ORIGINAL PAPER

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Phenotypic plasticity and priority rules for energy allocation in a freshwater clam: a field experiment

Abstract We studied resource allocation among maintenance, reproduction and growth in the freshwater clam *Anodonta piscinalis*. Recent theoretical and empirical studies imply that organisms with indeterminate growth may have priority rules for energy allocation. That being so, the traits involved should potentially be capable of considerable phenotypic modulation, as a mechanism to adjust allocation. We tested this hypothesis using a 1-year reciprocal transplant experiment at six sites. Experimental clams were caged at higher than natural densities in order to detect any phenotypic modulation of the traits and discover the putative priority rules in energy allocation. We recorded the survival and shell growth of clams during the experiment, and the reproductive output, somatic mass and fat content of clams at the end of the experiment. Shell growth, somatic mass, and the reproductive output of females varied more among transplant sites than among the populations of origin, suggesting a high capacity for phenotypic modulation. However, the reproductive investment, somatic mass and shell growth were also affected by origin; clams from productive habitats invested more in reproduction and were heavier. In comparison to undisturbed clams, the reproductive output of the experimental clams was similar and their fat content was higher, whereas their shell growth was considerably slower and their somatic mass lower. These results suggests that when resources are limiting (due to high density) reproductive allocation overrides allocation to somatic growth. The highest mortality during the experiment coincided with the period of reproductive stress in the spring. Additionally, the proportion of reproducing females was lower in those transplant groups where the survival rate was lowest, suggesting that maintenance allocation overrides allocation to reproduction when available resources are scarce. The results of this field experiment support theoretical predictions and results of previous laboratory experiments that suggest that there are priority rules for energy allocation in organisms with indeterminate growth.

Key words Unionidae · Growth · Reproduction · Survival · Trade-off

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Introduction

One of the central questions in life-history theory is how natural selection may produce the evolution of energy allocation patterns that lead to local maxima of individual fitness (Cody 1966; Williams 1966; Giesel 1976; Stearns 1976, 1989b; Tuomi et al. 1983; Perrin and Sibly 1993). Like other life-history traits, the traits involved in resource allocation among maintenance, growth and reproduction may also show phenotypic plasticity, i.e. the phenotypic expression of the traits depends on the environment. The two main types of phenotypic plasticity are developmental conversion and phenotypic modulation (Stearns 1989a). With developmental conversion, a trait is fixed in response to the environment during the early development of the individual, whereas phenotypic modulation refers to cases where the trait is flexible with respect to the environment. In other words, the observed energy allocation among the primary functions of the organism may follow from the responses to either the present (phenotypic modulation) or the past (developmental conversion) environment. Which type of plasticity is more beneficial depends on the dynamics of the environment; organisms facing large environmental variation during their life are expected to have flexible phenotypic traits (phenotypic modulation) (Bradshaw 1965; Levins 1968).

In nature, the availability of resources varies in time and space. Energy allocation among the different functions may, or may not, change as a response to this variation. Recent studies suggest that in organisms with inde-

a) b) c) d)

M = maintenance
G = growth
R = reproduction
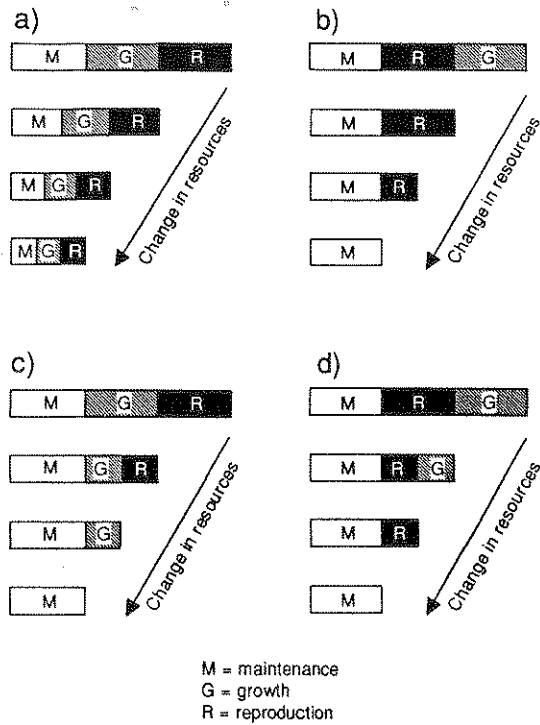
Fig. 1a–d Hypothesized changes in allocation pattern in response to changing resource levels. *Horizontal bars* represent the amount of assimilated energy available. *Sections* of the bar represent the amount allocated to maintenance, growth and reproduction. **a** A decrease in assimilated energy is divided equally among maintenance, growth and reproduction. **b** Growth is traded off against reproduction, allocation to maintenance remains constant. In panels **c** and **d** a decrease in assimilated energy affects both growth and reproduction, finally trading off against whichever function is less important in terms of fitness

