INTRODUCTION

There have been only a few studies focused on specific visual or olfactory functions of the pre-orbital gland in deer species, other than scent-marking in adult males (Schaller, 1967; Schaefer, 1972; Müller-Schwarze, 1975; Clutton-Brock et al., 1982; Macnamara and Eldridge, 1987; Burger, 2005; Rehorek et al., 2005).

ABSTRACT: Stressful manipulation by humans has been previously shown to result in opening the pre-orbital gland of the newborn red deer. We hypothesized that exposure of red deer to stressful handling would result in opening the pre-orbital gland. Further, we tested the potential factors associated with pre-orbital opening, including season, sex, age and struggling behavior. Pre-orbital gland status was observed in 76 red deer (48 males, 28 females) during 281 handling events with the animal isolated and fixed in a mechanical restraint cradle (crush) within 3 consecutive years. The deer age ranged from 1 to 5 yr in males, and from 1 to 11 yr in females at the beginning of observations. The proportion of handling events with an open gland was considerably greater during than before handling (27.76 vs. 0%, respectively, \( P < 0.001 \)). The deer that struggled or stiffened revealed 2-fold greater incidence of pre-orbital opening (\( P < 0.01 \)), regardless of sex. Thus, pre-orbital opening seemed to accompany the behaviors that have been found to be related to acute stress. The probability that the animal opens its pre-orbitals during handling sharply increased at the beginning of the rut (mating season, \( P < 0.01 \)). Deer that threatened the handler (10% out of 215 observations) opened their pre-orbitals 30 times more often than nonthreatening ones. These effects indicate that other motivations are likely involved in the opening of pre-orbitals, in addition to the perception of a stressful event. Incidence of pre-orbital opening decreased with age in both sexes (\( P < 0.05 \)) across 3 consecutive yr of observations, which suggests habituation to repeated handling. Sex of the animal did not influence the probability of pre-orbital opening. We found significant variability in pre-orbital opening across the individuals (\( P < 0.001 \)). In conclusion, we confirmed an association between opening of the pre-orbital gland and stressful handling in sub-adult and adult red deer, although repeated human handling did not elicit such incidence of pre-orbital opening as found in newborn red deer calves. Our results support multifactorial origins of pre-orbital opening and prompt the necessity of further research to distinguish between different motivations that might have been involved in opening of pre-orbitals.

Key words: Cervus elaphus, handling, pre-orbital gland opening, red deer, stress
In newborn red deer calves, the primary function of pre-orbital secretion is establishing mother–offspring bonds (Hatlapa, 1977). Pre-orbitals open in dominant adult stags when roaring during the rut (Volkman et al., 1978; Bartoš, 1983) and in both sexes during fighting or threatening (Bartoš, 1983). In red deer calves, pre-orbital opening has been associated with feeding and achieving satiety (Wölfel, 1976; 1983; Bartoš et al., 2005).

In our previous study, stressful manipulation by humans was shown to result in opening the pre-orbital gland of the newborn red deer (Bartošová-Víchová et al., 2007). This led to the suggestion that the pre-orbital opening could serve as a good indicator of the stress of the calf (Bartošová-Víchová et al., 2007). Individual handling in the restraint cradle (crush) produces both behavioral and physiological signs of acute (short-term) stress in adult deer even when manipulated repeatedly and habituation was likely to have occurred (Diverio et al., 1993; Carragher et al., 1997; Chaya et al., 2006). The deer strictly avoided the restraint cradle if they were given a free choice (Y-maze test; Pollard et al., 1994). Therefore, in the present study, we continued investigating the relationship between stress and pre-orbital opening in sub-adult and adult red deer when handled in the restraint cradle.

We hypothesized that exposure of red deer to stressful handling would result in opening the pre-orbital gland. If our hypothesis was true, pre-orbital opening would be of great importance for deer farming practice as a handy indicator of stress. Further, we tested the factors possibly associated with pre-orbital opening, including sex and age of the deer, season, duration of handling, and behavior of the animal during manipulation.

**MATERIALS AND METHODS**

This study received approval for animal use and care from the Institutional Animal Care and Use Committee of the Institute of Animal Science (No. 13803/2003-1020) in the Czech Republic and was conducted in accordance with Czech Central Commission for Animal Welfare.

