

Fluctuating asymmetry and mortality in cultured oysters (*Crassostrea gigas*) in Marennes-Oléron basin

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Abstract

Japanese cupped oysters suffer summer mortality in many culture sites along the French Atlantic coast. To ascertain whether mortality might be associated with morphological features of the shells, we estimated fluctuating asymmetry (FA) of hatchery/nursery-produced oysters kept in culture bags in the intertidal. FA is defined as random variations from a perfect symmetry plane (bilateral or radial) in some morphological trait. FA increases with disruption of homeostasy during ontogeny and has both genetic and environmental determinants. We found significant differences in FA between dead and live oysters in two of our three study groups. Therefore, lack of developmental stability may be involved in the summer mortality syndrome of oysters. We also found indirect evidence that once acquired, FA patterns of shells are retained at least partially. Since FA likely appears early during ontogeny and is recorded in the shells, we hypothesise that it might be useful for forecasting the ability of spat to resist environmental stress.

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Résumé

Dissymétrie fluctuante et mortalité chez l'huître creuse (*Crassostrea gigas*) dans le bassin de Marennes-Oléron. Les élevages d'huître creuse du littoral atlantique français sont victimes de mortalité estivale importante et variable. Dans le but d'examiner si la mortalité estivale est associée à la morphologie des coquilles, nous estimons la dissymétrie fluctuante (DF ; « fluctuating asymmetry ») d'huîtres produites en éclosure et élevées en milieu intertidal. On définit la DF comme étant les variations aléatoires d'un ou plusieurs caractères morphologiques autour d'un plan de symétrie corporelle (bilatérale ou radiale) ; la DF augmente avec le degré de perturbation de l'homéostasie au cours de l'ontogénie. Elle dépend des caractéristiques génétiques des spécimens et des effets de l'environnement. Nos résultats indiquent que dans 2 des 3 groupes étudiés, les spécimens morts présentent la DF différente de celle des spécimens vivants. En conséquence, il se peut que des perturbations de l'homéostasie survenues au cours de l'ontogénie ne soient pas étrangères au syndrome de mortalité estivale des huîtres. Nous observons également qu'une fois acquise, la signature de la DF est conservée au moins partiellement. Puisque DF apparaît vraisemblablement de façon précoce au cours de l'ontogénie et qu'elle est conservée, ce paramètre pourrait être un indicateur prévisionnel de la capacité du naissain à résister aux stress environnementaux.

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1. Introduction

Severe non-predatory mortality has been reported in a large number of cultured bivalve populations. These populations may differ by orders of magnitude in size. At the large

scale end of the spectrum, for instance, Japanese scallops *Patinopecten yessoensis* cultured in Mutsu Bay, suffered an estimated 40 000 t loss in the 1970s, presumably owing to competition-related mortality (Aoyama, 1989). In Marennes-Oléron basin (MOB), the oyster *Crassostrea angulata* supported large landings (ca. 40 000 t year⁻¹) before succumbing to parasitic outbreaks in the early 1970s and being replaced by a massive introduction of the Japanese

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cupped oyster, *Crassostrea gigas* (Thunberg) (Grizel and Héral, 1991). In contrast with the above, the small-scale mussel industry in Îles de la Madeleine, Québec, suffered more or less predictable summer mortality in the late 1980s and early 1990s (Myrand and Gaudreault, 1995).