terminate growth energy allocation is controlled by priority rules (Gurney et al. 1990; McCauley et al. 1990b; Bradley et al. 1991a; Glazier and Calow 1992; Perrin 1992). These "rules" may be envisioned as a group of physiological traits coadapted under natural selection. As a result, the traits reflecting the amount of allocation, e.g. to growth and reproduction, should show a high potential for phenotypic modulation, and covary to produce the allocation pattern determined by the underlying priority rules.

Figure 1 depicts some alternative responses to decreasing resources. If the availability of resources decreases, the proportion of assimilated energy that is allocated to each of the primary functions (maintenance, growth, reproduction) may remain the same (Fig. 1a). However, if decreased allocation to maintenance leads to decreased survival, allocation both to reproduction and growth may decrease relatively more than allocation to maintenance (Fig. 1b–d). This is predicted to occur in long-lived organisms in a variable environment, although "the value of survival" may decrease with age (the theory of increasing reproductive effort with age: Williams 1966). Furthermore, allocation to either growth or reproduction may be halted, whichever is least harmful in

terms of decrease in fitness (Fig. 1b). These models, and their combinations (Fig. 1c-d) have been empirically tested in the laboratory using cladocerans as model organisms (McCauley et al. 1990a; Bradley et al. 1991b; Glazier and Calow 1992).

Studies on energy allocation in natural populations of iteroparous marine bivalves suggest that if resources decrease, reproduction is often compromised for the sake of maintenance and somatic growth (corresponding to Fig. 1c) (Bayne et al. 1983; MacDonald and Thompson 1985). However, this is not a general rule. Based on a series of studies on the ecological energetics of island scallop *Chlamys islandica* (Vahl 1978, 1981b, c), Vahl (1981a) concluded that after maturity the priorities of energy allocation were maintenance>reproduction>growth (corresponding to Fig. 1d). Another aspect of energy allocation in marine bivalves was studied in a controlled field experiment by Peterson and Fegley (1986). They observed seasonal differences in the growth rates of adult hard clams *Mercenaria mercenaria* when compared to juveniles. The difference was most likely due to increased allocation to biochemical storage energy as adults prepared for the oncoming reproductive period. This study (Peterson and Fegley 1986) shows that *Mercenaria* clams may change their energy allocation among growth and reproduction in response to seasonal demands. Whether or not the documented variation in priorities of energy allocation in marine bivalves indicates "fine tuning" of allocation patterns by adaptive evolution has remained an open question in life-history studies.

In this paper we present the results of a 1-year field experiment planned to reveal the phenotypic plasticity and the possible priorities in the energy allocation pattern of a indeterminately growing freshwater clam *Anodonta piscinalis* Nilss. By transplanting the clams reciprocally among sites we set up a large range of past-present environments. To further increase the range of environments and to reveal putative allocation trade-offs we caged clams at unnaturally high densities. In a reciprocal design, a strong effect of transplant site would suggest phenotypic modulation of the allocation pattern, a strong effect of origin would indicate genetic canalization or developmental conversion, and a strong interaction effect between origin and transplant site would indicate local adaptation to the environment. We observed a large phenotypic modulation in most of the measured traits and gained evidence of the existence of priority rules for energy allocation.

