Journal of the Lepidopterists' Society
53(4), 1999, 138–141

METHOD OF HANDLING AFFECTS POST-CAPTURE ENCOUNTER PROBABILITIES IN MALE *HYPOLIMNAS BOLINA* (L.) (NYMPHALIDAE)

DARRELL J. KEMP
School of Tropical Biology, James Cook University, P.O. Box 6811 Cairns, Queensland, 4870, AUSTRALIA

AND

MYRON P. ZALUCKI
Department of Zoology and Entomology, The University of Queensland, St Lucia, Queensland, 4072, AUSTRALIA

ABSTRACT. Mark-recapture studies of butterfly populations are often plagued by low recapture rates, which make population estimation problematic. One reason for low recaptures is that the handling process of capture, marking and release contributes to low and unequal catchability of marked individuals. Here we report the results of an experiment conducted to evaluate the hypothesis that cooling individuals prior to release minimizes handling effects. The post-capture difference in site fidelity of territorial male *Hypolimnas bolina* (L.) (Lepidoptera: Nymphalidae) was compared among three groups: (1) males handled normally, (2) males chilled prior to release, and (3) uncaught controls. Unchilled males showed significantly reduced site fidelity compared to both control and chilled butterflies. Furthermore, chilled butterflies resumed activity after capture in a manner similar to uncaught controls. These results indicate that chilling has the potential to minimize the adverse effects of handling on subsequent butterfly catchability. Since 'equal catchability' of caught and uncaught individuals is a critical assumption of mark-release-recapture programs, this method has the potential to greatly increase the accuracy of subsequent population estimates. On this basis, in population studies on butterflies, the precise method of handling may prove a more meaningful consideration than the question of whether or not to handle.

Additional key words: catchability, mark-release-recapture, censusing, population estimation.

Mark-recapture methods represent a powerful tool for the estimation of animal population parameters, especially those of invertebrates (Seber 1973, Southwood 1978, Begon 1979). These methodologies have often been applied to the study of butterfly populations; however, on many occasions, such programs are plagued by low recapture rates, sometimes in the order of 1 to 10% (Brussard & Ehrlich 1970, Urquhart et al. 1970, Cook et al. 1971, Urquhart & Urquhart 1976, Watt et al. 1977, Cullenward et al. 1979). This makes the estimation of population size and related measures problematic (Rosenberg et al. 1995), and researchers must often resort to less powerful methods, such as transect-based surveys to census their populations (e.g., Pollard 1977, Eberhardt 1978, Thomas 1983, Dent 1997).

One of the many potential causes of low recapture rates in the Lepidoptera is that the actual process of capture and handling may affect the behaviour of marked individuals, reducing their chance of being recaptured (Gall 1984). This effect would contribute to low catchability in marked individuals, and lead to significant bias in subsequent population estimates (Gall 1984, Rosenberg et al. 1995). Although crucial to the application of mark-recapture methods, few authors attempt to validate the assumption of 'equal catchability' (Gall 1984). Several attempts to assay the effects of capture in butterflies have indicated that the capture and handling process may have strongly negative effects on subsequent catchability (Singer & Wedlake 1981, Lederhouse 1982, Morton 1984).

In recent times, many workers have taken to cooling individuals before their release (e.g., Bull et al. 1985, Zalucki & Kitching 1985, Suzuki & Zalucki 1986, Rutowski 1992, Zalucki 1993, Wickman & Jansson 1997). This technique stems from the idea that increased immobilization of individuals immediately prior to their release helps to reduce their subsequent degree of 'panic' (Wickman & Jansson 1997). In this way, individuals are supposedly more likely to remain in the immediate study area and behave normally, rather than disperse and avoid further capture (as found by Lederhouse 1982). The use of this technique, however, is based on mostly anecdotal information, and little, if any, published research has been conducted to evaluate its merit.

