Fluctuating asymmetry of the frog *Crinia signifera* in response to logging

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Abstract.   Fluctuating asymmetry has been proposed as an indicator of environmental stress and population health. However, a notable feature of research examining the relationship between fluctuating asymmetry and population fitness is that of inconsistency. Logging dramatically alters habitat and has the potential to increase or decrease environmental stress. To evaluate the response of fluctuating asymmetry to logging, I conducted research to determine whether fluctuating asymmetry differs in the frog *Crinia signifera* captured at logged and unlogged sites and if any differences were correlated with body size or body condition. There was a decrease in fluctuating asymmetry but also a decrease in body size and body condition as a result of logging. I also investigated whether any relationship existed between the subtle asymmetry of individuals and the following indicators of fitness: clutch size, clutch mass, average egg mass, testes size, body size and body condition. There was no significant relationship between subtle asymmetry and the fitness indicators.

Introduction

During growth and development, an organism is exposed to many stressors. The developmental stability of an organism is its ability to produce an 'ideal' form (Palmer 1994) or to produce a genetically predetermined phenotype (Alford *et al*. 1999) when exposed to these stressors. Conversely, developmental instability results when stressors interfere with the ability of organisms to correct against them (Alford *et al*. 1999), and, as a consequence, the 'ideal' form is not produced. Very often the 'ideal' form of an organism is not known; for bilaterally symmetrical organisms, however, or bilaterally symmetrical structures, the ideal form, by definition, is one of symmetry (Palmer and Strobeck 1986). Such symmetry provides a reference against which deviations from the norm can be compared.

Fluctuating asymmetry is a common indicator of developmental instability (Palmer 1994) because development of the two sides of a bilaterally symmetrical organism is influenced by *identical* genes (Clarke 1995) and differences in fluctuating asymmetry can be attributed to differences in developmental noise (Lajus *et al*. 2003). Both extrinsic (environmental) and intrinsic (primarily genetic inbreeding) stress have been shown to increase developmental noise (Palmer 1994).

Positive correlations between environmental stress and fluctuating asymmetry have been documented in the literature (Leary and Allendorf 1989) and consequently, changes in fluctuating asymmetry within a population may provide a sensitive indicator of changes in population health (Clarke 1995; Alford *et al*. 1999).

It has been suggested, however, that a change in fluctuating asymmetry may not be sufficient to make it a useful bioindicator of ecosystem health. Fluctuating asymmetry must reflect changes in fitness rather than changes in stress, otherwise the significance to be placed on that level of stress is debatable (Markow 1995; Floate and Fox 2000). In other words, environmental stress must affect the ability of a population's rate of increase (Floate and Fox 2000).

However, a notable feature revealed by reviews that have examined the link between fluctuating asymmetry and population fitness (Palmer and Strobeck 1986; Markow 1995; Leung and Forbes 1997; Clarke 1998) is inconsistency. For example, out of 14 studies adequately investigating the relationship between symmetry and survival reviewed by Clarke (1998), one showed no effect, two showed a positive relationship, three were ambivalent and eight demonstrated a negative relationship. Furthermore, it is likely that studies that contradict theoretical assumptions (i.e. those that show no effect or positive association between fluctuating asymmetry and fitness) are under-represented in the literature merely because they tend not to get published (Markow 1995).

Despite this, alternative indicators of population health are worth investigating because traditional indicators such as species richness or population estimates require large amounts of resources, take large amounts of time and are logistically difficult. These limitations apply especially to amphibian populations because abundance can fluctuate over short periods (Berven 1990; Berven and Grudzien 1990; Blaustein *et al*. 1994; deMaynadier and Hunter 1995), possibly making traditional monitoring relatively insensitive to the sometimes subtle factors leading to population decline.