Materials and methods

Study organism

A. piscinalis is a long-lived, annually reproducing, common European freshwater clam. The sexes are separate, and the larvae are parasites of fish. Fertilization takes place in mid summer. Glochidia larvae are produced during the late summer and brooded in the outer gill-blades of the females over the winter and released the next spring (Ökland 1963; Negus 1966; Jokela et al. 1991). The

clams are sedentary after the larval phase and inhabit a wide range of habitats, showing extensive among- and within-population variation in several life-history traits (Haukioja and Hakala 1978a). In their natural habitats, littoral areas of lakes and slow-flowing narrow channels, clams are exposed to annual and seasonal variation in resources.

Study sites

We chose our six study sites from among 31 populations sampled in summer 1988 and 1990 in southern and central Finland. We ranked the 31 populations according to mean length of the clams at the age of 3 years, which is a good indication of growth rate (Haukioja and Hakala 1978b). For the experiment, we chose three populations in which the clams grew slowly and three populations in which they grew rapidly (Fig. 2). Clams were aged by counting the annual rings, which is a reliable method for this species (Haukioja and Hakala 1978b; Pekkarinen 1991). As an additional criterion, we chose populations close to each other, i.e. populations that were subject to similar climatic conditions but were isolated from each other by dispersal barriers (coordinates for most distant sites: site F, 62°20′N, 25°55′E; site B, 63°07′N, 25°55′E). All the populations selected inhabited slow-flowing, sandy-bottomed sections of large water courses belonging to the Kymijoki river water system.
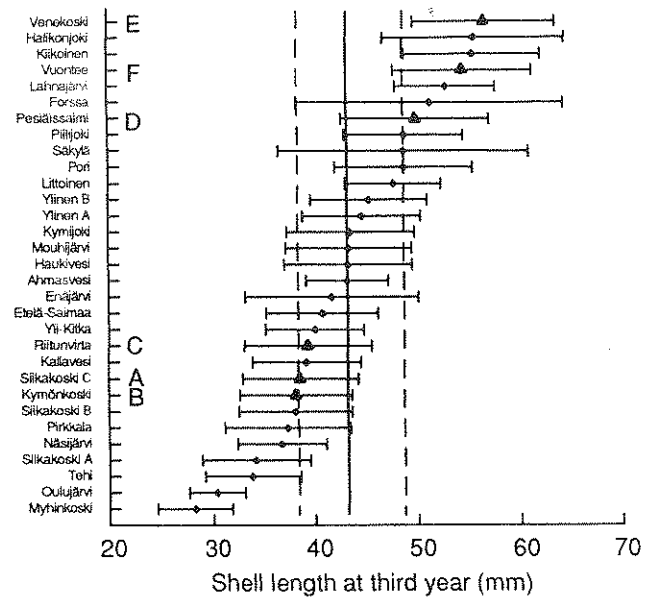
Experimental design

In late September 1990, we collected clams from the six study populations by scuba diving and transported them to the laboratory in water-filled buckets. In the laboratory the clams were kept for 1 week at +5°C in aerated lake water.

We chose 240 individuals (60 young females, 60 old females, 60 young males, 60 old males) from each origin for transplanting. "Young" clams were 5 years of age or younger (median age 3 years), and "old" clams were over 5 years of age (median age 7 years). Young clams had begun or were about to begin their reproductive life (age of maturation is 3–4 years). The sex of the clams was determined by gently prying the clam slightly open with a wedge-shaped tool gradually pushed between the valves from the posterior end, along the anterior-posterior axis of the clam. This causes the relaxation of adductor muscles without damaging them or the shell of the clam. We then checked whether the clam carried glochidia in the outer gill blades. In autumn, 95% of female clams brood glochidia in their outer gill blades (Jokela et al. 1991) which, consequently, swell markedly and change colour. We marked the clams individually by scraping a code on the shell (Hinch et al. 1985), and measured the length, height and width of the shell and the length of each year ring according to Haukioja and Hakala (1978b).