The aim of this paper is to evaluate the hypothesis that cooling individuals prior to release may increase their subsequent catchability relative to individuals handled in the normal manner. This hypothesis is investigated using territorial mate-locating males of the nymphalid species *Hypolimnas bolina* (L.).

MATERIALS AND METHODS

Male *H. bolina* in resident territories were censused along a 1020 m transect (Rutowski 1992) located on campus at James Cook University in Townsville, Australia (19°15′S, 146°45′E). Sampling was conducted in two rounds in 1998; from 9–12 February and 16–19 April, and once in 1999; from 22–25 February 1999. On each sample round, transects were censused

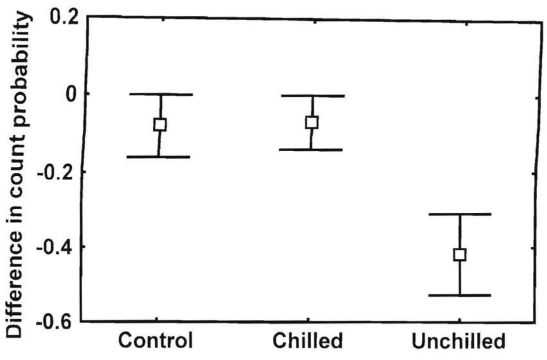

FIG. 1. The average (± SE) difference in the probability of encountering a male *H. bolina* between pre- and post-capture censuses, as a function of which treatment group he was assigned to. Control males were not caught, chilled males were caught and cooled before release, and unchilled males were caught but not chilled.

hourly for the first two days to identify individuals of high territory site fidelity (for census techniques see Pollard 1977). Notes on size and wing wear were taken to allow reliable identification of these individuals in subsequent censuses (Rutowski 1992). At 1000 h on the third day (termed the marking round), each resident was randomly assigned to one of three treatment groups. 'Unchilled' males were captured at their site, marked with an identification number on both hindwing undersides, and released immediately (total handling time of 40–50 seconds). 'Chilled' males were similarly captured and marked, but were held for a further 210 seconds in a paper envelope placed on a block of ice wrapped in newspaper (Wickman & Jansson 1997), then released directly back to their perch. Notes were taken on the immediate behavior of individuals in these two treatments upon release. Butterflies of the third group, designated as control, were not caught. Hourly transects were then conducted for the remainder of the third day and the next day to determine the behavior of males in all three groups. Censuses were conducted no sooner than 0900 h and no later than 1500 h, and only in the presence of sunshine before and during the entire transect. This was done to ensure that individuals were censused only at times of maximum site fidelity. During the course of each transect, individual males were deemed re-counted if they were within 20 meters of their designated territory, and actively engaged in defense of that site. No effort was made to search for individuals that were not immediately obvious at their sites following marking, hence the observation technique was kept similar before and after marking.

An index of site fidelity was calculated for each male by dividing his total counts by his total number of count opportunities (i.e. the number of times his territory was passed during transect censuses). Since the ratio of before: after-marking counts in each treatment group was homogenous between sampling rounds (Chi-squared homogeneity test on before- and after-counts across the three rounds: $\chi^2_2 < 2.79$, $p > 0.24$ for each group), data were pooled across all rounds. Initially, pre- and post-marking round count probabilities were compared for control group butterflies only. This was done to check that the activity of untreated butterflies was homogenous and that no other factor, for instance weather, affected the probability of counting butterflies between censuses. Mean differences (pre- and post marking round) in count probabilities between butterflies of each treatment group were then compared using a one-way analysis of variance (ANOVA). Three comparisons were planned prior to analysis; these were (1) between chilled and unchilled butterflies, (2) between chilled and control group butterflies, and (3) between unchilled and control group butterflies. These contrasts were evaluated using least significant difference (LSD) test for planned, non-orthogonal comparisons (Sokal & Rohlf 1995). Prior to conducting analyses, data sets were transformed using the angular transformation, and Kolmogorov-Smirnov goodness of fit tests were used to confirm that the transformed data were normally distributed (Kolmogorov-Smirnov $d < 0.24$, $p > 0.20$ for all groups). Levene tests were used to check homoscedasticity among ANOVA groups; these were non-significant in all cases ($p > 0.175$), which confirmed that data were homoscedastic. Two-tailed probabilities were used, and sample means throughout this paper are given ± 1 standard error.

