una piccola abrasione su cui si attacca una piccola etichetta col codice alfa-numerico stampato su carta idroresistente. Si riassumono e discutono i risultati di uno studio quadriennale su alcune specie di Dytiscidae di una torbiera montana. Si evidenzia la possibilità di applicazione del metodo per altri taxa.

**Abstract:** A new procedure for mark-recapture of Coleoptera Dytiscidae was developed. It consists of marking one elytron directly in the field using a battery driven mini-drill for making a small scratch, followed by attachment of a small label with an alpha-numeric code printed on hydro-resistant paper. The results of a four-year long study on some Dytiscidae species living in a montane peat-bog are summarized and discussed. The application of the method to other taxa is also outlined.

**Key words:** Mark-recapture methods, freshwater ecology, aquatic beetles, Arthropoda.

### INTRODUCTION

Mark-recapture methods are widely used to establish population size and movements of several animal taxa. For Arthropoda, the major challenge is the small size of the specimens, with consequent difficulties in marking, and, for some taxa, the habitat that they occupy during their active life or during overwinter or estivation, when they often shelter in the soil or roots, in situations where markings can be removed. In particular, for Arthropoda living in water, the difficulties are amplified by the necessity of finding a marking method that is stable in water for a long period. Indeed, to our knowledge, only a few studies that implemented mark-recapture methods have been carried out for water beetles. The first paper specifically dealing with marking Dytiscidae was provided by Brancucci (1975), who prepared a special tool, derived from dentist's equipment, to produce small notches in various positions on the pronotum and elytra. These notches could be coded and therefore a large number of combinations could be obtained. The major limits of this procedure are the relative complexity of the tools and the necessity of having very precise locations of the notches, to avoid the risk of confusion in reading the code during recapture. The total number of

combinations depends on the sequence of the notches - obviously the more complex the combination, the higher is the precision required in placing the notches, and hence the higher is the risk of misreading the mark of the recaptured specimens. Moreover, the total number of combinations is limited, and strongly related to the size of the beetle. According to Brancucci (1975), up to 2000 combinations are possible - a number that might not be enough for studies of coenoses rich in species and specimens. The manipulation of beetles in the marking procedure is delicate, since if an excessive pressure is given, the elytron can be perforated. Brancucci applied his methods with individual marking in a study on Swiss marshes (Brancucci 1980), but no more papers with individual marking using this procedure have been published.

A similar approach, consisting of carving a notch on the body, was applied by Sueselback (2002) to *Hydroporus incognitus*, a small-sized water beetle. However, he did not mark individuals - rather a mark was made for any animal caught for single dates or sites, thus only a few combinations were required. Moreover, the specimens were not marked in the field, but in the laboratory and returned to the study area after one day.

Svensson (1985), Nürnberger & Harrison

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(1995), Nürnberger (1996) used paint dots for marking Gyrinidae, whereas Aiken & Wilkinson (1985) and Aiken & Roughley (1985) applied a water-resistant tape to specimens of *Dytiscus alaskanus*. These latter methods proved to be useful for site-specific marking, but did not allow identification of individuals. Moreover, their application, as described, was quite lengthy. Davy-Bowker (2002) applied individual marking through small numbered labels to several species of Dytiscidae in a group of marshes in the UK, but his procedure required transportation of the collected specimens to the laboratory followed by marking the beetles when they were scarcely active, after maintaining them overnight in the dark and labelling them soon after. The labels themselves were relatively large, 3 x 1 mm, and written in pencil.

We aimed to set up a mark-recapture protocol for some aquatic Coleoptera as part of a survey of Arthropoda in an alpine peat-bog, composed of several more or less isolated ponds and streams. We chose the three species of Dytiscidae of the genus *Agabus* that were recorded in the study area: *Agabus congener* (Thunberg, 1794), *Agabus guttatus* (Paykull, 1798) and *Agabus bipustulatus* (Linnaeus, 1767), the latter species being present as a montane ecotype with reduced flight muscles and some minor morphologic differentiation with respect to the lowland forms, and previously considered to be a distinct species, *A. solieri* Aubé, 1837 (Drotz *et al.*, 2001; 2012).