We assigned a group of 40 individuals of each origin to be transplanted to each of the six sites (four subgroups: 10 randomly chosen young females, 10 young males, 10 old females and 10 old males). One group (40 individuals) served as a control group and was transplanted back to the original site. The balanced factorial design required 1440 individuals; however, we were unable to find the required number of old brooding females at origin E (47 instead of 60).

We started the experiment in early October 1990. All clams to be transplanted to a certain site were pooled (all six origins together, 240 clams) and then randomly assigned to six 1-m² metal frames. Thus each frame held 40 clams (40 individuals/m²). The frames were pushed 8 cm deep into the sediment and did not stick out from the bottom. This provided a sufficient barrier to prevent emigration or immigration of clams. Clams were positioned on a natural surface within the frames. Frames were covered with a wire mesh cage to prevent muskrat predation. The mesh cage was high enough (15 cm) to allow clams to crawl around in the cage. The mesh cage was 20 cm wider than the frame, preventing access to the proximity of the mesh wall. We used six cages at each site to provide the necessary replication, but did not design the experiment as a factorial block design (e.g. ten cages per site, one clam

Fig. 2 Individual shell growth rate of clams (mean length±SD at age of 3 years, from analysis of annual growth bands) in populations from which experimental populations were chosen. *Solid vertical line* indicates median growth rate. *Dashed vertical lines* on both sides of the median delimit the area in which 50% of the sampled populations fell. Populations chosen for the experiment are indicated by *black triangles*; *capital letters* indicate the code used in the text for each site

per subgroup per cage). We expected some mortality to occur, so executing the experiment as factorial blocks would have been inefficient. Instead, we chose the number of cages as a compromise between the needs of replication and the limitations set by scuba diving.

We visited the sites 12 times during the following year. Sites were visited more frequently during the summer than during the winter. At each visit we cleaned the wire cages to prevent filamentous algae from clogging the cages, and collected the shells of dead clams.

In October 1991, we collected all the remaining clams and brought them to the laboratory. Data were obtained for 1338 clams, since 89 (6.2%) were lost (not found either dead or alive) during the experiment. In the laboratory, we remeasured the shell to calculate growth during the experiment, and recorded the sex and the occurrence of trematode parasites by pressing a small piece of gonad between two glass plates under a dissection microscope (Taskinen et al. 1991). We dissected the body and gill blades and weighed them separately after drying at 60°C for 48 h. Fat content (%) of the body was estimated as weight loss during diethyl-ether extraction (Reznick 1983).

Several stress factors were intentionally included in this design. Firstly, the clams were kept in the laboratory without feeding for 1 week before the experiment was started. Although the clams were stored in cold water, they were kept at room temperature during marking for 1 day. The clams were not injured by marking and opening, but handling may still cause physical stress. Secondly, the cage affected the quantity of resources available. We cleaned the cages when visiting the sites, but at all sites periphytic algae attached to the wires and partly clogged the cage, and reduced the inflow of fresh water and food particles. The third, and perhaps the most important, factor is that the clam density in the experiment was more than twice as high as the highest natural densities recorded at the study sites (40 individuals/m² vs. 1–20 individuals/m²; J. Jokela, unpublished work).

To assess the overall effect of the experiment on the measured traits, we collected clams outside the frames at each study site

when the experiment was finished. We measured 16–20 brooding females per site in the same way as we measured brooding females in the experiment. We refer to these clams as "undisturbed samples" to distinguish them from the back-transplanted experimental controls. In the undisturbed samples, shell growth during the previous year was calculated by subtracting the length of the clam in the previous winter (measured from the last year-ring) from the total length. This corresponds to the study period since the growth season was over when the experiment was started (late October).