Identification of causes of mass mortality and implementation of appropriate remedy may be straightforward in cases where mortality events are linked in some way to culture methods and management. Overstocking, for instance, is readily checked by reducing stocking density (e.g., Aoyama, 1989). Adjusting cultivation depth, choosing appropriate culture techniques or even switching to sturdier and byssus-producing species (in the case of scallops) may help reducing negative effects of waves (Freites et al., 1999). Choosing appropriate depth may also help minimising effects of epibionts (e.g., Claereboudt et al., 1994; Fréchet et al., 2000). Mortality caused by the protistan parasite *Mikrocytos roughleyi* to intertidally grown oysters *Saccostrea glomerata* was greatly reduced by installing oyster trays at appropriate intertidal level (Lauckner, 1983, in Smith et al., 2000). Using the well-known stock-site methodology for testing stock and site influences on growth and survival (Dickie et al., 1984; Mallet et al., 1990), Myrand and Gaudreault (1995) found that summer mortality of cultured mussels *Mytilus edulis* in Îles de la Madeleine could be reduced to negligible levels simply by collecting spat in a local body of water where mussels were not prone to summer mortality, and growing them in the usual culture sites. Similar conclusions were reached by Imai et al. (English abstract in Imai et al., 1965), who found oysters from Hiroshima Bay to be more resistant to summer mortality than local oysters from Matsushima Bay. In some cases, however, the incidence of mortality may be variable within sites (Gouletquer et al., 1998).

In other situations, however, mortality is associated with large-scale environmental properties, and cultivation and restoration methods may not be easily amenable to adjustment. For instance, many phytoplankton blooms may have quite harmful effects. A *Ptychodiscus brevis* bloom caused the bay scallop *Argopecten irradians concentricus* population of Bogue Sound, North Carolina, to crash by about one order of magnitude (Peterson and Summerson, 1992). In the laboratory, the dinoflagellate *Gyrodinium aureolum* caused severe mortality in hatchery/nursery-grown bay scallops (Smolowitz and Shumway, 1997). In some instances, harmful phytoplankton probably kill the Japanese pearl oyster *Pinctada fucata* (Tomaru et al., 2001). Besides trophic factors, temperature can be a pervasive factor in massive summer mortality. Intense mortality in the oyster *Crassostrea virginica*, for instance, is restricted to high temperature periods, which are favourable to growth of pathological agents in high salinity sites (English abstract in Kanno et al., 1965; Powell et al., 1994). In MOB, summer mortality of *C. gigas* varies from year to year, but is generally more severe in southernmost sites than in others (Soletchnik et al., 1999). Furthermore, mortality is more severe on bottom leases than higher in the

water column. Off-bottom culture, however, is insufficient to avoid completely summer mortality.

Monitoring studies strongly suggest that summer mortality in *C. gigas* is a multi-factor process. Gouletquer et al. (1998), for instance, reported that summer mortality in MOB appeared to be linked to a sequence of situations involving first high food abundance leading to fast growth and high reproductive output, along with oxygen depletion and high water temperature during neap tides. During the following spring tides, long emersion time and stressful high air temperature, combined with exhaustion of glycogen reserves critically needed for anaerobiosis at low tide presumably added to the general stress level endured by the oysters. Parallel to this, the search of pathogenic causes have revealed a limited number of potential infectious agents, and challenge tests have shown that very similar strains may have quite different health consequences for oysters (Le Roux et al., 2002). Similar multi-factor processes in summer mortality of *C. gigas* were reported by Imai et al. (1968) and Cheney et al. (2000).

Since the oysters in MOB have little temporal or spatial refuge from summer mortality factors, it may be attempted to understand the role of environmental stress in summer mortality from the viewpoint of the environment only, a view which has support from results of case studies (e.g., Bricelj et al., 1992). This approach, however, assumes that the ability of the oysters to resist environmental stress is constant from year to year or independent of the individuals' developmental history. Oysters used for cultivation in MOB, however, are obtained from wild spat. In some years, stressful environmental conditions may increase developmental instability of spat and bear negative consequences for its future ability to resist environmental stress. Developmental instability is usually estimated using fluctuating asymmetry (FA; van Valen, 1962; Debat and David, 2001). FA is defined as random deviations from perfect symmetry (bilateral or radial) in some morphological trait. FA has both genetic and environmental determinants (Cadée, 2000). High FA has been related to lack of heterozygosity and disruption of coadapted gene complexes (Palmer and Strobeck, 1986; Clarke, 1993; Wilkins et al., 1995; Hochwender and Fritz, 1999). FA usually increases with increasing stress during ontogeny (Hochwender and Fritz, 1999; Badyaev et al., 2000; Hosken et al., 2000; Lens and Van Dongen, 2000; Kozlov et al., 2001) and is a convenient indicator of individual capacity to resist environmental stress (e.g., Brown and Bomberger Brown, 1998; Lens and Van Dongen, 2000; but see also Bjorksten et al., 2000). Recently, FA has been applied to the analysis of growth and survival of Iceland scallops *Chlamys islandica* grown in pearl nets for one year (Fréchet and Daigle, 2002). It was found that FA correlated with survivorship but not with growth patterns. Here we report preliminary observations of FA of *C. gigas* cultured in MOB in relation to resistance to summer mortality. In *C. gigas* shells are asymmetric, with the left valve being larger than the right valve (Legay et al., 2000). Therefore, shell