To evaluate the efficacy of fluctuating asymmetry as an indicator of increasing or decreasing environmental stress and amphibian population health I undertook an investigation

examining variations in fluctuating asymmetry in response to logging. Habitat changes as a result of logging have the potential to increase or decrease the environmental stress to which resident amphibians are exposed by dramatically altering the local habitat. Although the evidence is highly variable and species specific (e.g. Lemckert 1999), deMaynadier and Hunter (1995) found that recent logging led to a general decrease in the abundance of amphibians (although species richness was not similarly affected). Environmental changes from forest harvesting that are of documented importance to amphibians include the abundance of coarse woody debris, litter depth, understorey vegetation, large trees, canopy closure, moisture and soil pH (deMaynadier and Hunter 1995). Removal of the overstorey by timber harvesting may lead to higher temperatures on the forest floor (O'Connell 1987; Raymond and Hardy 1991; Vanderwoude and Lobry de Bruyn 2000). Reduced vegetative cover, forest harvesting and fire may also alter the abundance and assemblages of leaf litter invertebrates that constitute amphibian diets (Madden *et al.* 1976; Newman 1991).

My investigation was carried out in two parts. First, I conducted a study to determine whether fluctuating asymmetry differs in the common brown frog *Crinia signifera* (Girard, 1858) captured at logged and unlogged sites in Tasmania, Australia, and if any differences were correlated with indicators of population health. I then utilised *C. signifera* specimens obtained as bycatch from an existing litter invertebrate study undertaken in the same study area (Bashford *et al.* 2001) to further evaluate the relationship between subtle asymmetry (asymmetry of individuals rather than populations) and indicators of individual fitness.

Materials and methods

Study species

Crinia signifera was chosen as the model species for such an investigation because it is not excluded by logging and has a very flexible life history. It is a small frog with an adult snout–vent length of 20–30 mm (Robinson 1996). It is found in a wide range of habitats, is common throughout south-eastern Australia and is abundant at the study site. In Tasmania, it breeds in both permanent and ephemeral standing water and has its peak breeding season predominantly during spring and early summer (August–January).

Study site

The study area was centred on the commercial forests of the WARRA Long Term Ecological Research (LTER) site, Tasmania, Australia (43°3′S, 146°39′E). Vegetation at the site consists predominantly of temperate broad-leaf forest (mainly *Eucalyptus obliqua* wet forest). The logging and fire history at the site have resulted in a full range of successional stages of this forest type (www.warra.com, 2004). Standard logging practices in wet and mixed forests at the site consist of clearfell, burn and aerial sowing with eucalypt seed on a rotation of 90 years (Hickey and Neyland 2000).

Study 1. Fluctuating asymmetry

I collected frogs from ponds surrounded by logged (*n* = 4) and unlogged (*n* = 5) coupes. Logged sites were defined as those with more than two

years but less than five years since logging because the expression of fluctuating asymmetry requires a period of development during exposure to the 'stress' (Clarke 1995) and more recent logging may not have provided sufficient temporal exposure. Unlogged sites were defined as those sites with more than 35 years since logging but four of the five sites had never been logged. *C. signifera* has an extended breeding period and males, in particular, spend significant amounts of time at breeding sites (Lemckert and Shine 1993), thus ensuring extended exposure to the environment in each particular logging treatment.

Ponds were sampled once weekly between 20 September 2001 and 27 October 2001. Flat pieces of wood, obtained from the area surrounding ponds, were placed at the bank/water interface at the edge of each pond. Any frogs found beneath these cover-boards were placed in individually labelled containers with a small amount of water. Because the ventral markings of each frog are unique, digital photographs allowed identification of individuals. Recaptured frogs were not remeasured.

Six metrical traits were measured for each frog: length of forearm, 1st phalange, 2nd phalange, 3rd phalange, femur and tibio-fibula. Three replicate measurements were conducted of each trait, on each side.

Palmer (1994) recommended taking replicate measurements of each trait 'blind' (i.e. without reference to previous measurements) to minimise measurer bias. This was achieved by measuring four animals simultaneously and undertaking one round of measurements (all traits and sides of all frogs once) before repeating measurements on all frogs for the second and third replicate measurements. This way, any measurements taken on the first frog had been forgotten after having measured three other frogs. Measurements were taken using a light microscope fitted with an eyepiece micrometer at a magnification of ×6.7. Temporal separation of replicate measurements (Palmer 1994) were not undertaken because I anaesthetised animals in order to improve measurement precision.