We excluded clams that were heavily infected by trematode parasites from all analyses (172 clams, 23.7% of survivors), since heavy infection castrates females (Taskinen 1992) and affects the somatic mass of the clams (Jokela et al. 1993). In heavily infected individuals >80% of the gonad was invaded by the parasite. The prevalence of early trematode infections among the remaining survivors was 6.2%.

Data analysis

We analysed the data using generalized linear models (logit-analysis, multivariate and univariate analysis of covariance) (McCullagh and Nelder 1983; Hosmer and Lemeshow 1989; Trexler and Travis 1993). Our main interest was to study the effect of population (ORIGIN) and transplant site (SITE) on the survival and performance of clams, and how this effect was modified by age (AGE) and sex (SEX) of the clams. We assessed the performance of clams by shell growth during the experiment, somatic mass and fat content; and additionally for females, by the proportion of reproductive females, weight of reproductive output (weight of outer gill blades of brooding females) and reproductive investment (outer gill blade/somatic mass ratio: Harper and Ogden 1970). The weight of the gill blades is not an absolute measure of the weight of the glochidia, but it gives a relative measure of offspring production (Fig. 3).

If more than one individual per transplant group was alive in a cage, we used cage means of dependent variables in all analyses of covariance. Therefore, the maximum number of independent observations per site is six (number of cages). In all analyses of covariance the shell length was used as a covariate. Mortality of
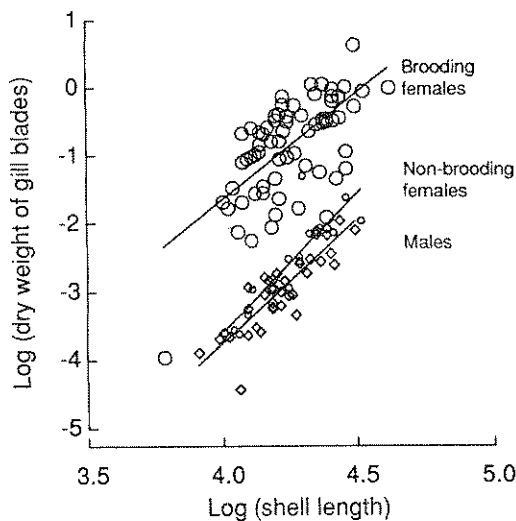

Fig. 3 Dry mass of gill blades of brooding females (*large circles*), non-brooding females (*small circles*) and males (*diamonds*) in relation to log-transformed shell length of clams in experimental controls (*n*=122). The difference between regression lines of males and non-breeding females and of breeding females indicates the mass of glochidia. Since the slopes of the regression lines are not significantly different (ANCOVA, $F_{2,116}$=0.61, P=0.546), weight of the gill blades can be used as a relative measure of reproductive output

young clams impaired the design (low number of survivors), so that age could not be used as a factor. Therefore, age groups were combined for these analyses. However, since length is strongly correlated with age, it corrects for most of the variation caused by age. If necessary, we used ln-transformed values of the response variables and the covariate to meet the requirements of normally distributed and homoscedastic residuals. The assumption of parallel slopes was fulfilled in all analyses of covariance. When interpreting the results, we use length-adjusted means, calculated with MANCOVA. Bonferroni tests were used for unplanned comparisons of the means if needed for the accurate interpretation of the results. The Bonferroni method corrects for experiment wise type I error, is a conservative test, and can be used with adjusted means (Day and Quinn 1989). The statistical analyses were performed with the SPSS (Norusis 1990) and SYSTAT (Wilkinson 1990) statistical packages. More details of statistical analyses are given in Results.

