Environmental monitoring and fluctuating asymmetry

Study design

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ABSTRACT

This report identifies the critical issues, including associated risks, that will need to be considered if fluctuating asymmetry (FA) is to be tested as an environmental monitoring technique, for example in measuring stress in birds and other animals induced by environmental disturbances. After a brief introduction to the biological theory behind FA, issues surrounding experimental design are examined, and areas where it could be of use to the Department of Conservation are identified: heterozygosity; environmental stress; competitive stress; pathogen stress; and toxicity. Choosing appropriate study populations that provide suitably large sample sizes will be critical. Methodological issues considered include: multiple traits; measurement accuracy; measurement reliability; unbiased data recording; and other environmental indicators. Recommendations are given for several areas of concern: taking FA measurements; software; FA assumptions; size dependency; injury; calculating indices. A proposal is outlined for a pilot study as the next stage in evaluating FA. This should be done under field conditions, using a suitable sample population being assessed under other environmental monitoring techniques.

Keywords: environmental monitoring, risk management, environmental stress, indigenous birds, monitoring techniques, fluctuating asymmetry

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1. Introduction

1.1 PURPOSE

The purpose of this report is to provide operational scientific advice to the Department of Conservation (DOC) on the merits and uses of fluctuating asymmetry (FA) as an environmental monitoring technique.

1.2 OBJECTIVES

1. To consider issues surrounding the design of an experimental programme that can convincingly test the usefulness of FA.
2. To consider an experimental methodology that can both accurately and reliably assess levels of FA in populations of native organisms.
3. To consider issues of data collection, data treatment, and appropriate statistical analyses.
4. To make recommendations on issues to address when designing a study to test the utility of FA as an environmental monitor.

1.3 THEORETICAL BACKGROUND TO FLUCTUATING ASYMMETRY

Fluctuating asymmetry (FA) is defined as the non-directional difference between left- and right-sides of paired bilateral traits (Van Valen 1962), and is often used as a measure of an organism's ability to buffer its development against disturbances (developmental stability; Palmer & Strobeck 1986; Parsons 1990). Because the same genes control paired morphological characters on both the left- and right-sides of an organism, the random deviations from bilateral symmetry that produce FA may be used as an indicator of developmental stability.

For example, imagine a population that is experiencing temperature extremes in their environment. In this population there will be variation between individuals in the ability to cope with fluctuations between very high and very low temperatures. Individuals that cope poorly with temperature stress will experience disruptions to their development that could negatively affect health and survivorship; and these individuals will also manifest relatively large physical asymmetry as a result of their body attempting to cope with the stress. However, individuals that cope well in the extreme environment are less likely to experience negative health consequences, and will have lower asymmetry.

FA provides a useful measure of variation because the effects of environmental stress can be compared to the morphological ideal of perfect symmetry (Watson & Thornhill 1994).

1.4 USING FLUCTUATING ASYMMETRY IN ENVIRONMENTAL MONITORING

Support for FA as an indicator of stress has been found in studies where FA increases with environmental disturbances such as extreme temperatures (Parsons 1990), pollutants (Parsons 1990; Alekperov & Gashimova 1997), parasites (Bailit et al. 1970; Polak 1993), and homozygosity (Mitton 1993).

In addition to the predominantly natural experiments cited above, some researchers have conducted stress-inducing manipulation experiments that also demonstrate a relationship between FA and environmental stress. For example, Fair et al. (1999) elicited an immune-response in juvenile Japanese quail (*Coturnix coturnix japonica*) and found that health was reduced in stressed birds relative to unstressed controls (e.g. lower body mass and wing length in stressed birds). They also found that stressed birds showed greater FA in their primary wing feathers than did controls.

Using FA to assess organism quality in response to environmental factors is useful because FA provides a window into developmental stability and fitness before any changes in more direct measures of fitness can be detected (Clarke 1995). For example, population declines in some amphibians can be predicted by patterns of FA (Waldman & Tocher 1998). In addition, the relative ease of measuring FA is especially important in studying species that respond negatively to being bled, or to being handled for long periods of time. These features of FA are particularly interesting in a conservation context.