Frogs were anaesthetised before measurements using benzocaine (200 mL saturated solution of ethy-p-aminobenzoate in ethanol diluted to 1 L in water). Kaiser and Green (2001) found the teething ointment Oragel® (with benzocaine as the active ingredient) to be a safe anaesthetic for frogs. A shallow layer of benzocaine was poured into the bottom of four Petri dishes. A frog was placed in each dish and the lid replaced until it was anaesthetised to the point where its limbs could be moved by a researcher with little defensive counter-movement. The frog was then rinsed in distilled water and placed, stomach down, on a piece of glass. Care was taken to minimise the spread of any disease between frogs by covering the glass with plastic wrap before new measurements and sterilising all equipment between measurements. Each frog was positioned to allow the easy measurement of each trait on both sides of its body. Each frog was measured once, lifted and then replaced on the glass to make sure that the previous position didn't bias measurements. Frogs were moistened with drops of water throughout the measuring process to reduce dehydration stress. Upon completion of all measurements, frogs were returned to their original field-collection container with a small amount of water in the bottom, and allowed to recover (a process that usually took ~30 min). All animals were released at their point of capture on subsequent field visits.

Study 1. Population health

Body size and body condition were used as indicators of population health and required the measurement of snout–vent length (SVL) and mass (M) of each frog. Body condition was calculated as the cubic root of $M*SVL^{-1}$ (R. Alford, personal communication). Individuals with greater body size are likely to be less susceptible to desiccation (Bellis 1962; Ponsero and Joly 1998) and have increased fecundity, clutch mass and testes size (Kaplan and Salthe 1979; Berven and Gill 1983; Semlitsch *et al.* 1988; Berven 1990; Williamson and Bull 1995; Lauck 2005) . Because *C. signifera* is an extended breeder and calling is energetically costly (Mac Nally 1981) male mating success is likely to be related to body condition.

Study 2. Subtle asymmetry

Crinia signifera specimens were obtained as bycatch from an existing litter invertebrate study at the Warra LTER using lethal pitfall traps (Bashford *et al*. 2001). Each specimen was patted dry with paper towelling before mass was measured using an electronic balance (±0.5 µg) and SVL was measured using Vernier callipers (±0.05 mm). Specimens were dissected and testes, eggs and left and right femur and tibio-fibula were removed for further analysis. Eggs were counted and dry mass determined by drying for ~4 weeks at 55°C to constant mass. The length of both testes was measured. To determine the degree of fluctuating asymmetry, the length of both left and right femur and tibio-fibula were measured for each individual at a magnification of ×15 using a binocular microscope fitted with an eyepiece micrometer. Three replicate measurements were made and measurements were taken blind (i.e. with intervals of more than one day between replicates) as recommended by Palmer and Strobeck (1986). Body condition was calculated as in Study 1 (above).

Data analysis

Statistical analyses of fluctuating asymmetry were conducted as described by Palmer and Strobeck (1986), Palmer (1994) and Alford *et al*. (1999). The significance of obscuring factors in fluctuating asymmetry analysis (i.e. measurement error, directional asymmetry, antisymmetry, size) was determined using a Model III ANOVA with sides (fixed) × individual (random) for each trait (see Palmer 1994 for definitions). Visual inspection of scatterplots and regressions between |R–L| and SVL were used to assess dependence of fluctuating asymmetry indices on trait size *within* samples. Regression between log(var(R–L)) and mean (R+L) was used to indicate dependence of fluctuating asymmetry indices on trait size *among* samples. Two indices of fluctuating asymmetry are reported, FA1 and FA10 (Palmer 1994). FA1 (|R–L|) is the simplest index and provides a good intuitive understanding of what is happening within the data. It can be biased by directional asymmetry and antisymmetry and is dependent on overall size if errors are multiplicative (Palmer 1994). FA10 is a variance-based index:

