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FORUM

Laterality in octopus eye use?

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In recent papers, Byrne et al. (2002, 2004) have published interesting results suggesting lateralization of (monocular) eye use in octopuses. They point out that, 'lateralization is thought to have evolved only in vertebrates' (Byrne et al. 2002, page 461), and that any demonstration of lateralization in cephalopods would suggest that lateralization 'might be a common principle that evolves when bilaterally symmetrical neuronal systems must cope with complex sensory inputs' (Byrne et al. 2004, page 1107). This is an interesting claim, plausible in that octopuses are noted for their complex nervous systems (Boyle 1986) and behaviour (Hanlon & Messenger 1996). But did the authors show laterality in octopuses? This critique focuses on their data analyses because it appears that a common statistical error in the analyses make it difficult to verify the authors' claims.

In both papers, octopuses were individually filmed for 1-h sessions on 5 different days ($N = 8$ octopuses in Byrne et al. 2002; $N = 25$ octopuses in Byrne et al. 2002). Single frames were selected at 10-s intervals for analysis; only frames in which the octopus was attached to the front of the tank were used. Each paper presents graphically the proportion of time each octopus used its left eye, its right eye, or (rarely) both eyes (Byrne et al. 2002 only). Other behavioural data collected by Byrne et al. (2002, 2004) were analysed similarly and are subject to the same concerns as raised below.

To evaluate the significance of the octopuses' eye preference, chi-square tests were carried out, comparing the number of instances of use of each eye, overall. Based on these tests, the authors concluded that all eight octopuses in Byrne et al. (2002) and 23 of 25 octopuses in Byrne et al. (2004) showed a significant preference for one eye. (Note: some subjects were used in both papers.)

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The sampling regime in these two experiments was, in effect, a form of scan sampling in which behaviour was recorded for analysis every 10 s. Each 10-s sampling interval was then used as a separate data point and added to a cell count for a chi-square analysis (left eye used versus right eye used). According to Martin & Bateson (1993, page 86), 'If scanned samples are to be used as separate data points… they must be statistically independent of one another…'. They go on to say, 'clearly, scan samples taken at, say 30-s intervals would not constitute independent measures.' The 10-s sampling interval used by the authors could well lead to data points that are not independent, therefore. Byrne et al. (2004, page 1109) state, 'The shortest bouts of left, right or binocular eye use were 11 s long, so we feel confident that, with an interval of 10 s, the independence of data (Martin & Bateson 2000 [sic]) was ensured'. This argument appears to be based on a misreading of Martin & Bateson's text. Martin & Bateson's (1993, page 91) suggestion that sample intervals should be as short as possible and the duration of the behaviour pattern should be long relative to the sample interval were suggestions for instantaneous sampling. Both instantaneous and one-zero sampling yield proportions that Martin & Bateson caution cannot be treated as statistically independent measures. Certainly, if the bouts were always longer than the sampling interval (in this case, bouts at least 11 s, sampling interval 10 s), then adjacent points could not possibly have been independent of one another.

How important is statistical independence for chi-square analyses? Kramer & Schmidhammer (1992) published a clear explanation of appropriate use of the chi-square statistic in ethological studies. A key pitfall, they point out, is lack of independence: 'the observations that are summed to yield the cell frequencies must be independent of each other' (page 834). And, violations '…will result in rejecting true null hypotheses more often than assumed with the selected alpha level' (page 837). Violations of independence, then, are an important problem for chi-square analyses (as well as for other statistical analyses; see any standard statistics text).

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Table 1. Sample size needed to detect a significant lateral bias for $\alpha = 0.05$ and power of 0.80

Expected bias of individuals	Minimum sample size needed
0.90	10
0.80	20
0.70	48
0.60	200

So have the authors showed laterality in eye use? Based on these two papers, the answer appears to be no. The published proportions of eye use in individuals (Figure 3a in Byrne et al. 2002; Figure 5 in Byrne et al. 2004) provide enough data to reject any population-level bias for use of a particular eye, as the authors recognized (Byrne et al. 2004), but do not provide enough information to evaluate individual preference. The authors' conclusion that octopuses show antisymmetrical distribution of eye preference (Byrne et al. 2004) hinges on whether or not individual octopuses have significant preferences in eye use, so this population-level conclusion is also in question.

One solution to this analytical problem would be for the authors to reanalyse their own data. Unfortunately, their sampling design lends itself most directly to a sample size of five, the number of sessions videotaped per octopus, which is too small a sample size for detecting lateralization. How large a sample size is needed? Table 1 provides some guidelines, assuming a null hypothesis of no preference versus a two-tailed alternative and the range of reported biases (Byrne et al. 2002, 2004). Entries were computed by interpolating from the master table given in Kraemer & Thiemann (1987); for greater depth, see Cohen (1977).

If the authors wish to subsample the taped data set differently, they will have to show that the behaviour at one sampling point is truly independent of the behaviour at the next sampling point. How can we be sure the individual observations are independent? One reasonable criterion would be that the individual animal changed position completely and then repositioned itself between observations. Octopus eye use is likely to be sensitive to den orientation, the location of the octopus within its tank and within the laboratory, the location of recent activity in the laboratory, and probably a number of other variables. These variables also would need to be addressed; for example, by controlling detectable activity and noise (Hanlon & Budelmann 1987) within the laboratory, systematically varying den orientation and tank position within the room, and recording not just which eye the octopus was using, but also where within the tank the octopus was sitting.

We conclude that lateralization in octopuses would be a truly interesting finding, but it will be challenging to establish.

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