We had to face various difficulties, and all previously described methods did not appear to be applicable. In particular, the peat-bog was located at 2200 m (Orco Valley, Gran Paradiso National Park) and was only accessible by a foot path, so the entire marking procedure had to be completed in the field immediately after sampling. Due to the altitude, the seasonal timing that was available for the study was limited to the summer months; moreover, some of the ponds are temporary, and can dry up completely for several weeks. During these drought occurrences, the beetles hide themselves in the dry mud at the bottom of the pond, so that marking could be eroded by movement through the soil. During winter, the specimens shelter in the mud and amid grasses, so that the same possible problems for resistance of the labels were foreseen. One more point to be considered was that the beetles' activity is always in a slightly acidic aquatic environment, and since we expected their lifespan to exceed one year, marking had to remain readable for a

long time. Needless to say, the mark had to be placed so that the beetles could fly.

#### MATERIALS AND METHODS

Several methods were trialed prior to the beginning of the main study on specimens of *Agabus* maintained in water tanks in the laboratory. Marking with dots of paint, lacquer, nail varnish in several colors and in various parts of the pronotum and elytra could potentially result in a large number of combinations, but in all cases the colored marks were lost after some days, or at most some weeks. Moreover, these marking methods were quite unpractical to be applied in the field, since the markings required several minutes to dry before the beetle could be released. We tried to fix labels on the elytra with various types of glue, but the elytral wax coating prevented the label from adhering to the body for more than a few days. The use of any solvent to help the adherence of the glue was excluded since possible long-term toxicity could affect the beetles' life expectancy, even if no immediate effects were seen. After many attempts, we found a method that could be very easily, safely and quickly applied and, in the preliminary experiments in the laboratory on some related species, appeared to last indefinitely.

A small grinding stone (Dremel, code 83322, Fig. 1) was mounted on a Dremel battery-driven multi-tool (Bosch GmbH), and a small part of an elytron was lightly scraped to remove wax and render integument slightly rough. On this small eroded part of the elytron, the label could be attached very firmly.

Considering that labels had to remain in aquatic conditions for the entire length of a beetle's life, and had to maintain readability, a water-resistant medium was chosen. This was so-called "stone paper", composed of 80% calcium carbonate and 20% non-toxic resin (Ogami/Repap) and sold as thin sheets as thick as a normal sheet of paper, but very resistant and flexible. Labels were prepared with a small font (Arial 3) and were printed on the stone paper with a laser printer set at the highest resolution. A two- and three-digit alphanumeric code was used, so that, using alternatively the left or right elytron, a very large number of unique combinations was possible (several thousands). In case of equivocal combinations (*i.e.*, 666 and 999) one of the two was discarded. The labels were pre-cut to a size of about 1.5 x 1 mm. They were very light, weighting about 40 µg, whereas specimens of *Agabus congener* weight about 40 mg, thus they could not interfere with flight.

The described methodology was applied for the first time in 2014 in eleven reciprocally isolated aquatic areas, selected inside the previously cited peat bog. Most of these were ponds of various depth - usually not deeper than 50 cm - and a surface area of a few square meters, whereas two were small streams, partially covered with vegetation and with very slow water flow. Small creeks present in the peat bog with strongly running water did not host specimens of Dytiscidae and were therefore not considered.

The marking sessions were carried out for two years (2014-2015), with 8 sampling sessions between late June and early October for each year. Starting from the second session of the first year, recaptures were simultaneously recorded during the sessions. For four more years (2016-2019), 2 recapture sessions per year were carried out.



Fig. 1. The Dremel grinding stone used to erase the elytron.

The marking procedure was usually carried out by three people. As far as possible, exhaustive sampling was carried out in the various sites by sweep-netting the entire pond, with particular attention to the margins, where the beetles often hide, until no more specimens were collected. During each session, all specimens sampled were provisionally stored in small trays with water and moss, trying to avoid excessive warming of the water. Previously marked specimens were recorded, provisionally stored in a different tray, and released when the sampling in the site was completed.

After the sampling on the site was completed, each unmarked beetle was taken from the tray, one elytron was slightly milled with the Dremel tool and the beetle was then placed in another tray with water (Fig. 2A). When all specimens had been milled, the marking procedure was started. Each beetle was quickly dried, one label was taken with a sleeve needle (prepared with an entomological pin), lightly laid on a drop of cyanoacrylate glue (Attack, Saratoga), drained to avoid excess liquid, and then put onto the milled part of the elytron. The label was pressed against the elytron for about 30 seconds, trying to place it evenly and fully adhering to the integument. The code was then recorded and the specimen released (Figs 2B,C). Care was taken to avoid any glue to expand to the suture since the beetles had to retain the possibility to fly.

Juvenile specimens with a very tender integument were difficult to treat, and the risk of creating severe damage was very high. For this reason, we avoided to marking juveniles.