$$\sigma^2_i = (MS_{sj} - MS_m)/M,$$

where σ^2 is the variance of individual *i*, MS_{sj} is the sides × individual interaction Mean Squares, MS_m is the measurement error Mean Squares and m is the number of replicate measurements per side (Palmer 1994). FA10 is one indicator that can partition out measurement error from the overall between-sides variances. This index is not biased by directional asymmetry, or heavily biased by antisymmetry, but is sensitive to overall size if errors are multiplicative. Study 1 had a nested design: ponds nested within logging treatments. MANOVA was conducted on |R–L| to determine differences in fluctuating asymmetry with logging treatment (dependent variables = traits, model effects = logging and logging(pond)). Despite the fact that scatter plots between |R–L| and SVL did not indicate size scaling (see previous), this may have been because of a restriction in size range of frogs within logged or unlogged treatments. Since size scaling is typical of morphometric traits that exhibit multiplicative errors such as those used in this study (see Graham *et al*. 2003), data analysis was also undertaken, after size scaling was removed using |logR – logL|.

Nested ANOVA (logging(pond)) was used to analyse differences in body size and body condition between logging treatments in Study 1. Owing to sexual size dimorphism and because relatively few females were captured, analyses in Study 1 were conducted for males only. Scatter plots were used to determine any correlation between fitness traits and unsigned asymmetry (|R–L|) in Study 2. Asymmetry–fitness relationships are expected to have a triangular bivariate distribution (Leung and Forbes 1997; Ross Alford, personal communication) because individuals with high asymmetry are expected to have low fitness whereas individuals with low asymmetry can have both high

fitness (because they are developmentally stable) or low fitness (because they are developmentally unstable but have low asymmetry by chance) (see Fig. 1). Since total eggs per clutch, average egg mass, total clutch mass and testes size were significantly correlated with body size (Lauck 2005) the effect of body size was removed by dividing all fitness traits by SVL in the graphs relating to Study 2. Factor analysis (PCA) with Varimax rotation was undertaken to group fitness correlates into related components that explained most variance in fitness. Factor scores were retained for subsequent regression with total subtle asymmetry. Separate analyses were conducted for males and females owing to significant sexual size dimorphism. Body condition could not be calculated for females in Study 2 because total clutch mass was measured as dry mass and body mass was measured as a wet mass.

Results

Study 1. Fluctuating asymmetry at logged and unlogged sites

The interaction variance term (i.e. sides × individual) indicated that non-directional asymmetry (antisymmetry + fluctuating asymmetry) could be distinguished from measurement error for all traits (Table 1). A significant 'sides' variance term indicates the presence of directional asymmetry (DA; which may artificially inflate some indices of fluctuating asymmetry and may indicate the presence of a genetic component of between sides variation) (Palmer 1994). The forearm, 1st phalange and 2nd phalange were found to have insignificant DA; however, DA was significant in the 3rd phalange, femur and tibio-fibula (Table 1). The presence of DA was removed statistically from the latter three traits by subtracting (mean (R–L))/2 from the side with the larger mean and adding it to the smaller side of all individuals in the sample (Palmer 1994). Reanalysis of data verified that DA had been successfully removed (Table 1).

Visual inspection of frequency distributions of (R–L) did not indicate skewness, platykurtosis or significant departure from normality for any traits. Kolmogorov–Smirnov tests confirmed these patterns (Table 2). This demonstrates that antisymmetry (which may inflate some fluctuating asymmetry indices) is an insignificant component of non-directional asymmetry (i.e. antisymmetry + fluctuating asymmetry) and,
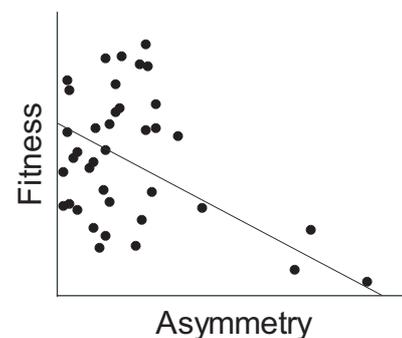

Fig. 1. The expected pattern between unsigned asymmetry (|FA1|) and fitness, if fitness is negatively related to asymmetry within a population (adapted from Leung and Forbes 1997).