1.5 LIMITATIONS

It must be noted that not all studies support the use of FA as an environmental monitor. For example, refer to the mixed findings of studies summarised in supplementary tables for Møller & Swaddle (1997).

This report outlines some of the limitations of using FA that include:

- The technique provides useful results only in certain circumstances.
- The technique is relatively intensive in terms of labour, sample size, and data analysis.
- The technique needs to be confirmed as operationally feasible in comparison with existing environmental monitoring techniques.

2. Experimental design

2.1 STUDY POPULATIONS

2.1.1 Species types

FA research can be conducted on many different species. Researchers have investigated FA in taxonomic groups as varied as birds, reptiles, insects, plants, and amphibians. However, the choice of study populations *is* important, especially as it relates to the number of individuals available for study and the environmental conditions where they occur. Although researchers study FA as a measure of individual quality, the asymmetry profile of a population is made up of the FA characteristics of each individual. Therefore, studying FA can provide useful comparisons between populations that experience different environmental conditions.

2.1.2 Sample size

When choosing study populations, researchers *must* collect data from at least 30 individuals per sample, and preferably 40 or 50 if differences between study populations are likely to be small (Palmer 1994). Using smaller samples makes it unlikely that any differences in FA will be detected. In addition to requiring a relatively large sample size, within each sample at least three replicates are desirable. Researchers are more likely to detect levels of FA variation within and between study populations by using relatively large sample sizes and replicates.

2.1.3 Environmental parameters

Design of the overall research programme is driven by a number of issues that are relevant to sample selection. It is essential that only the environmental parameter of interest differs greatly between study populations. The following are examples of population differences that may be of interest in a conservation context:

- Heterozygosity: e.g. inbred island v. outbred mainland populations; reintroductions with few founders v. source populations.
- Environmental stress: e.g. high food and low predator abundance v. low food and high predator abundance; high v. low densities of defoliators; high v. low flow and temperature regimes in rivers.
- Competitive stress: e.g. low v. high levels of species competition (especially in island restoration contexts).
- Pathogen stress: e.g. low v. high incidence of pathogens and/or parasites.
- Toxicity: e.g. low v. high levels of pollution/contamination.

2.1.4 Natural and manipulated samples

These environmental parameters can be tested on both natural and experimentally manipulated populations. Study populations that are naturally occurring in appropriate conditions for the study are convenient in that there are fewer ethical concerns about manipulating organism stress. However, these natural populations rarely occur with all other parameters consistent between study populations as is necessary for a good test of FA as a tool for environmental monitoring. In contrast, it is easier to ensure consistency between study populations in a manipulation experimental design, but often more costly due to special housing requirements and maintenance costs. A comprehensive study design should include aspects of natural and manipulation experiments.

2.1.5 Joint research programmes

One way to maximise effort and minimise cost is to form a cooperative relationship with a research programme that is either already intending to collect FA information or working on a suitable project and receptive to collecting FA information with DOC's assistance.

The following researchers (and/or their students) have been conducting FA research in New Zealand (either currently, or within the last five years):

- James Briskie (University of Canterbury) on South Island robin.
- Charles Daugherty (Victoria University) on tuatara.
- Stephen Sarre (Massey University) and Mandy Tocher (DOC, Dunedin) on grand skinks.
- Bruce Waldman (University of Canterbury) and Mandy Tocher (DOC, Dunedin) on various species of amphibians.
- Steve Wratten (Lincoln University) on damselflies.
- Hugh Best (DOC) and Neil Gemmell (University of Canterbury) on New Zealand fur seals.

2.1.6 Pilot study

It is recommended that the experimental design be developed through a pilot study. A pilot study enables the assumptions of a design to be tested on a smaller scale without the capital investment necessary for a full programme. However, the sample requirements for a pilot study would be the minimum requirements for the larger programme. Further details about the value of a pilot study are covered in Section 5.

2.2 RISK MANAGEMENT

TABLE 1. SUMMARY OF THE POSSIBLE RISKS OF HAVING A POORLY DESIGNED STUDY. A CORRESPONDING RISK CONSEQUENCE AND RECOMMENDATION IS GIVEN FOR EACH RISK SCENARIO.