**Animals and Observations**

In total, 76 red deer (48 males, 28 females) were observed at the experimental farm of the Institute of Animal Science at Podlesko (N 50°3.027′ E 14°35.625′, Prague, Czech Republic) within 3 consecutive years (2008 to 2010). At the beginning of observation, the hinds (females) were aged from 1 to 11 yr and males from 1 to 5 yr; maximal age of the deer within the 3 yr of observations was 14 yr in females and 5 yr in males, due to farm management and animal selection. Most deer were observed repeatedly, up to 9 times in males, depending on management needs of the deer at the farm. The deer were accustomed to human presence and daily routine. They were recognized individually according to colored and numbered collars and ear tags. All the deer were born and spent their entire life at the same farm. They had been handled in the same way and intensity from birth.

Pre-orbital gland opening was observed and recorded using a dictaphone by 1 observer during manipulations in which the animal was isolated and fixed in a mechanical restraint cradle (crush), which worked as follows: A group of deer (ranging from 1 to 15 individuals) was driven by 3 to 4 people from the paddock through a corridor and into a small pen with solid wooden walls. Up to 6 deer were then moved to the sheltered distributor adjacent to the restraint cradle by 1 trained handler (always the same person) and individually pushed or lifted (in the case of an animal stiffening to the restraint cradle. Handling in the restraint cradle consisted of various sets of procedures according to purposes of deer management or other experiments. Each handling included weighing of the deer, blood sampling (8 mL of blood were collected via jugular venipuncture) and collar adjustment or change. Additional manipulations included rectal fecal sampling, ear-re-tagging or antler measuring (in males). Each deer rejoined the group in the enclosure after it was released from the restraint cradle. No additional stressors were applied to the deer beyond the routine practice.

Because the set of manipulations within 1 handling event varied, we used duration of handling (i.e., time from entering to leaving the restraint cradle in seconds) as the variable describing amount of manipulations the individual received.

Pre-orbital gland opening (open or closed) was recorded: in the distributor before the deer was pushed to enter the restraint cradle, at the time the deer entered the restraint cradle, during handling and when leaving the restraint cradle. In addition, we recorded the behavior of the deer in the restraint cradle. We adjusted the method used by Chaya et al. (2006) in calves to our observational capacity, so that we classified deer as struggling (at least 1 struggle after being fixed into the restraint cradle) or quiet (no movement). We also recorded the duration of each particular handling event (for details see Statistics section), and if the deer threatened the handler in the distributor by direct gazing, by head threat or by teeth grinding.

Females were handled routinely 3 times per yr at our farm: in early spring (March), at the very beginning of the rut (early September) and after the rut (November). In contrast, males were handled once a month between April and November, except during the full rut (late September and October). The handling of the males covered periods of antler growth from April to August, the beginning of the rut in early September and after the rut in November. All the females or all the males at the farm were handled on the same day. Within 3 yr, the status of the pre-orbital gland opening was confirmed in each animal.
was observed during 6 handling sessions for females and 9 handling sessions for males. Handling sessions throughout the year were further classified into seasons (spring/summer, early rut and after rut).

The pre-orbital gland was considered as open when the back of the gland (a pale spot) was visible and was considered closed when no slit could be distinguished. (For pictures of closed and open pre-orbital glands, see Bartošová-Vichová et al., 2007). Although we recorded different levels of pre-orbital opening (e.g., blinking, one-shot brief openings), only 2 summary categories could be analyzed according to the sparse data distribution: whether the deer opened its pre-orbitals during handling or not (variable pre-orbital gland opening with levels yes or no).

Statistics

Data were analyzed using the SAS system (SAS Inst. Inc., Cary, NC). We applied GLM for categorical data analysis based on the generalized estimating equation (GEE) approach processed in the GENMOD procedure (Stokes et al., 2000) to test the effect of explanatory variables (see below) on pre-orbital gland opening (dependent variable). As it was a binary response (yes or no), we chose the logistic regression model with appropriate parameters (i.e., binomial distribution and logit link function). The $\chi^2$ tests (using FREQ procedure in SAS) were calculated for simple associations between variables and whenever the data did not allow fitting the GLM models. Such was the case of assessing individual variability and consistency in pre-orbital opening across the sessions when the model did not converge. For this we only used deer with at least 5 records of pre-orbital gland status ($n = 23$ deer, from 5 to 9 observations per animal). We set the level of consistent response at the 75% quantile (Q3) of one pre-orbital response (either open or closed). We used a 0.05 level of significance; otherwise the results were reported as non-significant (n.s.).