Table 1.   Results of ANOVAs (side = fixed factor, individual = random factor) testing the significance of factors confounding fluctuating asymmetry analysis

Significant 'side' effects indicate the presence of directional asymmetry. A non-significant interaction between 'side' and 'individual' indicates that non-directional asymmetry (antisymmetry + fluctuating asymmetry) can be separated from measurement error

Trait	n	Source of variation	SS	d.f.	F	P
Forearm	122	Side	4.87×10^{-3}	1,482	0.453	0.502
		Side × individual	1.293	120,482	1.604	<0.001
1st phalange	122	Side	5.56×10^{-5}	1,480	0.004	0.948
		Side × individual	1.557	119,480	2.691	<0.001
2nd phalange	123	Side	1.240×10^{-4}	1,484	0.020	0.920
		Side × individual	1.487	120,484	2.570	<0.001
3rd phalange	119	Side[A]	0.188	1,475	23.915	<0.001
		Side[B]	3.382×10^{-3}	1,475	0.429	0.514
		Side × individual	0.930	118,476	2.253	<0.001
Femur	121	Side[A]	4.491	1,484	22.654	<0.001
		Side[B]	2.172×10^{-2}	1,484	0.110	0.741
		Side × individual	23.789	120,484	15.602	<0.001
Tibio-fibula	120	Side[A]	0.211	1,478	10.853	0.001
		Side[B]	8.994×10^{-3}	1,478	0.471	0.494
		Side × individual	2.274	119,478	4.146	<0.001

[A]Before statistical adjustment for directional asymmetry.
[B]After statistical adjustment for directional asymmetry.

therefore, non-directional asymmetry can be used as a valid estimate of the presence of fluctuating asymmetry.

Trait size may also confound estimates of fluctuating asymmetry. To check for size dependence of fluctuating asymmetry within samples, |R–L| was plotted against an independent measure of body size (SVL) to test for any relationship. Both the plots and the slopes of these regressions (Table 3) provide no evidence of a size-dependent component to fluctuating asymmetry.

Since fluctuating asymmetry was not obscured by measurement error, directional asymmetry, antisymmetry or size (i.e. all traits exhibited ideal fluctuating asymmetry), it could be used in comparisons between logged and control sites.

For FA10, replicate measurements were used to partition out measurement error, as in Palmer (1994) (Table 4). Also presented in Table 4 is the measurement error index ME2, a descriptor of measurement error calculated as the square root of the error MS (Palmer and Strobeck 2003).

Fluctuating asymmetry was significantly higher in un-logged than logged sites (Pillai trace = 0.064, $F_{6,349} = 3.980$, $P = 0.001$), a fact also confirmed for five of the six traits with FA1 (Table 4). Reanalysis after size scaling was removed confirmed that size scaling did not confound the logging effect (Pillai trace = 0.058, $F_{6,349} = 3.407$, $P = 0.003$). Ponds within the same logging treatments also demonstrated significant variation in fluctuating asymmetry (Pillai trace = 0.177, $F_{42,2124} = 1.533$, $P = 0.016$).

Table 2.   Summary statistics indicating the presence/absence of directional asymmetry in samples from both logging treatments

Statistics were computed after taking average of replicate measurements, as in Palmer (1994)

Sample	Trait	n	(R–L)					
			Mean	±s.e.	Skew	±s.e.	Kurtosis	±s.e.
Logged	Forearm	69	0.013	0.009	0.160	0.289	0.569	0.570
	1st phalange	67	0.005	0.013	−0.641	0.293	2.782	0.578
	2nd phalange	68	0.018	0.010	0.386	0.291	−0.400	0.574
	3rd phalange	66	0.004	0.008	−0.289	0.295	0.144	0.582
	Femur	69	−0.018	0.038	0.286	0.289	0.056	0.570
	Tibio-fibula	69	−0.010	0.013	0.045	0.289	0.873	0.570
Control	Forearm	53	−0.005	0.014	0.228	0.327	0.692	0.644
	1st phalange	53	−0.006	0.011	1.137	0.327	3.363	0.644
	2nd phalange	53	−0.016	0.014	−0.038	0.327	0.058	0.644
	3rd phalange	53	−0.015	0.011	−0.883	0.327	1.278	0.644
	Femur	52	0.049	0.059	0.232	0.330	−0.078	0.650
	Tibio-fibula	51	0.031	0.016	−0.521	0.333	1.290	0.656