asymmetry includes directional asymmetry and FA components. We focussed on FA patterns. Our study was done as a part of MOREST programme, a multi-disciplinary effort aiming at a better understanding of environmental, genetic, ecophysiological, immunological and pathological aspects of summer mortality of *C. gigas* along the French Atlantic coast (<http://www.ifremer.fr/morest/>).

2. Methods

C. gigas adults from MOB were spawned in the laboratory (one female, one male per family). Spat was raised in the laboratory (one larval tank per family), placed in culture bags (one bag per family) in June 2001 and grown in the intertidal near Ronce-les-Bains (45.8° N, 1.2° W), France, on a single culture table. The oysters were brought back to the laboratory on 19 September 2001. We studied oysters from two families (families A and B) separately and from a blend of gametes of the 24 females and six males used to obtain the 16 families studied in MOREST programme (group C). Individuals with abnormal shells due to chamerling or shells with culture bag mesh marks were rejected. All remaining oysters were studied, but dead oysters were retained in the study only if their valves were still articulated.

Asymmetry studies usually involve linear measurements of individuals. Oysters, however, are notoriously variable morphologically (Imai and Sakai, 1961). Therefore linear measurements are likely to be useless. Legay et al. (2000), however, found an elegant solution to this problem by using mass of the valves instead of linear measurements. In doing so, they implicitly integrated in a single estimate all possible estimations of asymmetry based on linear measurements. Our analysis was based on a similar strategy. The oysters were killed by heat shock at 70 °C for 2 h and washed in 47–50% sodium hypochlorite for 2 h. The shells were dried at 70 °C for 2 h. The same treatment was applied a second and even a third time in cases where individuals did not open after a single treatment run. Shells were subsequently gently scraped to remove remaining epibionts without damaging delicate parts of the shells. The hinge ligament also was removed. All oysters, dead or alive, were treated as above. Each valve was weighted with 0.0001 g precision. Measurement error was examined by comparing FA as estimated by two independent measurements of valve mass (FA1 and FA2, respectively). We estimated the parameters of the relationship $FA2 = a + bFA1$ and found $a = 0$ and $b = 1.0013$ ($N = 44$, $R^2 = 0.9997$, $P < 0.0001$). Therefore measurement error was negligible.

Two general growth models have been recognised (Mosimann and Campbell, 1988), which have critical implications for estimating FA (Graham et al., in press). The additive growth model occurs in cases where the tissue that generates growth does not change in size, as found in nails, feathers, teeth, scales, bristles, etc. In this case random growth errors may be additive (Mosimann and Campbell, 1988). The multiplicative growth model is appropriate in cases where

growth results from tissue actively participating in the production of new tissue. In this case random growth errors are multiplicative. With the additive growth model, FA is appropriately measured as e.g. $FA = (L - R)/(L + R)$, where L and R are the size of the left (cupped valve) and right (flat valve) trait measured, respectively. With the multiplicative growth model, however, the appropriate formulation for FA is $FA = \ln(L/R)$, which removes size dependence in FA estimates (Graham et al., in press). The appropriate growth model may be ascertained from the analysis of the residuals of the relationship between the right and left sides. Positive scaling of residuals (tested following White, 1980) is strong evidence that growth is multiplicative (Graham et al., in press). Because shell asymmetry was directional, we estimated FA as the factor scores on the second principal component analysis carried out on unstandardised log-scale data, as proposed by Graham et al. (1998).