The factors with potential influence on the pre-orbital gland opening during handling were analyzed in 2 steps.

First, on the whole data set ($n = 281$), we set up a generalized linear model (“overall model,” PROC GENMOD, SAS) to test the effects of the deer sex (male or female), age at handling (years) and season (spring/summer, early rut or after the rut) on the probability that the deer opens its pre-orbitals during handling (dependent variable; the probability was estimated from the binary records of pre-orbital gland opening: yes or no). The deer identity entered the model as a random effect via the REPEATED statement so that the repeated measures on the same individuals were properly treated (all measures from 1 individual across the years of observations were taken as repeated measures rather than the measures within each year, because of obvious intra-individual correlation of the data collected on 1 subject; Chaya et al., 2006).

Tests for specified hypotheses concerning the model parameters for explanatory variables were constructed via the ESTIMATE statement. The effect of the deer age was explored as a continual variable (as a covariate). The classification of the age by maturity to yearlings, sub-adults (up to 3 yr of age) and adults (Lincoln, 1971; e.g., Adam and Robinson, 1994) did not work in most of the analyses due to sparse data in particular classes; thus, it was applied only once in the case of separate analysis of pre-orbital gland opening in males (see below).

Second, the data set was reduced to observations with known information about animal behavior in the restraint cradle and duration of handling ($n = 201$; 101 for males, 100 for females). The effects of year of observation, waiting time (from closing the group of deer in the pen to the beginning of pushing an animal into the restraint cradle), loading time (from the beginning of pushing the deer into the restraint cradle to the deer entering the restraint cradle), handling duration (from entering to leaving the restraint cradle) and behavior in the restraint cradle (struggling or quiet) were added to the model estimating the probability of open pre-orbital gland (dependent variable). Separate models were fitted for each sex because of unbalanced data collected for males and females according to particular behavioral variables. Threatening behavior of the deer toward the human handler was not included in the model due to low incidence (20 cases; 7 in males and 13 in females) and mainly sparse distribution (the effect failed to be estimated by the model).

A multivariate general linear mixed model (GLMM, PROC MIXED, SAS) treated with repeated measures on a particular individual (RANDOM statement) was fitted to detect factors associated with loading time (dependent variable; i.e., variable characterizing avoidance of the deer to enter the restraint cradle). The tested fixed effects were sex of the deer, year of observation, pre-orbital opening during handling (yes or no), behavior (struggling or not) and threatening the handler (yes or no). Age of the deer and waiting time were entered in the model as covariates.

The effects associated with threatening the handler in manipulated animals (yes or no) were tested using a logistic regression model (PROC GENMOD, SAS). We modeled the probability that the deer threatens the handler (dependent variable). The explanatory variables were deer age and sex (age nested in sex, due to large differences in age of males of females due to farm reasons), pre-orbital gland opening during the particular handling (yes or no) and season.

RESULTS

Altogether we recorded 281 observations of the pre-orbital gland status (154 in males and 127 in females) on 76 individuals (48 males, 28 females). The overall incidence of opened pre-orbitals during an handling event was
27.8% and did not differ between the 2 sexes (27.9% for males, 27.6% for hinds; \( \chi^2_{(1)} = 0.05 \), n.s.).

All of the deer had their pre-orbitals closed before the handler started to move them from the distributor to the restraint cradle. Thus, the proportion of observations with an open gland was considerably greater during than before handling (27.7 vs. 0%, respectively; \( \chi^2_{(1)} = 90.41, P < 0.001 \)).

Most of the deer that opened their pre-orbitals (n = 78 handlings) did so in the beginning of handling (i.e., when entering the restraint cradle). Only in 8 cases did an animal open its pre-orbitals later during handling. We recorded only 4 cases when the animal did not close its pre-orbitals before the handling was finished.