Table 3.   Dependence of fluctuating asymmetry indices on trait size
Statistics were computed after taking the average of replicate measurements, as in Palmer (1994). (R+L)/2 indicates the size of the trait. Regression between |R–L| and SVL was used to assess dependence of FA indices on trait size *within* samples. Regression between log(var(R–L)) and mean (R+L) was used to indicate dependence of FA indices on trait size *among* samples

Sample	Trait	n	(R+L)/2		Regression |R–L| *v.* SVL		Regression log(var(R–L)) *v.* mean (R+L)	
			Mean	±s.e.	Slope	±s.e.	Slope	±s.e.
Logged	Forearm	69	3.985	0.063	–0.003	0.004	–0.153	0.139
	1st phalange	67	1.498	0.018	0.002	0.005	0.500	0.407
	2nd phalange	68	2.515	0.027	0.001	0.004	0.069	0.258
	3rd phalange	66	1.441	0.018	0.002	0.003	0.078	0.273
	Femur	69	7.099	0.057	0.002	0.012	0.108	0.112
	Tibio-fibula	69	7.504	0.060	0.010	0.005	–0.056	0.083
Control	Forearm	53	4.086	0.044	0.003	0.004	0.242	0.163
	1st phalange	53	1.553	0.024	0.003	0.003	0.822	0.318
	2nd phalange	53	2.610	0.028	0.005	0.003	–0.107	0.293
	3rd phalange	53	1.501	0.019	0.006	0.003	0.159	0.356
	Femur	52	7.275	0.060	–0.003	0.015	–0.043	0.162
	Tibio-fibula	51	7.642	0.060	–0.004	0.004	0.084	0.086

Study 1. Population health

Comparisons of population health at logged and unlogged sites are presented in Table 5. Analysis showed that SVL was greater at unlogged than logged sites (mean and s.e. of 25.9 ± 0.234 mm compared with 25.0 ± 0.210 mm). Similarly, males at unlogged sites had better body condition than those at logged sites (mean and s.e. of 0.39 ± 0.003, compared with 0.376 ± 0.002). Body size and condition between ponds within the same logging treatment did not differ.

Study 2. Subtle asymmetry

Factor analysis reduced the four female and three male fitness correlates to one 'fitness' component for each sex. Scatterplots (Fig. 2) did not correspond to the expected pattern between unsigned asymmetry (|FA1|) and fitness, if fitness is negatively related to asymmetry within a population (Fig. 1). Linear regression between these 'fitness' components and subtle asymmetry showed no significant relationship for both males ($R^2 = 0.002$, $F_{1,38} = 0.078$, $P = 0.782$) and females ($R^2 = 0.021$, $F_{1,29} = 0.602$, $P = 0.444$).

Discussion

One of the challenges of conservation biology is to identify and measure the ecological effects of anthropogenic land management. In this study I investigated the value of fluctuating asymmetry as an indicator of changing ecosystem stress and population health for the amphibian *Crinia signifera* in response to recent logging. I tested differences in fluctuating asymmetry and indicators of population health among populations exposed to two logging treatments: recently logged

Table 4.   Results for the fluctuating asymmetry indices FA1 and FA10
FA1 was computed after taking the average of replicate measurements, as in Palmer (1994). For FA10, replicate measurements were used to partition out measurement error. MS_m = measurement error mean square; σ^2 = non-directional asymmetry; d.f. = approximate degrees of freedom for non-directional asymmetry after partitioning out measurement error; ME2 = measurement error index from Palmer and Strobeck (2003)