#### RESULTS

During the two years of marking, a total number of 3342 specimens was marked (2970 *Agabus congener*, 163 *Agabus guttatus*, 209 *Agabus solieri*). The maximum number of beetles marked in one day was 280. The total number of recapture events was 2248, with several specimens recaptured more than once (Tab. 1). In both 2017 and 2018, the oldest

Tab. 1. Recapture events in the 6 years of the study.

Recapture events	2014	2015	2016	2017	2018	2019	Total recapture events
<i>Agabus congener</i>	273	870	804	51	34	4	2036
<i>Agabus guttatus</i>	8	19	13	1	0	0	41
<i>Agabus solieri</i>	72	76	19	2	2	0	171

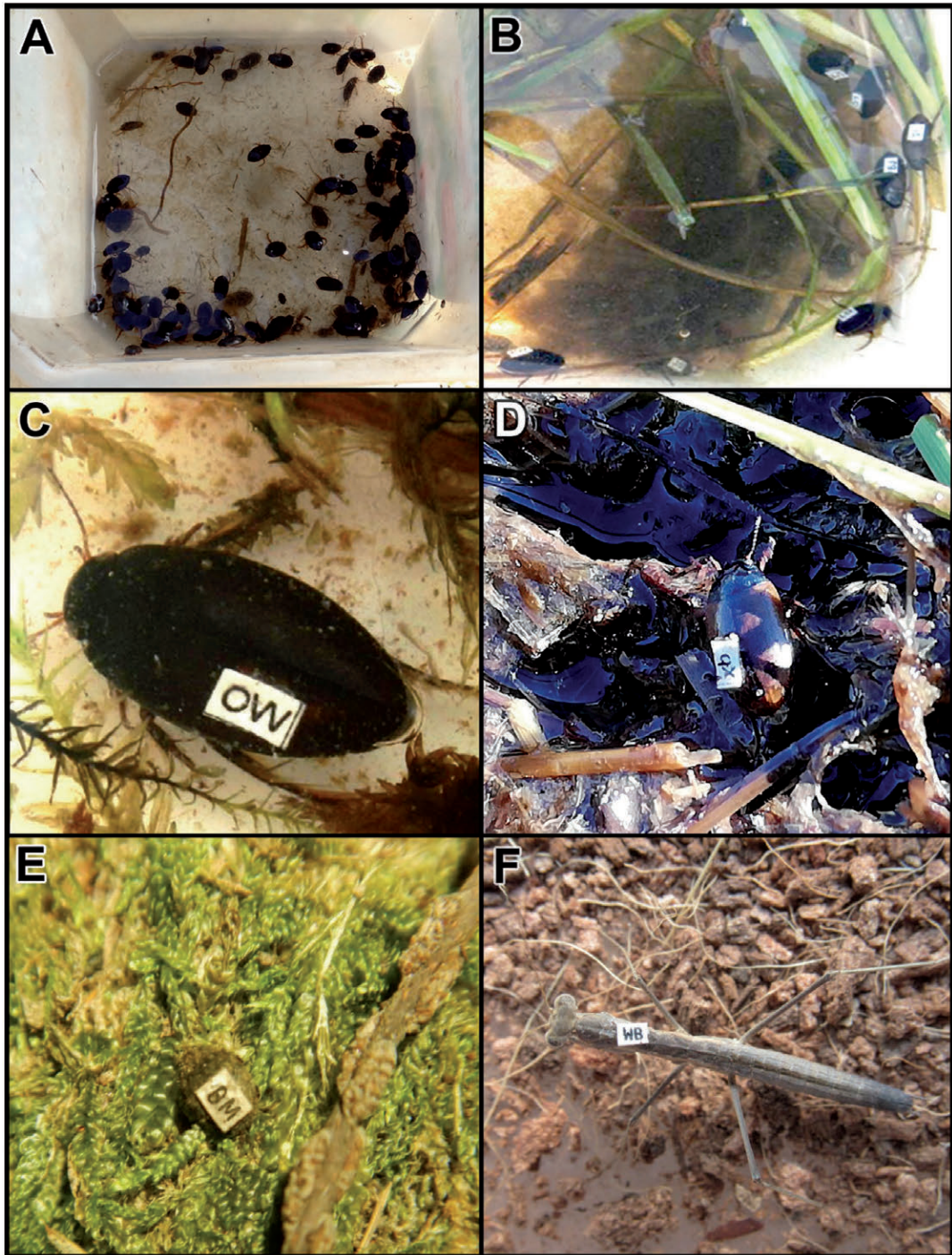


Fig. 2. The sampled specimens in the tray prior to the marking procedure (A); the specimens freshly marked, before being released (B); a specimen of *Agabus bipustulatus* freshly marked (C); a specimen of *Agabus congener*, originally marked in 2015, recaptured in 2019: the label (qx) is still perfectly readable after 4 years (D); a specimen of *Curimus sp. cf. lariensis* (Coleoptera: Byrrhidae), originally marked in 2017 and recollected in 2019: the label (BM) is perfectly readable (E); a marked specimen of *Geomantis larvoides* (Mantodea: Mantidae, photo Maioglio) (F).

specimens recaptured were marked in 2014. The 4 specimens recaptured in 2019 were marked in 2015.

Mortality or damage to the beetles during the process of marking was extremely limited. Only 4-5 specimens were damaged in the 2 years (usually loss of one leg during manipulation) and 3 were recorded to have died because of overheating during a marking session held in a particularly warm day.

During the sampling, 19 marked specimens were found dead, even two years after being marked, without any apparent sign of damage. In some cases, these specimens had been previously re-collected, so we suppose that they died of natural causes and were re-collected by chance.

#### DISCUSSION

The marking method for aquatic Coleoptera that we have developed proved to be very efficient, not invasive, easily applicable in the field, and very fast. Once the method has been practiced, the entire procedure of milling the elytron and attaching the pre-cut label requires less than 2 minutes for each specimen.

Regarding the possible influence of the marking on predation risk, it is true that marked specimens with the label are potentially quite visible. However, these species generally remain hidden among grasses and mud in the bottom of the ponds, and also water turbidity is generally quite high, so that they are always scarcely visible. In many cases, the labels are quickly covered by a slight layer of mud that reduces contrast. For the same reasons, the marking is not likely to influence the probability of recapture. Moreover, sampling for these species is carried out by repeated sweep-netting of the entire pond, including its bottom and margins, so that there is an equal possibility of collecting marked and unmarked individuals (*i.e.* the method is not dependent on visual detection). Quite interestingly, in some rare instances the marking and sampling method found dead marked specimens, providing potentially useful additional information that can be used when data are analyzed.