To check for any relationship between growth and FA, we tested the correlation between an index of individual size (V) and FA. V was calculated as $V = H[(A_1 + A_2)/2][(B_1 + B_2)/2]$, where H is thickness of the shell with both valves closed, A and B are the length and height of the valves, respectively, and the subscripts 1 and 2 stand for the left and the right valves, respectively. Linear measurements were made according to Fig. 1. Correlations between V and FA were not significant (Table 1). Therefore the data were tested using a two-way ANOVA with group and status (dead vs. alive) as fixed main effects.

To control for possible effects of repeated cleaning operations on shell mass, we washed a set of shells (right and left

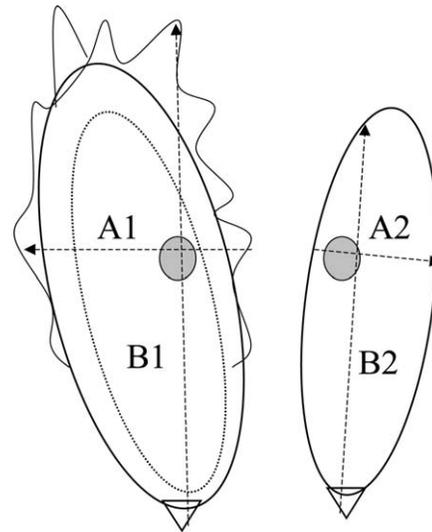


Fig. 1. Methodology for measuring the linear dimensions of the shells. A1, B1: length and height, respectively, of the left (cupped) valve. A2, B2: length and height, respectively, of the right (flat) valve. Height was measured from the umbo to the most distant point of the shell, provided that the axis of measurement overlapped with the muscle scar. Length was the widest dimension of the shell perpendicular to height, provided that the axis of measurement overlapped with the muscle scar. Width was determined by closing the valves and measuring the thickness of the whole oyster, with the caliper oriented in the height axis. This procedure allowed to make standardised measurements in spite of variability in shell shape.

Table 1
Correlations (Pearson's r) between individual volume index and asymmetry of the shells according to group and status of oysters

Group	Dead			Live		
	N	r	P	N	r	P
Family A	16	0.2579	0.3349	82	-0.1226	0.2727
Family B	99	0.0228	0.8224	126	-0.0954	0.2839
Group C	74	-0.1980	0.0888	108	-0.1414	0.1424

valves) three times and measured the mass of the valves separately each time. We compared FA after the second and third washes with FA after a single wash. The effect of successive washes was tested using a repeated measures design with a first order autoregressive model for error structure. We found a significant effect of washing (test of main effect, $F = 71.59$; $df = 2, 140$; $P < 0.0001$). Each wash had a significant effect (first wash vs. second wash, $t = -10.43$, $df = 140$, $P < 0.0001$; first wash vs. third wash, $t = -11.54$, $df = 140$, $P < 0.0001$). Therefore FA increased with successive washes. Consequently we adjusted a correction factor to the mass of each valve separately to account for mass loss of each valve after successive washes. The correction factor was computed as $\Delta m = m_b - m_a$, with m_b being valve mass after the first wash and m_a being valve mass after the second or the third wash, depending on the case. Knowing m_a and the number of washes given, the corrected initial valve mass was easily computed as the sum of Δm and m_a . Further details are given in Table 2.

3. Results

The relationship between the mass of the right and left valves is shown in Fig. 2. Clearly the relationships were linear for all groups. Residuals increased with increasing valve mass in family B ($\chi^2 = 10.50$, $P = 0.0052$ and $\chi^2 = 10.15$, $P = 0.0062$ for dead and live oysters, respectively; $df = 2$) and group C ($\chi^2 = 6.85$, $P = 0.0326$, and $\chi^2 = 8.94$, $P = 0.0115$ for dead and live oysters, respectively; $df = 2$). The trend was similar but not significant in family A