In 54 (24.8%) of 218 observations for which we recorded behavior of an animal in the restraint cradle, the animal struggled. Moreover, for 36 occasions (17.3%) the deer exhibited strong resistance to entering the restraint cradle by stiffening (taking from 1 to 12 min of loading time until it was successfully moved to the restraint cradle). Only one third of observed handlings (n = 12) contained both struggling and stiffening. Struggling deer compared with not struggling revealed 2-fold incidence of pre-orbital opening (46.3 vs. 23.2%; \( \chi^2_{(1)} = 10.52, P < 0.01 \)), with no significant differences between the sexes (27.9 vs. 27.6%). We found very similar results for reluctance to enter the restraint cradle (stiffening; data not shown).

We found significant variability in pre-orbital opening across the individuals (\( \chi^2_{(22)} = 61.98, P < 0.001 \)). From 23 deer that were observed at least 5 times (5 males, 18 females), 16 (69.6%) revealed consistency in pre-orbital opening (at least 75% of 1 response, open or closed), with 7 deer having 0 incidence of the alternative response (2 animals opened their pre-orbitals in all observations, 5 in none). Due to low numbers and thus low reliability, we abandoned the idea of detailed analysis on this matter. Nevertheless, individuality of the animal on which the observations were made repeatedly should be taken into account when analyzing pre-orbital gland opening.

When treated for repeated observations on the same individuals (overall model), the probability that the animal would open its pre-orbitals during handling was significantly affected by the season (\( \chi^2_{(2)} = 11.33, P < 0.01 \), year of observation \( \chi^2_{(2)} = 6.93, P < 0.05 \) and age \( \chi^2_{(1)} = 4.56, P < 0.05 \)). Probability of pre-orbital opening was greater in early rut than either in spring/summer or after the rut (Figure 1a), regardless of the deer sex, and it decreased during 3 consecutive years of observations (Figure 1b). In both sexes, probability of pre-orbital opening decreased with age (In males: slope = -0.30; \( Z = -2.04, P < 0.05 \). In females: slope = -0.13; \( Z = -1.98, P < 0.05 \)); the difference between males and females only approached statistical significance (\( \chi^2_{(2)} = 4.99, P = 0.08 \); see Figure 2). Sex of the animal did not influence the probability of pre-orbital opening (\( P = 0.87 \)).

![Figure 1.](image)

**Figure 1.** Probability that the deer opens its pre-orbitals during handling according to the (a) season and (b) year of observation [binomial generalized linear model, PROC GENMOD (SAS Inst., Cary, NC), n = 281]. n.s. = non-significant.

### Pre-orbital Opening in Males and Females

In males, probability of pre-orbital opening during handling was associated with season (\( \chi^2_{(2)} = 10.20, P < 0.01 \); Figure 3) and behavior (\( \chi^2_{(1)} = 4.83, P < 0.03; n = 110 \)). Quiet males (n = 80 observations) had a reduced probability \( (0.26 \pm 0.07) \) of opening their pre-orbitals than struggling ones \( (0.51 \pm 0.13, n = 30) \). However, the probability of pre-orbital opening predicted for males followed the same declining trend within 3 subsequent years of observation as found in the overall model \( (0.42 \pm 0.10, 0.20 \pm 0.13 \text{ and } 0.18 \pm 0.14) \) but did not reach significance \( (P = 0.23) \). This was most likely due to extended SE indicating large individual variability among males or other factors in the model being of greater influence on pre-orbital opening. The effect of age was not significant; however, the probability of opening in adults tended to be less than in sub-adult deer when treated as class variable \( (0.20 \pm 0.09 \text{ vs. } 0.32 \pm 0.13, P = 0.21) \).

In females, the probability that the hind opened its pre-orbitals during handling was not influenced by any of the tested factors describing behavior or duration of handling. It was only marginally affected by year of observations \( (\chi^2_{(2)} = 5.04, P = 0.08, n = 106) \), with decreasing trend over the consecutive years (for 2008, 2009 and 2010, respectively,
0.40 ± 0.10, 0.30 ± 0.11 and 0.14 ± 0.14). Compared with the overall model, the season effect in hinds contained only 2 amounts for which we had behavioral observations (early rut and after the rut; predicted probabilities were almost the same as those computed by the overall model: 0.28 ± 0.10 in early rut, and 0.20 ± 0.06 after the rut).