RESULTS

A total of 30 primary territory residents were identified during the three sampling occasions, and these were randomly distributed amongst treatments as follows: 11 chilled, 11 unchilled, and eight control. Of the total 226 count opportunities before the marking round, 191 counts were registered, and primary residents exhibited a mean site fidelity of 0.87 ± 0.02 (each being present on approximately 87% of all count opportunities). Mean pre-marking site fidelity did not differ significantly among the three butterfly groups (control, chilled, unchilled; ANOVA on angular-transformed data, $F_{2,27} = 0.15$, $p = 0.86$). In addition, the mean site fidelity of control group butterflies was not significantly different between pre- and post-marking censuses (paired *t*-test on angular-transformed data, $t = 0.63$, df = 7, $p = 0.55$).

Post-marking censuses revealed that four marked individuals (one chilled and three unchilled) had deserted their territories. All other males were counted at least once defending their pre-designated territory area. The mean difference between pre- and post-marking round fidelity varied significantly among treatment groups (ANOVA on angular-transformed data, $F_{2, 27} = 3.97$, $p < 0.05$; Fig. 1). Fidelity was reduced to a varying degree in all groups following the marking round. The reduction in site fidelity of unchilled butterflies was significantly greater than that of either control or chilled group butterflies (LSD test for planned comparisons, $p < 0.05$). The significant difference between the two captured treatment groups demonstrated an effect due to the method of handling individual males before their release. Furthermore, the change in fidelity among chilled butterflies did not differ significantly from that shown by control group butterflies (LSD test, $p = 0.85$). Hence, relative to uncaught controls, the process of capture and marking had no appreciable effect on the subsequent territorial fidelity of butterflies that were chilled prior to their release.

Captured and marked butterflies in each treatment group showed clear behavioral differences following their release. While all chilled males remained perched on the substrate where they were placed, non-chilled males either resumed active territory defense (2 males), roosted on the underside of shaded foliage near the site (2 males), or flew quickly out of the area (7 males). Hence, while all chilled butterflies remained in the vicinity of their territory (i.e., less than 20 meters away) in the first instance, only 4 (36.4%) of the 11 non-chilled butterflies did so.

DISCUSSION

Although based on relatively small sample sizes, the results of this experiment clearly support the hypothesis that cooling may reduce capture effects and increase post-handling catchability of territorial male *H. bolina*. Not only did the process of chilling affect the probability of resighting captured males, but chilled males also resumed activity in a manner similar to that of butterflies that were never caught. This result is significant, since it shows that chilling may not simply reduce or 'manage' the adverse effect of handling, but that this process has the potential to actually nullify short-term effects of capture. To our knowledge, this has not been demonstrated previously for any butterfly species.

These results contrast with the generally negative effects of handling of butterflies obtained in previous studies, for example Singer and Wedlake (1981), Lederhouse (1982), and Morton (1984). In these studies, however, no reference is given to any method of chilling butterflies prior to their release, and it must be assumed that butterflies were simply released immediately following marking. The experiment conducted here on *H. bolina* corroborates the finding that butterflies handled in this manner may be less likely to be recaptured, but that this adverse handling effect may be mediated by a chilling treatment prior to release.