Table 2
Parameter values for correcting for loss of valve mass following successive washes. Change in valve mass (Δm) is fitted the statistical model $\Delta m = b + cm_a^d$, where b , c and d are parameters and m_a is valve mass after washing. MSE is mean square error and N is number of observations. Numbers in parentheses are standard error of the estimates

	Left valve		Right valve	
	Second wash	Third wash	Second wash	Third wash
b	0.00555 (0.00289)	0.00489 (0.00633)	-0.00694 (0.00849)	-0.00773 (0.0109)
c	0.00154 (0.00101)	0.00477 (0.00330)	0.00245 (0.00354)	0.00208 (0.00311)
d	1.9226 (0.3427)	1.5109 (0.3567)	1.1549 (0.5325)	1.4244 (0.5673)
MSE	0.000103	0.000266	0.000213	0.000643
N	72	72	70	70

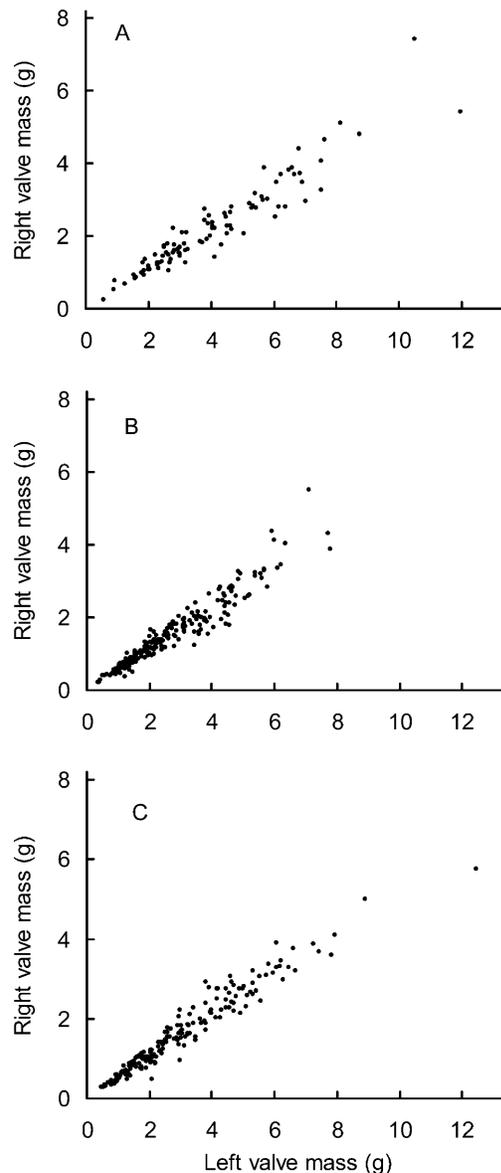


Fig. 2. Plot of right valve mass as a function of left valve mass for (A) family A, (B) family B and (C) group C. Data not corrected for successive washes.

($\chi^2 = 2.62$, $P = 0.2762$, and $\chi^2 = 4.05$, $P = 0.1320$ for dead and live oysters, respectively; $df = 2$). With log-transformed data, all groups were homoskedastic. The frequency of occurrence of asymmetry values of dead and live oysters is shown in Fig. 3A–C. In family A, there was clear evidence of differences in FA between dead and live oysters. Family B, however, appeared to show no differences in FA between dead and live individuals. Finally, in group C live oysters