Pre-orbital Opening and Behavior during Handling

Probability of struggling during handling varied significantly only with season ($\chi^2 (2) = 14.13, P < 0.001; n = 201$). About one third of the deer struggled during spring/summer and also in early rut (0.34 ± 0.08 and 0.32 ± 0.1, respectively), whereas this behavior significantly declined after the rut (0.07 ± 0.06). We recorded the same pattern when stiffening was added to struggling and modeled as 1 variable ($\chi^2 (2) = 8.81, P < 0.02$); the deer exhibited a similar level of struggling and reluctance behavior during spring/summer and when the rut had started (0.40 ± 0.08 and 0.44 ± 0.08, respectively), whereas it was only 0.21 ± 0.06 in post-rut period. Thus, it seems that the deer expressed stiffening rather than struggling after the rutting period. The length of stiffening (loading time to the restraint cradle) was not associated with any of the observed variables. As such, it most likely expressed individual deer differences in the level of fear or resistance to being handled.

Threatening the handler was observed in 20 occasions (13 in males, 7 in females) out of 215 observations with known information. The probability that the animal would threaten the handler was significantly associated with its age and sex (age nested in sex, $\chi^2 (2) = 6.83, P < 0.04$) and also with pre-orbital opening ($\chi^2 (1) = 4.75, P < 0.03$). Males threatened the handler considerably more often (0.28 ± 0.09) than females (0.01 ± 0.01), in both cases with increasing incidence as the deer aged (In males: slope = 0.61; $Z = 2.89, P < 0.01$. In females: slope = 0.33; $Z = 2.70, P < 0.01$; Figure 4). Threatening deer had their pre-orbitals open more often (0.16 ± 0.06) than non-threatening ones (0.05 ± 0.05). Threats were seen only once in a particular deer, except for 2 females that threatened repeatedly (4 times out of 4 and twice out of 3 observations of the pre-orbital gland status). There was no relationship between threatening the handler in the distributor and subsequent behavior (struggling or stiffening) in the restraint cradle.

DISCUSSION

In agreement with our expectations, opening of the pre-orbital gland in red deer was associated with handling of the animal. The pre-orbital gland was closed before the manipulation started, whereas the deer in more than one fourth of the observed handling events opened their pre-orbitals when subjected to a set of stressful manipulations, including isolation from other herd members by the human handler, pressing it to the restraint cradle, weighing and blood sampling. Further, we found individual differences in pre-orbital response to handling among deer, as well as significant effects of other factors, such as season, year of observation and age of the animal.

In most cases (90%) the animal opened its pre-orbitals at the very beginning of handling. Only in 8 cases did the pre-orbital opening emerge later during handling as reaction to a painful procedure (ear perforation during re-tagging or blood sampling). It indicates that pre-orbital opening was not associated exclusively with painful intervention, but it also reflected human presence in proximity to the animal and manipulation per se.

Pre-orbital opening apparently varied in its length and intensity. In all but 4 cases, pre-orbital opening vanished during handling, and the gland was closed by the end of handling. Also struggling, if expressed, started at the beginning and disappeared within initial period of handling. It may reflect habituation to the handling event. In both
sexes, incidence of pre-orbital opening was significantly associated with struggling and also with the other repeatedly observed behavior, stiffening. When struggling or stiffening (only one third of handlings included both behaviors), the deer opened their pre-orbitals twice as often as the quiet deer and those taking less time to enter the restraint cradle. Thus, pre-orbital opening seemed to be similarly expressed as the behaviors that have been found to be related to acute stress in animals (Morgan and Tromborg, 2007), including red deer (e.g., Diverio et al., 1999; Carragher et al., 1997). In our study, the significance of struggling as an indicator of aversive perception of a handling procedure was supported by the more minor influence of tested factors [i.e. age and sex of the animal, year of observation or duration of handling event, and the probability of whether the animal would struggle when handled or not (except for the period after the rut; see later in discussion)].

Opening of pre-orbital gland and behavior (struggling) showed different seasonal patterns, however. Struggling in males kept similar incidence between spring and early rut and dropped after the rut in both sexes. It seems that struggling during handling reflected exhaustion of the deer from the rutting period. Pre-orbital opening, as distinct from struggling, increased sharply only for rutting time, and it was not different from that observed during spring/summer period. Despite the very low amount of struggling when the rut is over, the animal may still perceive even routine handling as stressful or at least a discomfort, provoking a pre-orbital opening response. Unlike struggling, pre-orbital opening does not seem to be costly behavior in terms of energy input. Thus, comparable incidence of pre-orbital opening after the rut and in spring/summer may reflect similar amounts of stress in handled deer throughout the year (except for the rut).