RISK TO ACHIEVING GOAL	CONSEQUENCE	RECOMMENDATION
No natural populations that differ in only one environmental stress parameter can be found.	Major	Mitigate by developing an alternative experimental design based on manipulation of environmental stress parameters.
Sample sizes of suitable natural or manipulated populations are too small to yield useful FA data (i.e. < 30).	Major	Prevent costs escalating with poor likelihood of goal being reached by not progressing further until suitable sample sizes can be found.
Operating costs of manipulation experiments are very expensive.	Moderate	Mitigate by forming a cooperative relationship with individuals involved in compatible research.
Ethical concerns with manipulation experiments prove prohibitive.	Major	Protect against by pursuing populations suitable for natural experiments. Mitigate by modifying the degree of stress manipulated.
The pilot study does not show any differences between samples that vary in level of exposure to environmental stress.	Terminal	Reconsider the usefulness of using FA as an environmental indicator.

3. Methodology

3.1 ISSUES

Researchers have been measuring FA for more than 35 years, but recent methodological and technological advancements have improved the ability to both accurately and reliably assess levels of FA. The differences between left and right sides are generally in the order of 1% of the overall trait measured (Møller & Pomiankowski 1993), thus making measurement precision especially important. Important factors to take into account when designing any methodology for FA research include:

- Use of multiple traits
- Acceptable measurement accuracy
- Acceptable measurement reliability (i.e. repeatability)
- Unbiased data recording

Designing a suitable experimental programme and collecting informative FA data is only part of the task of testing FA as a tool for environmental monitoring. A robust test of the utility of FA in this context requires that data using existing environmental monitoring techniques be collected concurrently. The only convincing test of the utility of FA as an environmental monitor for DOC is if the different types of data suggest the same environmental trends in a population. Therefore, only after a correlation is statistically verified between the results of existing environmental monitoring techniques and results of FA analysis can FA be considered to be an informative environmental monitor. Once such a relationship has been established, DOC can consider using the technique in the absence of other monitors.

3.1.1 Multiple traits

It has been hypothesized that different types of traits may manifest responses to environmental stresses through FA to varying degrees (for discussion see Palmer & Strobeck 1986; Palmer 1994; Leung & Forbes 1997). To this end, many researchers suggest measuring asymmetry between left and right sides in a number of different types of traits (Table 2).

TABLE 2. SELECTED TRAITS MEASURED IN PREVIOUS STUDIES OF FA.

SKELETAL	SOFT TISSUE	OTHER
Tibia length	Flipper length	Scale counts
Wing chord length	Digit length	Bristle counts
Tarsal segment	Wrist width	Various feather lengths
Tarsus length	Ear length	Dermatoglyphic measures

When a number of research staff are collecting data it is important that they all have a clear and consistent operational definition of each trait. For example, wing chord measurement can be defined as the length of the extended wing from the bend at the wrist to the tip of the longest primary (Fig. 1).

Composite indices of asymmetry have been found to indicate the underlying levels of responses to stress better than single traits (Dufour & Weatherhead 1996; Leung & Forbes 1997; Gangestad & Thornhill 1999) and should be calculated for all subsequent analyses (guidance on how to calculate composite indices is given in Section 4.1.6).