In our study, the recaptures were relatively limited, and varied according to the species. In *A. congener*, however, recaptures were higher than in the other studies on water beetles previously cited. In this regard, it must also be considered that an exhaustive sampling for these species that live hidden in the mud and among the roots of the grasses, and that are good swimmers that tend to swim away from the sampling

net, is virtually impossible. Moreover, it should be noted that once an elytron is milled, the scar remains visible for the entire life of the individual. Even if the labels were lost, we would have found unlabeled specimens with a milled elytron, which did not occur. Thus, loss of labels seems very unlikely under this method.

#### CONCLUSIONS

The new developed marking method for aquatic Coleoptera is important since it greatly simplifies the technical procedure, allowing studies of population dynamics and evaluation of inter-site dispersal.

It is extremely reliable, quickly applied and ideal for marking species that spend part or all of their life in extreme conditions and environments that are difficult to study.

The labels printed on “stone-paper” maintain an apparently unlimited readability, as demonstrated by labels of specimens recaptured in 2019 that were marked in 2015: these labels remained four years under water and among mud and roots of plants, including three overwintering periods in frozen ponds beneath snow, yet they were still perfectly readable (Fig. 2D).

The necessary training for the procedure is very quickly obtained, and the general cost is practically nil, excepting the Dremel mini-drill, that is anyway quite economic. With a single sheet of stone-paper, of the cost of a few cents, several hundred labels can be printed.

The procedure can be applied to other taxa other than aquatic beetles. Depending on the taxon, the preliminary milling can be avoided, since the label can be glued on the integument if there is not an excessive amount of cuticular wax. However, the almost indestructible stone-paper associated with the laser-printed alphanumeric code allows an efficient general use, including on quite small taxa. Studies are in progress on a wingless species of Coleoptera Byrrhidae, only 4 mm long, living inside mosses on rocks in often extremely wet conditions and with mud covering the body during rainy periods. These conditions imply a high risk of erosion or encrusting of anything stuck onto the body. However, the labels, glued onto both elytra, remained perfectly readable two years after marking (Fig. 2E) (Cerrato & Meregalli, work in progress).

Also, taxa other than Coleoptera were marked with this procedure, such as Mantodea (O. Maioglio, personal communication, Fig. 2F).

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