Increase in pre-orbital opening with forthcoming rut was particularly prominent in males and had been already observed in red deer males in a social context (Bartoš, 1983). This increase most likely arose along with a greater general excitability and sensitivity of the deer during the reproductive season. Regardless of the season of the year, pre-orbital opening was more frequent in observations where the deer was recorded as threatening the handler (via head posture, direct gaze, characteristic teeth grinding, etc.). It demonstrates a different approach of the animal, in terms of fight-or-flight reaction, and it likely indicates an assessment of the dominance position toward the handler. Threatening behavior toward the human handler seems to correspond to intraspecific behavior in adult deer, showing a clear, positive relationship between pre-orbital opening and dominant behavior (Bartoš, 1983).

These findings (seasonal effect and threatening the handler) support our earlier suggestion of the multifactorial origin of pre-orbital opening (Bartoš, 1983; Bartoš et al., 2005) and prompt the necessity of further research to distinguish between different motivations that might have been involved in opening of pre-orbitals. Nevertheless, repeated human handling does not seem to elicit such incidence of pre-orbital opening as found under other motivations. The proportion of handling events with open pre-orbitals in our study (28%) was less than that observed not only in handled newborn calves (100%, Bartošová-Vichová et al., 2007) but also in feeding context in bottle-fed calves (77%, Bartoš et al., 2005) or during rutting behavior in adult males (72%, Bartoš, 1983).

Incidence of pre-orbital opening was almost the same in both sexes (27.9 vs. 27.6%). Nevertheless, in both sexes incidence of pre-orbital opening decreased with the age of the animal, decreasing more sharply in males. We are, however, far from being able to make definitive conclusions about sex-dependent differences, at least because males and females at our farm have been managed and manipulated in different ways (males with greater frequency than females). In addition, our males and females substantially diverged in age range (males from 1 to 5 yr, females from 1 to 14 yr).

Age of the animal reflected the number of handlings it had been given through its life. Thus, it indicates that—at least in terms of pre-orbital opening—habitation to repeated handling that was found in red deer hinds (Diverio et al., 1996) also occurred in our deer. The role of habituation is further supported by considerable decrease in pre-orbital opening during 3 consecutive yr of observations. Our observations began, coincidentally, when the extensive handling of the deer had just started.

Unlike pre-orbital opening, struggling revealed no significant relationship with age or yr of observation, regardless of the sex of the animal. This was also the case with passive avoidance toward entering the restraint cradle via stiffening. However, there was, even if non-significant, a trend of changing the proportion of struggling in favor of stiffening with the age of the animal. Thus, it indicates subtle behavioral changes in repeatedly handled deer,
which should be addressed in further research. We speculate that pre-orbital opening might be a better indicator of the animal’s perception of human handling than behavior itself. An in-depth study that also includes physiological indicators of the stress of the animals is needed in this area.

As already mentioned, attention also should be paid to aggressive behavior toward the handler, which was demonstrated in nearly 10% of handlings in our study. It may signify potentially serious danger of injury for the handler. Therefore, it is very important to detect predictors of such a behavior in the deer. As expected, threatening the handler was more frequent in older deer and predominantly occurred in males. However, we noted 2 adult females routinely threatening the handler. Additional indicators of potentially risky behavior could also be an open pre-orbital gland of the animal. In context of social interaction, pre-orbital opening in dominant deer was detected only when investigating a series of quick-shot photographs taken during individual interactions (Bartoš, 1983). Therefore, pre-orbital glands should be video-recorded for purposes of future studies. If the results of this study are to be confirmed, the handler should be thoroughly trained to recognize even quick pre-orbital opening in a handled animal.

In addition to the tested factors, we found significant individual differences in pre-orbital opening among the observed animals. The proportion of handlings with pre-orbital opening among individuals ranged from 0 to 100%. However, 70% of individuals were consistent in their pre-orbital response.

In conclusion, we confirmed an association between opening of the pre-orbital gland and stressful handling in red deer. Our results support the presumption that pre-orbital opening might work as a simple and easily recognized indicator of deer stress for farmers. However, this should be addressed in further research, as motivations other than stress or fear during handling were apparently involved in pre-orbital gland opening elicitation.

LITERATURE CITED