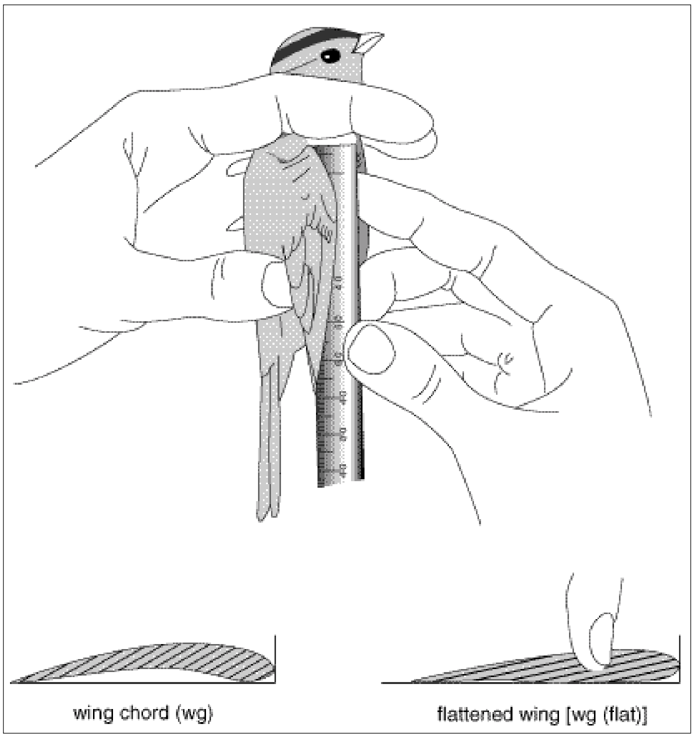

Figure 1. Measurement of the wing chord.

(Illustration taken from: US Department of Agriculture Forest Service General Technical Report PSW-GTR-144-Web: www2.psw.fs.fed.us/publications/Documents/gtr-144/04-constant.html)

3.1.2 Measurement accuracy

There are two basic types of traits: meristic (e.g. bristle counts) and metrical (e.g. measurement of bristle length). Meristic traits are easier to measure with little error and are often not sensitive to variations in body size or age (Palmer 1994). In contrast, metrical traits are necessarily measured with error, but variation is continuous and many more traits are available to the researcher.

Traditionally, callipers have been used to make metrical measurements. However, even trained researchers may show hand-bias in their measuring (i.e. always measuring a bird by holding it in the left hand and measuring the left wing or leg), thus yielding FA measurements that are more a result of their bias or experience than of trait asymmetry (Yezerinac et al. 1992). A recent innovation has provided means to avoid this bias by using images taken with a digital camera (refer Section 4.1.2 for related software). This digital method enables the researcher to take multiple photos of a trait (giving potential to check for data collection error), have measurements made at a different time by a measurer blind to the organism's identity, and to potentially decrease handling stress to the study organism. When using a digital camera it is important to always include a scale for calibration when making measurements digitally (a grid of about 100 mm square is usually suitable).

3.1.3 Measurement reliability

Because FA is usually manifest in small deviations from symmetry, measurement error can make collecting meaningful FA data impossible. Therefore, repeatability analyses (Krebs 1989) must be conducted to ensure that the between sample variation is significantly greater than the within sample variation. Traits that do not have significant repeatability should be excluded, as their inclusion will add noise to the statistical analyses. Measurement repeatability should be calculated for both absolute asymmetries (i.e. $|L - R|$) and composite indices. Measurements should be made at different times if possible, or nonsequentially if time does not permit otherwise. To minimise the effects of measurement error, analyses can be conducted on the mean asymmetry of three repeated measures for each trait (as recommended by Yezerinac et al. 1992).

3.1.4 Unbiased data recording

If life history information is to be used in concert with FA data, members of the population must be individually identified. If no life history information is available (or required), individuals must at least be marked after measurement to ensure that they are only measured once. Previous studies have used samples of birds with coloured leg-bands, reptiles with clipped toes, or fish with subcutaneous paint markings. Known populations ensure a minimum chance of confusing individuals and introduce the possibility of integrating individual histories into later analyses if desired (e.g. age, sex, past health and/or injury).

Blind collecting and recording of data is preferred, but it is often impractical in the field. An alternative is to have the data collector separate from the asymmetry measurer (an attractive characteristic of using digital cameras for data collection). Asymmetry measurers should be naïve to the specific identity and history of individuals sampled, and to the environmental treatment conditions.

After data collection it is often usual to identify outliers prior to analysis (using techniques like Mahalanobis outlier analysis*). However, in FA analysis it is inappropriate to exclude outliers because they are important for assessing variation in symmetry due to stress and fitness (Leung & Forbes 1997). One exception to this rule would be if the trait appeared to be injured (at measuring or in the past). In cases of injury refer to Section 4.1.5.

3.1.5 Other environmental indicators

A number of traditional environmental indicators are tabulated in Table 3. If we take fecundity in a bird species as an example, there are a number of quantifiable measures that could be assessed: number of eggs laid, hatching success, number of young fledged, number of clutches in a season, number of young recruited into the reproductive population.