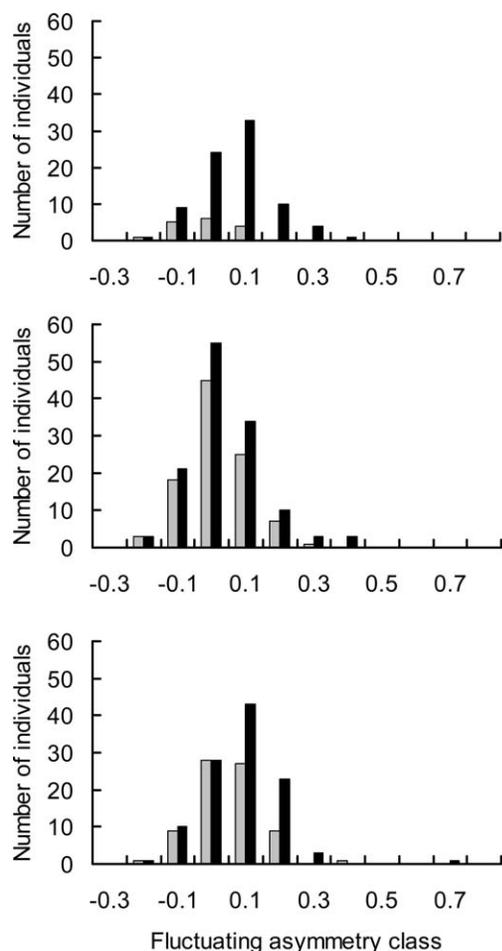


Fig. 3. Frequency of occurrence of asymmetry values in (A) family A, (B) family B and (C) group C. Light-grey bars are dead oysters. Black bars are live oysters. Data not corrected for successive washes. See Table 2 for statistical information on corrected data.

exhibited a tendency toward higher values than dead individuals. There were three individuals with high FA values in groups B and C. With all data included, residuals of the ANOVA were not normally distributed in some groups (Shapiro-Wilk test; family B, live oysters: $W = 0.964004$, $N = 129$, $P = 0.0017$; group C, live oysters: $W = 0.929907$, $N = 109$, $P < 0.0001$; for all groups pooled, $W = 0.972558$, $P < 0.0001$, $N = 510$). With these outliers removed, the assumption of normality was satisfied in all groups (Shapiro-Wilk test; family B, live oysters: $W = 0.985883$, $N = 127$, $P = 0.2129$; group C, live oysters: $W = 0.995905$, $N = 108$, $P = 0.9885$; for all groups pooled, $W = 0.993617$, $P = 0.0309$, $N = 507$). These data were kept in Figs. 2 and 3. ANOVA revealed a borderline interaction between group and status ($P = 0.0569$; Table 3). With the three outliers removed, the interaction was significant ($P = 0.0242$). Therefore we accepted the interaction as significant and tested the least squares means of all sets of oysters using all the data. FA of dead and live oysters was different for family A and group C, but not for family B (Table 4; with the three outliers removed, results of statistical analyses were essentially the same).

Table 3
Effect of group and status of oysters on asymmetry of the shells. ANOVA (SAS, PROC MIXED)

	Effect		
	df	F^a	P
Group	2	9.89	<0.0001
Status	1	23.80	<0.0001
Group \times status	2	2.88	0.0569

^aDenominator degree of freedom is 504 for all tests.

Table 4

Mean of shell fluctuating asymmetry (least squares means; ANOVA). Groups with common symbols are not significantly different from each other ($\alpha = 0.05$). Numbers in parentheses are standard errors

	Family A	Family B	Group C
Dead	-0.07905* (0.02568)	-0.03661*, ✕ (0.01032)	-0.00121 ^{†, ‡} (0.01186)
Live	0.02268 ^{#, ‡} (0.01134)	-0.00991 ^{†, ✕} (0.00905)	0.04036 [#] (0.00984)

4. Discussion

The results found in the present study clearly suggest that the fate of hatchery/nursery produced *C. gigas* grown in the intertidal is related to FA to some extent, with dead oysters displaying lower FA. Since FA is related to disruption of homeostasy, lack of developmental stability may be involved in the summer mortality syndrome of oysters. This may explain the difficulty of pointing out single mechanistic causes to the syndrome (e.g., Goulletquer et al., 1998; Le Roux et al., 2002).