Results

Survival of young and old clams

Young clams had on average lower survival (40.1%) during the experiment than the old ones (65.8%) (Table 1), as indicated by the significance of the parameter for age (Z=7.94, P<0.001) in a saturated logit model in which

Table 1 Survival (%) of young (median 3 years) and old (median 7 years) clams from different study populations (*origin*) at different transplant sites (*site*) during the reciprocal transplant experiment. Values for back-transplanted control groups are shown along the diagonal in bold. Note that at site F none of the clams from site A survived. In young clams both origin and site affected survival; in old clams the interaction was also statistically significant (logit analysis, see Results). Adjusted residuals (difference between expected and observed counts divided by an estimate of standard error) are given in parenthesis if residual of the 'no-interaction' model was larger than |2|. Distribution of adjusted residuals approaches standard normal distribution for large sample sizes (5% of values exceed 1.96)

| | | Site | | | | | | |
		A	B	C	D	E	F	$\bar{X}$
Young clams								
Origin	A	**30.0** (2.23)	21.1	5.6	28.6	30.8	0	18.6
	B	31.6 (2.26)	**25.0**	5.0	27.8	12.5	5.3	17.9
	C	17.6 (−2.24)	58.8	**36.8**	81.3	64.7	6.7	44.6
	D	47.4	63.2	30.0	**75.0**	73.3	23.5	51.8
	E	50.0	60.0	57.9	72.2	**84.2**	33.3	56.6
	F	35.0	52.4	20.0	72.2	73.7	**26.3**	46.2
	$\bar{X}$	35.7	46.6	25.9	60.6	58.6	16.0	40.1
Old clams								
Origin	A	**75.0** (2.15)	50.0	45.0	82.4	66.7	0 (−2.49)	53.0
	B	42.1	**47.4**	47.4	81.3	77.8	5.0	48.6
	C	95.0	97.7	**82.4**	83.3	90.0	47.6	81.7
	D	70.0	89.5	65.0	**100**	89.5	41.2	76.7
	E	61.1	77.8	83.3	94.1	**76.5**	38.9	71.7
	F	73.7	44.4 (−2.22)	63.6	61.6 (−2.90)	65.0	**70.0** (4.49)	63.2
	$\bar{X}$	69.8	67.3	63.8	84.1	77.4	33.9	65.8
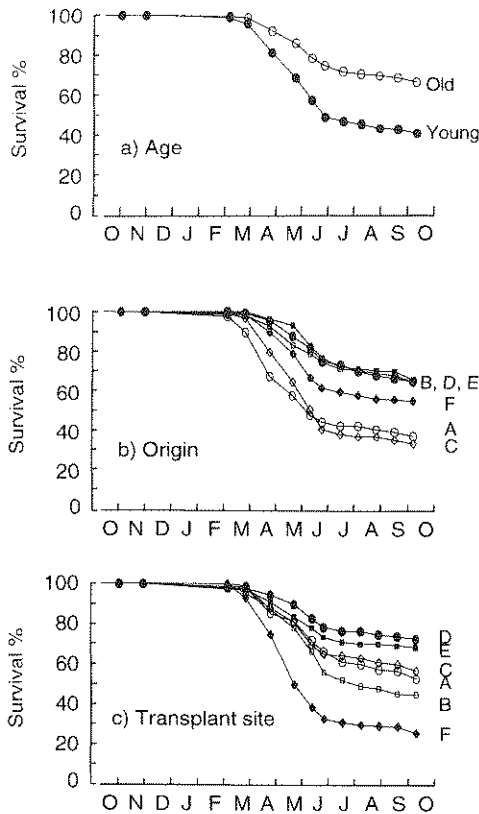
Fig. 4 Cumulative survival of clams in the experiment by **a** age group, **b** origin and **c** transplant site (includes clams from all origins). *Letters* on the *x*-axis indicate months, starting from early October 1990. In **b** and **c** *open symbols* indicate "poor" sites (slow growth, Fig. 2) and *solid symbols* indicate "good" sites (rapid growth, Fig. 2)

planted to different sites, but the effect of the transplant site was large (Table 1). The survival of old clams was affected by origin, site and their interaction (ORIGIN, $\chi^2=52.07$, $df=5$, $P<0.001$; SITE, $\chi^2=68.96$, $df=5$, $P<0.001$; ORIGIN×SITE, $\chi^2=59.21$, $df=25$, $P<0.001$). Analysis of adjusted residuals of the model including only main effects indicated that the high survival of back-transplanted control clams at site F (survival 70%, adjusted residual 4.49) contributed greatly to the significant interaction term (Table 1). Except for this control group, the survival of young and old clams of all origins was low at site F (Table 1). Environmental conditions were exceptionally harsh at site F due to dredging of the channel upstream (see Discussion).