TABLE 3. SELECTED CHARACTERISTICS USED IN ASSESSING LEVELS OF ORGANISM STRESS. THE SIGN IN THE BRACKETS INDICATES THE DIRECTION OF CORRELATION WITH THE WELL-BEING OF AN ORGANISM.

PHYSICAL	ENVIRONMENTAL
Blood stress hormones (–)	Food abundance (+)
Growth rate (+)	Inbreeding (–)
Parasite load (–)	Competition (–)
Mortality (–)	Percentage browse (–)
Fecundity (+)	Predator abundance (–)
Behavioural observations (–)	
Condition ratings (+)	
Heterozygosity (+)	
Pathogen infection (–)	

3.2 RISK MANAGEMENT

Predictions should be made about the relationships expected between FA and traditional environmental monitoring techniques. If these predictions are supported, and the experimental design is robust, DOC can consider further using FA as an environmental monitoring technique. However, if the predicted relationships between FA and other indicators of stress are not supported by the data, consideration of alternative explanations must be made; some examples given below:

- FA as measured is not a good indicator of environmental stress in the context investigated.
- The differences in FA in response to environmental stress are too small to be detected by the methods employed.
- There is unidentified noise in the data.
- The traits selected may not manifest FA in the manner predicted.

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* The following web site provides background to outlier analysis using Mahalanobis distances: http://www.jmpdiscovery.com/product/jmppoints/sixthdim/sixthdim.shtml

TABLE 4. SUMMARY OF THE POSSIBLE RISKS OF HAVING A POORLY DESIGNED STUDY. A CORRESPONDING RISK CONSEQUENCE AND RECOMMENDATION IS GIVEN FOR EACH RISK SCENARIO.

RISK TO ACHIEVING GOAL	CONSEQUENCE	RECOMMENDATION
The nature of the study species does not lend itself to collecting data on a number of traits.	Major	Reconsider selection of the study species, or think more creatively about available traits.
Research staff are inconsistent, inaccurate and unreliable when collecting data.	Terminal	Detect by testing repeatability of measurements early in the study. Prevent by ensuring that research staff are suitably trained in the techniques used. Reassess the nature of traits being measured and exclude traits that cannot be measured with significant repeatability. Prevent through measuring techniques that are less likely to introduce error (e.g. digital).
Skilled research staff are collecting biased measurements.	Terminal	Detect by testing repeatability of measurements early in the study. Prevent by ensuring blind procedures for data collection.
Study organisms are overly stressed during data collection.	Moderate	Use data collection techniques that are less intrusive to the species (e.g. using a digital camera for data collection).
Members of the study species are being unknowingly remeasured.	Terminal	Prevent by using a marked sample, or mark each individual after measurement.

4. Data collection and treatment

4.1 ISSUES

Not all variation in bilateral symmetry are incidences of FA (asymmetries normally distributed around a mean of zero), nor are all asymmetries useful in examining responses to stresses in the environment (Palmer & Strobeck 1992; Palmer 1994). There are two other major patterns of statistical variation in bilateral symmetry: *directional asymmetry* describes when asymmetries do not have a mean of zero; *antisymmetry* also describes bilateral difference, but the side that is larger varies at random.

Traits that do not conform to the assumptions of FA are generally considered inappropriate for use in examining hypotheses of environmental stress (Palmer & Strobeck 1992; Palmer 1994). However, recently some researchers have proposed that traits showing leptokurtosis (Gangestad & Thornhill 1999) and slight directional asymmetry (Leung & Forbes 1997; Leamy 1999) may also be appropriate for use in analyses.

Effects of unsuitable data treatment may confound study findings. For example, failing to assess measurement error is problematic in studies of FA because it can cause a FA-like pattern or artificially inflate observed FA (Palmer 1994). In addition, scaling data for trait size is necessary when a relationship between asymmetry and trait size is detected (Palmer 1994).