Considering that group C, which was a genetic pool of the spawners used in the MOREST programme in 2001 exhibited a significant link between FA and status, it seems reasonable to conclude that the link between FA and status has some generality. There were, however, differences among groups, with status having no effect in family B, as opposed to family A and group C (Table 4). There is conflicting evidence about the relative importance of genetic and environmental causes in generating FA. On the one hand, genetic factors such as disruption of co-adapted gene complexes (Clarke, 1993; Wilkins et al., 1995; Hochwender and Fritz, 1999) and lack of heterozygosity (see references in Palmer and Strobeck, 1986) contribute to higher FA, although Vøllestad et al. (1999) found no relationship between FA and heterozygosity. Cadée (2000) found variability in the relative importance of genetic and environmental effects, with more stressful conditions leading to a greater relative importance of environmental effects. On the other hand, FA has also been found to correlate with parasitism (Møller, 1996; but see Ward et al., 1998). Severity of parasitic infections is probably more a consequence of developmental instability (as estimated by FA) than a cause (Thomas et al., 1998). FA has been found to increase in response to environmental stress during ontogeny (Hochwender and Fritz, 1999; Badyaev et al., 2000; Hosken et al., 2000; Lens and Van Dongen, 2000). A further mechanism for increasing FA, at least in plants, is competition for

resources because of limited energy for maintaining homeostasis (as estimated by FA) (Alados et al., 2001; Kozlov et al., 2001). Therefore, FA appears to reflect the general quality of the genetic makeup of individuals but also epigenetic effects of stressful conditions during growth. The oysters in the present study were produced in common hatchery/nursery facilities and grown on a single culture table at constant intertidal height. These conditions are likely to minimise heterogeneity in growth conditions among groups and appear to support the hypothesis that variability in FA and status were related to genetic differences among groups. This is inconclusive, however, first because the bags and larval tanks were not duplicated, and second because no attempt was made to manipulate FA through the application of variable environmental stresses. Therefore, both genetic and non-genetic factors might have influenced FA and resistance to summer mortality. This is not to say that genetic influences do not impact the ability to resist summer mortality. Mortality levels of *C. gigas* families selected for heat resistance correlated well between the field and the laboratory (Beattie et al., 1980). In the present situation, however, environmental and genetic influences cannot be sorted out.

A further point concerns the time at which FA is generated—assuming that environmental control is dominant over genetic influences—and its stability through time. In many cases, compensatory growth may occur and damp individual asymmetry events through time (e.g., Collin, 1997; see also references in Freeman et al., 2003, p. 372; Palmer and Strobeck, 2003, p. 284). Other cases have been found where asymmetry is stable through time (e.g., snow crab: Chippindale and Palmer, 1993; barn swallow: Shykoff and Møller, 1999). In the present study, FA appeared to be generated during early ontogeny and to persist to some extent throughout life, because heteroskedasticity in the plots of right valve mass vs. left valve mass (Fig. 2) imply a multiplicative growth model, with shell growth patterns depending on past growth events (Mosimann and Campbell, 1988; Graham et al., 1998). In this case, FA patterns established early in life will be reflected in future FA patterns. If the relationship between FA and survivorship holds tightly enough, it follows that future survivorship of adults may be forecasted from FA of spat. Our results provide some support to the hypothesis that FA is stable once acquired in family B and group C, but is inconclusive in the case of family A. Further evidence is needed to provide a firm basis for forecasting survivorship from FA patterns.

The significance of early events, however, is supported by evidence that spatial organisation of molecular processes early after fertilisation is central to normal embryonic development (Atlan, 1999; Simmonds et al., 2001; Wilkie and Davis, 2001). Any disruption of spatial organisation of molecular gradients within early embryos is likely to be reflected in embryological processes and in later anatomical characteristics, which will translate into increased FA. Internal organisation of oocytes and early embryos may be disrupted by small gradients in a number of environmental