One concern with capturing and marking butterflies is that individuals will suffer from an increased level of predation owing to the wing mark and subsequent loss of crypsis or aposematism (Gall 1984, Reynolds et al. 1997). If present, such an effect is often difficult to distinguish from any potential effects arising from capture and handling. Fortunately, in this study, the contrasting results between treatment groups allowed the separation of specific marking effects. Since both treatment groups were given similar marks, chilled males provided an adequate control for the effects of wing marks in the unchilled group. That only the unchilled group of butterflies were less likely to be recounted than controls clearly suggests that, at least in the short term, these were affected by the method of handling and not by the marks placed upon their wings. A similar conclusion was reached by Morton (1984), who found that colored wing marks did not significantly affect recapture of the highly cryptic woodland butterfly *Melanargia galathea* (L.). The limitation with the current experiment, however, is that post-marking site fidelity was only measured for a period of two days. On this basis, the presence of a longer term effect due to wing marks in *H. bolina* cannot be completely discounted.

The question of why captured butterflies may be less likely to be resighted is not specifically addressed by this study, but some insight is afforded by casual behavioral observations made on released individuals. Clear differences in behavior were evident between treatment groups, and this resulted in more unchilled butterflies leaving their immediate area of capture in the short term. This difference alone may be responsible for differences in encounter probabilities of unchilled and chilled individuals, especially in the case of a site-tenacious butterfly such as male *H. bolina* (Rutowski 1992). In unchilled *H. bolina*, the decision to leave the territory is more likely to be made as a consequence of immediate 'panic' upon release. This behavior appears similar to the defensive or evasive response of butterflies to failed predatory attacks, or failed capture attempts. Chilled butterflies, however, remain in their territory area for quite some time whilst warming up, and therefore any active decision to abandon the site must be made at some later stage. On average, the difference between groups after marking is that unchilled butterflies must decide whether to return to their former territory, and the chilled butterflies must decide whether to leave. If both these decisions are sufficiently unlikely, then discrepancies will exist between the recounts of chilled and unchilled group individuals, leading to the results observed in this study.

In theory (depending on specific post-capture behavioural responses), this principle may hold for a wide variety of butterfly species being sampled in a wide variety of circumstances. Indeed, both Lederhouse (1982) and Singer and Wedlake (1981) suggested that initial dispersal or displacement from the site of capture may have accounted for their observed capture effects in butterflies handled without chilling. The advantages of chilling may therefore lie in the prevention of early dispersal immediately following capture, as implied by Wickman and Jansson (1997). Since 'equal catchability' of caught and uncaught individuals is a critical assumption of mark-release-recapture programs, this method has the potential to greatly increase the accuracy of subsequent population estimates. On this basis, in studies that intend to employ mark-release-recapture techniques, the actual method of handling may prove a more meaningful consideration than the question of whether or not to handle.

ACKNOWLEDGMENTS

Thanks are due to Dr. C. J. Hill and Prof. F .S. Chew for first suggesting the potential benefits of cooling butterflies before release to D. J. K. Prof. R. E. Jones kindly offered suggestions and criticized an earlier manuscript. This research was supported by an Australian Postgraduate Research Award to D. J. K.

LITERATURE CITED

BEGON, M. 1979. Investigating animal abundance: capture-recapture for biologists. University Park Press, Baltimore.

BRUSSARD, P. F. & P. R. EHRLICH. 1970. The population structure of *Erebia epipsodea* (Lepidoptera: Satyrinae). Ecology 51:119–129.

BULL, C. M., M. P. ZALUCKI, Y. SUZUKI, D. MACKAY & R. L. KITCHING. 1985. An experimental investigation of resource use by female monarch butterflies, *Danaus plexippus* (L.). Aust. J. Ecol. 10:391–98.

COOK, L. M., K. FRANK & L. P. BROWER. 1971. Experiments on the demography of tropical butterflies I. Survival rate and density in two species of *Parides*. Biotropica 3:17–20.

CULLENWARD, M .J., P. R. EHRLICH, R. R. WHITE & C. E. HOLDREN. 1979. The ecology and population genetics of an alpine checkerspot butterfly, *Euphydryas anica*. Oecologia 38:1–12.

DENT, D. R. 1997. Quantifying insect parameters, pp. 57–109. *In* D. R. Dent & M. P. Walton (eds.), Methods in ecological and agricultural entomology. CAB International, Wallingford.