4.1.1 Making FA measurements

Measurements should be made by adhering to the principles of blind data collection and repeatability that have been discussed previously. Left and right sides of the same trait must be measured irrespective of whether data are collected by callipers, counts or digital camera. The research staff collecting the data should be well trained in the measurement techniques being applied. If data collection involves a number of people it is important to assess inter-individual consistency between techniques and measurements.

Data should be entered into spreadsheets with columns dedicated to subject identification and left and right trait size for all traits measured (including multiple measurements if taken). Any information on subject history and sample group should be entered into a separate spreadsheet in a corresponding fashion. Having two sources of data that can be merged later in the analysis maintains blind data collection. All subsequent data manipulations can be made from the data in the form we have outlined here. Palmer presents an alternative method (see http://www.biology.ualberta.ca/palmer.hp/palmer.html), but some researchers have found his method difficult to put into practice.

4.1.2 Software

In the event that digital images are being used, there are a number of software packages that are free to download from the internet and that are designed to make and record measurements from digital images (four good examples of measurement software are given below):

- **NIH *Image*** is an image processing and analysis program for the Macintosh (available at http://rsb.info.nih.gov/nih-image/).
- **Object-Image** is an extended version of *NIH Image*. This Macintosh software integrates measurements, macros, graphical objects and image names in a closed unit (available at http://simon.bio.uva.nl/object-image.html).
- **Scion Image** is a package for Microsoft Windows 95, 98, ME, NT and 2000 that captures, displays, analyses, enhances, measures, annotates, and outputs graphic images (available at http://www.scioncorp.com/).
- **UTHSCSA ImageTool (IT)** is an image processing and analysis program for Microsoft Windows 95 or Windows NT that acquires, displays, edits, analyses, processes, compresses, saves and prints both greyscale and colour graphic images (available at http://www.uthscsa.edu/dig/itdesc.html).

4.1.3 FA assumptions

It is necessary to test for directional asymmetry and antisymmetry by examining whether the distribution of the signed asymmetries for each trait is significantly different from normal (using a test like Shapiro-Wilk W-test, refer Sokal & Rohlf 1981) and whether the mean of each signed asymmetry measure is significantly different from zero (using a test like the two-tailed *t*-test).

4.1.4 Size dependency

To ensure that data is suitable for FA analysis it is necessary to test for size dependency by correlating the unsigned asymmetry value for each trait with the size of the trait. Significant correlations indicate size dependency and should be corrected by dividing the unsigned asymmetry of the trait by the mean size of that trait:

$$|L - R| / 0.5\,[L + R]$$

4.1.5 Injury

It is necessary to control for unnatural trait asymmetries resulting from injury by allocating the mean asymmetry for that trait in the population to all individuals with known or suspected injury.

4.1.6 Calculating indices

It is necessary to standardise asymmetry values within traits because all traits should contribute equally to the composite asymmetry indices. There are a number of methods available to standardise asymmetry values, but the following method was found to be the most robust and powerful of six tested (Leung et al. 2000):

$$|L - R| / \text{sample mean of } |L - R|$$

4.2 RISK MANAGEMENT

TABLE 5. SUMMARY OF THE POSSIBLE RISKS OF HAVING A POORLY DESIGNED STUDY. A CORRESPONDING RISK CONSEQUENCE AND RECOMMENDATION IS GIVEN FOR EACH RISK SCENARIO.

RISK TO ACHIEVING GOAL	CONSEQUENCE	RECOMMENDATION
Data are recorded in such a way that it requires extensive reorganisation prior to analysis.	Minor	Prevent by ensuring that research staff are suitably trained in the techniques and datasheets used
Making asymmetry measurements is labour intensive, expensive and limits number of individuals that can be measured in the field season.	Moderate	Mitigate by using efficient data collection and measurement techniques. For example, using a digital camera to collect the data ensures that organism handling time is very short.
Poor data treatment means that the FA analyses are invalid.	Terminal	Prevent by following accepted protocols for data treatment. Also discard any traits that cannot be measured with significant repeatability or data that are known to be poor indicators of individual FA (e.g. injured traits).
New information implies that the techniques previously used are not useful for assessing how organism FA relates to environmental stress.	Moderate	Maintain excellent data records that enable the data to be re-analysed at a later date (even by somebody not previously involved in the work).