variables. One such variable may be shear stress exerted on gametes and young embryos by turbulent eddies (Mead and Denny, 1995). They found that high shear stress could reduce fertilisation efficiency but also had negative effects on development and survival of embryos, especially if shear stress was applied at fertilisation. Shear stress may impact cell functioning of a number of similar-sized organisms (Juhl et al., 2001). Therefore, effects of shear stress on similar-sized organisms potentially may be widespread. Mixing of gamete solutions to promote fertilisation is standard practice in hatcheries. Increasing turbulence in larval cultures is also standard practice. The amount of shear stress applied in such operations may be generally profitable at low shear stress levels, but clearly could be detrimental at high levels. Future resistance to environmental stress (e.g., temperature) may unknowingly be jeopardised because of loss of homeostasis. On the other hand, later developmental processes may depend on effects induced by shear stress and related mechanical forces (as seen in lung development, for instance; Wirtz and Dobbs, 2000). Finally there is evidence that FA may respond to environmental stress or to intraspecific competition (Kozlov et al., 2001) later in an individual's life cycle. The issue could be critical for quality control of hatchery spat batches. If FA is determined early in life and if the link holds in the long term, early determination of FA may provide a convenient tool for early warning of potential homeostatic problems. Furthermore, early determination of FA may provide a basis for pricing various spat batches according to their expected capability to resist summer mortality. Cost of spat may amount to a significant portion of total production costs (e.g., Spencer et al., 1985).

Abnormal shell calcification appears to be part of the summer mortality syndrome in many cases (see references in Comps et al., 2001). In a case study of summer mortality in *C. virginica*, for instance, Bricelj et al. (1992) found anomalous conchiolin deposits on either the right or the left valve, or on both valves, and a tendency of the left (cupped) valve to extend beyond the edge of the right valve. However, we did not notice anomalous shell deposition in our samples—besides chambering or meshing. Whether anomalous extension of either valve occurred in our samples and was involved in mortality and FA is unclear. In a context of forecasting spat survival, the critical issue here is that early measurements of FA be correlated with future anomalous extension of valves, a point which cannot be ascertained with the present data.

A source of concern in our study may be the unknown, but potentially long (i.e., possibly nearly two months) period that dead shells remained in the culture bags before being sampled. A possible bias likely to occur in the case of differential longevity is that dead shells may have eroded after death. If right and left valves eroded at different rates, this might have caused bias in comparisons of the effect of status. It is clear, however, that successive washes resulted in increased FA values. Assuming that successive washes acted as a proxy of the effect of progressive shell dissolution and

erosion, we conclude that these processes acted to increase apparent FA, if anything. Given that significant effects were attributable to mean FA being lower in dead oysters than in live oysters, we conclude that any redissolution bias in FA of dead shells would have resulted in decreasing differences between dead and live individuals, thus favouring a conservative interpretation of the statistical analyses. The MSE values in Table 2 suggest that the correction for the effect of washing was increasingly variable with successive washes. This indicates that successive washes not only change the average FA, but also its variability, presumably owing to added valve wear caused by extra manipulations. Therefore, if washes reflect the effect of shell dissolution of dead oysters accurately, the variability of FA values of dead oysters was probably inflated, but to an unknown extent. This potential effect again favoured a conservative interpretation of the statistical analyses.

Our results may relate to carrying capacity studies in two ways. First, carrying capacity models usually incorporate mortality as a constant process (e.g., Bacher et al., 1998). If the relationship between FA, environmental stress and mortality were known, environmental forcing on density-independent mortality could be included in such models. Second, Alados et al. (2001) and Kozlov et al. (2001) reported significant relationships between FA and growth. Therefore, knowledge of FA distribution in modelled populations may allow to include individual variability in growth rate. As noted earlier, however, the relationship between FA and individual size was not significant. Legay et al. (2000) reported a positive relationship between a commonly used measure of FA (which they called “dissymétrie relative”) and individual size in *C. gigas*. This is inconsistent with our results and may have to do with the methodology used by Legay et al. (2000). They estimated FA as $(L - R)/0.5(L + R)$, which is inappropriate with multiplicative growth (which is probably the rule in *C. gigas*; Fig. 2B,C) and directional asymmetry (Graham et al., 1998, in press). Therefore, for the time being we conclude that there is no relationship between FA and growth in *C. gigas*.

FA appears particularly appealing for forecasting spat ability to resist environmental stress because it seems to appear early in ontogeny, to be stable through time and is based on simple measurements. A further point is that shells of dead individuals retain useful information. At the present time, however, further evidence is required to support the inferences relative to timing and stability of FA in *C. gigas* and establish the link between survivorship and FA on more solid ground.

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