EBERHARDT, L. L. 1978. Transect methods for population studies. J. Wild. Mgt. 42:1–31.

GALL, L. F. 1984. The effects of capturing and marking on subsequent activity in *Boloria acrocnema* (Lepidoptera:Nymphalidae), with a comparison of different numerical methods that estimate population size. Biol. Cons. 28:139–154.

LEDERHOUSE, R. C. 1982. Factors affecting equal catchability in two swallowtail butterflies, *Papilio polyxenes* and *P.glaucus*. Ecol. Entomol. 7:379–383.

MORTON, A. C. 1984. The effects of marking and handling on recapture frequencies of butterflies, pp. 55–58. *In* R. I. Vane-Wright and P. R. Ackery (eds.), The biology of butterflies. Academic Press, London.

POLLARD, E. 1977. A method for assessing changes in the abundance of butterflies. Biol. Cons.:115–134.

REYNOLDS, D. R., J R. RILEY, N J ARMES, R. J. COOTER, M. R. TUCKER & J. COLVIN. 1997. Techniques for quantifying insect migration, pp. 111–145. *In* D. R. Dent, & M. P. Walton (eds.), Methods in ecological and agricultural entomology. CAB International, Wallingford.

ROSENBERG, D. K., W. S. OVERTON & R. G. ANTHONY. 1995. Estimation of animal abundance when capture probabilities are low and heterogeneous. J. Wild. Mgt. 59:252–261.

RUTOWSKI, R. L. 1992. Male mate-locating behaviour in the common eggfly, *Hypolimnas bolina* (Nymphalidae). J. Lepid. Soc. 46:24–38.

SEBER, G. A. F. 1973. The estimation of animal abundance and related parameters. 2nd ed. Griffin, London.

SINGER, M. C. & P. WEDLAKE. 1981. Capture does affect the probability of recapture in a butterfly species. Ecol. Entomol. 6:215–216.

SOKAL, R. R. & F. J. ROHLF. 1995. Biometry. 3rd ed. Freeman, New York.

SOUTHWOOD, T. R. E. 1978. Ecological methods with particular reference to the study of insect populations. 2nd ed. Chapman & Hall, London.

SUZUKI, Y. & M. P. ZALUCKI. 1986. Mate acquisition as a factor influencing female dispersal in *Danaus plexippus* (L.). J. Aust. Entomol. Soc. 25:31–35.

THOMAS, J. A. 1983. A quick method for estimating butterfly numbers during surveys. Biol. Cons. 27:195–211.

URQUHART, F. A. & N. R. URQUHART. 1976. A study of the peninsular Florida populations of the monarch butterfly (*Danaus p.plexippus*; Danaidae). J. Lepid. Soc. 30:73–87.

URQUHART, F. A., N. R. URQUHART & F. MUNGER. 1970. A study of a continuously breeding population of *Danaus plexippus* in southern California compared to a migratory population and its significance in the study of insect movement. J. Res. Lepid. 7:169–181.

WATT, W. B., F. S. CHEW, L. R. G. SNYDER, A. G. WATT & D. E. ROTHSCHILD. 1977. Population structure of pierid butterflies. I. Numbers and movements of some montane *Colias* species. Oecologia 27:1–22.

WICKMAN, P & P. JANSSON. 1997. An estimate of female mate searching costs in the lekking butterfly *Coenonympha pamphilus*. Behav. Ecol. Sociobiol. 40:312–328.

ZALUCKI, M. P. 1993. Sex around the milkweed patch—the significance of patches in host plants in monarch reproduction. *In* S. B. Malcolm & M. P. Zalucki (eds.), The biology and conservation of the Monarch butterfly. Museum of Natural History Contributions to Science, Los Angeles.

ZALUCKI, M. P. & R. L. KITCHING. 1985. The dynamics of adult *Danaus plexippus* L. around patches of its host plant *Asclepias* spp. J. Lepid. Soc. 38:209–19.

Received for publication 5 April 1999; revised and accepted 16 December 1999.