Visual inspection of cumulative survival curves indicates a peak in mortality in the spring (March–July). Survival was high during the winter (October–February) and at the autumn (late July–October) (Fig. 4).

Comparison of experimental and undisturbed clams

Comparison of the shell growth, reproductive output, somatic mass and percent fat content of the brooding females between the back-transplanted experimental controls and the undisturbed population indicated a significant experimental effect. In the two-way MANCOVA, both the effect of the experiment (EXP, undisturbed vs. back-transplanted experimental controls) and population (POP), as well as their interaction, were statistically highly significant (Table 2). Univariate statistics indicate that the significant interaction term is solely due to differences in the shell growth (Table 2). The undisturbed clams grew more than those in the experiment, and the effect of the experiment differed in magnitude among populations (Fig. 5a). Reproductive output was not affected by the experiment, but differed significantly among the populations (Fig. 5b, Table 2). The experimental control clams were lighter (Fig. 5c) but contained a higher percent fat than the undisturbed ones (Fig. 5d). The experiment did not affect reproductive investment, calculated as gill blade-somatic mass ratio (ANCOVA, $F_{1, 108}=0.87$, $P=0.353$, Fig. 5e), but the differences among the populations were statistically significant (ANCOVA, $F_{5, 108}=3.33$, $P=0.008$), as was clam length as a covariate (ANCOVA, $F_{1, 108}=5.34$, $P=0.023$). The in-

origin, site and age were used as independent variables. In further analysis the age groups were treated separately.

We analysed the effect of origin and site on the survival probability of clams in the experiment using a logit-analysis (Hosmer and Lemeshow 1989). For young clams both origin and site had significant effects on survival probability during the experiment ($\chi^2=77.81$, $df=5$, $P<0.001$; $\chi^2=77.65$, $df=5$, $P<0.001$, respectively), whereas the interaction term did not ($\chi^2=27.57$, $df=25$, $P=0.312$). Thus, for young clams the differences in survival among origins remained when they were trans-

Table 2 Multivariate analysis of covariance for differences in shell growth, reproductive output, somatic mass and fat content of brooding females among different populations (*POP*) and between undisturbed samples and back-transplanted experimental controls (*EXP*). Length of clam was used as a covariate

Effect	Multivariate (Wilks Λ)			Univ.	Shell growth		Reproductive output		Somatic mass		Percent body fat	
	df	F	P	df	F	P	F	P	F	P	F	P
EXP	4, 101	41.78	<0.001	1	159.17	<0.001	0.78	0.379	13.82	<0.001	5.94	0.016
POP	20, 336	5.83	<0.001	5	19.88	<0.001	6.30	<0.001	2.64	0.027	0.38	0.858
EXP×POP	20, 336	2.22	0.006	5	6.64	<0.001	1.50	0.195	0.55	0.736	0.03	1.000
LENGTH	4, 101	85.85	<0.001	1	29.78	<0.001	101.62	<0.001	213.33	<0.001	1.35	0.270
Error MS				104	0.139		0.178		0.031		0.119	