5. Next stage: pilot study

5.1 ACTION PLAN

This report provides basic information for use by DOC when designing a specific research programme to test the utility of FA as a tool for environmental monitoring. The factors that are identified as having *terminal* or *major* consequences in the Risk Management sections (Tables 1, 4, and 5) should be attended to first. The study can only proceed when the uncertainty of these factors is resolved. However, even when the criteria recommended for the study are fulfilled, it is necessary to conduct a pilot study to test the feasibility of the study programme under field conditions.

The process necessary to design a pilot study has been partly referred to previously and can be further described as:

1. Consult with DOC staff, and other research scientists, that are likely to be interested and involved in the implications of FA for environmental monitoring. This consultation may take the form of a workshop with this report treated as a discussion document for facilitating the group developing a feasible pilot study.
2. With consultation, choose a sample population that consists of suitable numbers of marked study organisms. The sample must be chosen so that the environmental factors of interest vary along a single dimension.
3. Investigate the relative merits and possibilities of conducting both natural and manipulation experiments.
4. Ensure a number of traditional environmental monitoring techniques are carried out in concert with the FA measurements to test for consistency of findings between new and existing techniques.
5. Follow the methodology that has been outlined earlier in this report to facilitate accurate and reliable data collection, as well as appropriate data treatment and analyses.
6. Independently evaluate the success of the pilot study (see Section 5.4).

5.2 RESOURCING DETAILS

There are different resource needs for natural and experimentally manipulated programmes. For example, if manipulation experiments are to be attempted, it is necessary to purchase and construct some experimental equipment (e.g. aviaries for housing captive birds). However, both natural and manipulation experiments require a substantial number of hours of labour. In addition to the basic labour costs, investment in research staff upskilling will be necessary. Research assistants will require training in data collection techniques, and familiarisation with data sheets and protocols.

A digital camera will need to be purchased if the merits of collecting data using this technology were viewed as substantial. Experience in measuring FA prompts me to recommend a camera that has both high and adjustable resolution settings, and a zoom capacity. We recommend a camera that has the facility to save images to disk (i.e. without the necessity to periodically download images to another machine) to cope with the special requirements of working in the field.

If the decision were made to use digital cameras for data collection it would be necessary to acquire software suitable for making FA measurements. Commonly used software packages are excellent and available for free download from the internet for both Macintosh- and Microsoft-based systems. We recommend Object-Image for Macintosh or Scion Image for PC. Irrespective of the measurement technique employed there is a need for specialised statistical software capable of conducting the necessary statistical analyses.

5.3 TIMEFRAME

The timeframe depends on the specifics of the experimental design of the pilot study. However, in the early stages of the study there should be allowance made for preliminary analysis of the asymmetry data collected in order to assess, and respond to, possible issues surrounding accuracy and reliability of data collection.

5.4 INDEPENDENT EVALUATION FRAMEWORK

To assess whether the pilot study was successful in addressing the overall goal, it is necessary to establish an independent evaluation framework. This framework would be best designed and implemented by the people that are likely to be affected by the widespread use of FA in assessing the stress an organism experiences in its environment.

The evaluation framework should contain comparisons of the traditional environmental monitoring techniques in relation to the newly developed FA technique along some or all of the following dimensions:

- Quantity and cost of the physical resources required.
- Quantity and cost of the human resources required.
- Operational practicality.
- Accuracy and reliability of the data collected.
- Ability to be applied across a number of environmental contexts.
- Ease of application across locations and species.
- Potential for use as the sole environmental monitoring technique.
- Ability to minimise actual or potential negative impacts of handling study organisms.
- Ease of upskilling field staff to effectively use the technique.
- International acceptance of the technique in environmental monitoring.

The design of the evaluation framework needs to provide useful benchmarks for the people considering the performance of the pilot study to allow them to assess the success of the trial. Some of these performance indicators can be assessed at the beginning of the pilot study, while others require completion of the pilot study prior to consideration. The decision about whether DOC further pursues the use of FA as an environmental monitor depends on how the technique fares under the sort of scrutiny provided for by the evaluation framework.

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