Sample	Trait	n	|R–L| = FA1			FA10		
			Mean	±s.e.	MS_m	σ^2	d.f.	ME2
Logged	Forearm	69	0.069	0.006	0.005	0.00076	7	0.073
	1st phalange	67	0.083	0.009	0.005	0.00358	35	0.070
	2nd phalange	68	0.077	0.007	0.005	0.00159	19	0.069
	3rd phalange	66	0.070	0.005	0.003	0.00121	20	0.057
	Femur	69	0.272	0.020	0.014	0.04780	64	0.120
	Tibio-fibula	69	0.103	0.008	0.005	0.00458	42	0.070
Control	Forearm	53	0.092	0.009	0.008	0.00179	9	0.090
	1st phalange	53	0.071	0.007	0.005	0.00163	14	0.072
	2nd phalange	53	0.098	0.007	0.004	0.00381	31	0.066
	3rd phalange	53	0.084	0.006	0.004	0.00203	22	0.063
	Femur	52	0.353	0.033	0.015	0.07943	51	0.123
	Tibio-fibula	51	0.106	0.009	0.004	0.00484	35	0.064

and unlogged (Study 1). Although appropriate for monitoring the response of populations, this can add little understanding to the specific relationships between subtle asymmetry and individual fitness because there is no way of determining whether developmentally unstable individuals are less fit than stable ones (Clarke 1998). As a result I also examined the specific relationship between indicators of fitness and subtle asymmetry in *C. signifera within* a population (Study 2).

In Study 2, increases in fluctuating asymmetry were not correlated with fitness indicators relating to reproductive output (i.e. size-adjusted average egg mass, total clutch mass, clutch size and testes size) or the indicators also used in Study 1 (body size and body condition). Within populations, therefore, less developmentally stable individuals do not exhibit decreased fitness for any measured trait.

However, comparisons among populations identified lower fluctuating asymmetry at logged habitats (i.e. an increase in developmental stability associated with logging) and smaller body size and lower body condition in populations from logged habitats (i.e. a decrease in population health as a result of logging).

By itself, a reduction in developmental instability associated with logging is not entirely surprising, as *C. signifera* is not excluded by logging and readily colonises disturbed habitat (a characteristic making it an ideal model species for such an examination). However, given that no relationship was found between fitness traits and the subtle asymmetry of individuals within a population, what do differences between fluctuating asymmetry and population health associated with logging treatment indicate?

The negative relationship between fluctuating asymmetry and population health associated with logging treatment may be a consequence of trait choice. First, the indicators of population health used in Study 1 (body size and body condition) were an inference and not a direct measure of population health and may simply be a reflection of increased food abundance and/or decreased temperature (see Introduction) at unlogged sites independent of any change in stress correlated with logging treatment.

Second, traits chosen for the assessment of fluctuating asymmetry may not have influenced individual fitness and population health, i.e. differences in limb length may be statistically significant but not physiologically limiting. All of the traits used compared the lengths of limbs (a restriction

governed by the limited number of bilateral traits that could be measured on live specimens without harming them). Since variations in fluctuating asymmetry are small (Markow 1995) it may be that they are easily compensated for and have few fitness consequences.

For amphibians the relationship between fitness and fluctuating asymmetry is also unlikely to be simple (Palmer and Strobeck 1986). In Study 1, the biphasic life history of amphibians is an obvious potential complication. The underlying assumption is that life-history traits measured on adults are a reflection of the terrestrial habitat to which they have been exposed. However, key periods of larval development may subsequently be expressed as adult asymmetries and population health correlates. For example, reduced shading around breeding sites as a result of logging might result in increased rates of pond desiccation. Many amphibian species (especially those adapted to development in temporary ponds) are able to increase the rate of development (i.e. decrease time to metamorphosis) in response to decreasing pond water volumes but do so at the expense of size at metamorphosis (Newman 1988; Crump 1989; Rowe and Dunson 1995; Blaustein *et al*. 1999; Loman and Claesson 2003). Increased growth rates have been shown to increase developmental stability (Danzmann *et al*. 1986; McKenzie and
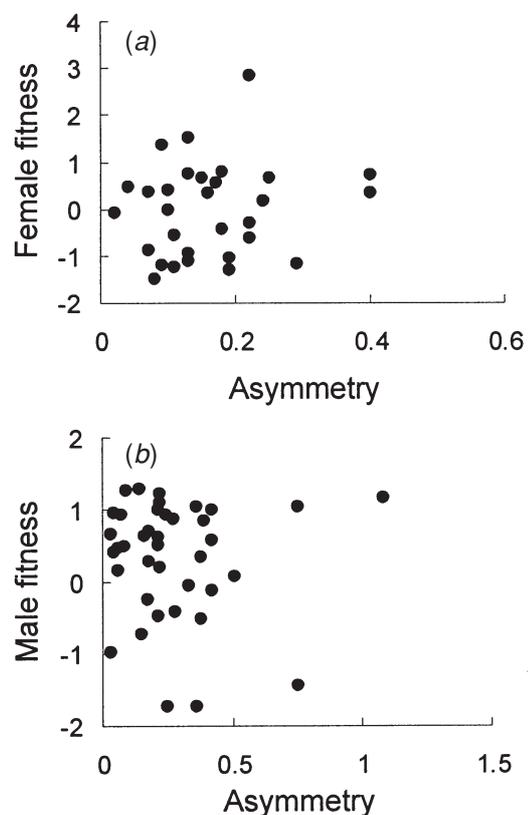

Fig. 2. Scatter plots showing the relationship between unsigned asymmetry and 'fitness' components (derived from factor analysis) in (*a*) males and (*b*) females.

Table 5.   Results of nested ANOVA (logging(pond)) testing for differences in indicators of population health between logged and unlogged sites

Source of variation	Dependent variables	d.f.	F	P
SVL	Logging	1,16	5.208	0.036
	Logging(pond)	7,111	0.974	0.454
Body condition	Logging	1,12	5.363	0.039
	Logging(pond)	7,111	1.748	0.105

O'Farrell 1993) and size at metamorphosis is known to predict body size in adulthood (Halliday and Verrell 1988). Therefore, the negative relationship between fluctuating asymmetry and body size associated with logging treatment may be a consequence of larval plasticity. Although Kellner and Alford (2003) found that asymmetry reflects recent growth history in the domestic fowl, *Gallus gallus domesticus*, such patterns may vary with species (or even genetic strain) and the type of environmental stress. In amphibians, the persistence of asymmetry through to later life stages is unknown (see McCoy and Harris 2003).

Similarly, it may also be possible that variations in phenotypic traits and fluctuating asymmetry are a response to different environmental mechanisms. For example, logging may increase forest floor temperatures (Rothermel and Semlitsch 2002), resulting in smaller postmetamorphic body size (Berven 1982) and reduced body condition (Reading and Clarke 1995). Although the range of temperatures experienced by amphibians at logged and unlogged sites may differ, neither may be more stressful than the other because resident amphibians may be able to mediate extreme temperatures through behavioural adaptations such as microhabitat use (Navas 1996) and nocturnal activity. The fitness trait of body size may, therefore, be independent of environmental stress (i.e. fluctuating asymmetry) but, nevertheless, dependent on logging treatment.

It is also important to highlight the influence of natural selection on the relationship between fitness and fluctuating asymmetry (Markow 1995). If natural selection is in operation, then individuals being measured within fluctuating asymmetry studies may not represent the whole population but rather those that have *survived* exposure to stress. To test such a relationship, Floate and Fox (2000) reasoned that the frequency distributions of highly stressed populations and low-stress populations would differ. In their study, the frequency distribution of fluctuating asymmetry in house flies (*Musca domestica*) was normal for populations exposed to high stress but deviated significantly from normal for populations exposed to low stress levels. Exploratory frequency distributions of fluctuating asymmetry for *C. signifera* populations at logged and unlogged sites indicate that environmental stress associated with logging treatment may not be sufficient to cause the mortality of developmentally unstable individuals. Such possibilities will, however, need further consideration if relationships between fluctuating asymmetry and fitness are to be understood and fluctuating asymmetry proposed as a monitoring tool (Floate and Fox 2000).

Clearly, the positive relationship between fluctuating asymmetry and population health with logging treatment for *C. signifera* does not conform to expected patterns and fluctuating asymmetry and population health correlates are not disrupted by logging in a predictable manner. As a result, it is not possible to determine the significance of the reduced fluctuating asymmetry found at logged sites. Further research is required before fluctuating asymmetry can be used as a surrogate of population health by land managers monitoring the response of amphibians to logging.

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