☐ Experimental control

▨ Undisturbed population
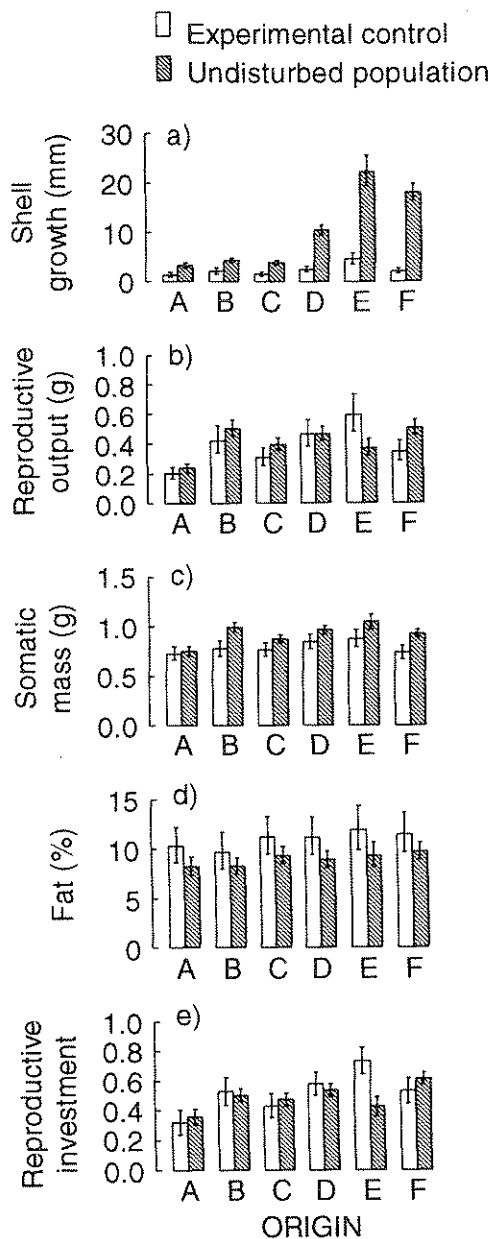
Fig. 5 Length-adjusted mean (±SE) of **a** shell growth, **b** reproductive output, **c** somatic mass, **d** body fat content and **e** reproductive investment of brooding female clams in experimental controls and undisturbed samples originating from different populations. *Error bars* are slightly asymmetrical due to back-transformation from logarithmic means

teraction was not significant (ANCOVA, $F_{5,108}=1.81$, $P=0.117$, error MS=0.03). To conclude, the experiment affected shell growth and somatic mass more than it affected the percent fat content and the reproductive performance of the clams (Table 2, Fig. 5).

Shell growth, somatic mass and fat content of clams in the experiment

Because of low survival at site F (all old clams transplanted to site F from site A died during the experiment:

Table 1) there were not enough data for the analysis at this site. Clams transplanted to and originating from site F were therefore excluded from all further analyses.

In MANCOVA, using the shell growth, somatic mass and percent fat content as dependent variables, the multivariate main effects of origin, site and sex and the interaction origin×site were statistically significant (Table 3). Overall variation among transplant sites (multivariate $F_{12,477}=14.44$, Table 3) was greater than that among populations (multivariate $F_{12,477}=2.69$, Table 3). Clams were lightest and grew most poorly at transplant sites A and C (Fig. 6). At site E, where the clams were heaviest and grew most, the clams were on average 1.7 times heavier and their shell growth was 4.4 times faster than at site A. All pairwise comparisons of mean shell growth and somatic mass between sites were statistically significant (Bonferroni tests, $P<0.05$) except differences between sites A and C, and B and D (Bonferroni tests, $P>0.05$)(Fig. 6). The significant multivariate interaction was caused by a strong interaction effect on somatic mass (univariate $F_{16,171}=2.12$, $P=0.009$) together with a weaker effect on shell growth (univariate $F_{16,171}=1.65$, $P=0.060$) (Table 3). This indicates that the effects of clam origin on somatic mass, and to some extent also on shell growth, depended on where they were transplanted to (Fig. 6). For example, at sites A, C and E the effect of origin on somatic mass was negligible, but at sites B and D it was statistically significant (within-site ANOVA, see Fig. 6). Likewise, the effect of origin on shell growth was significant at sites B and E (within-site ANOVA, see Fig. 6). At site B clams from population E were significantly lighter than clams from populations A, B, or C, and they grew less than clams from population A (Bonferroni tests, $P<0.05$, Fig. 6). At site D clams from population E were lighter than clams from population A (Bonferroni test, $P<0.05$, Fig. 6). The significant multivariate main effect of sex was due to differences between sexes in somatic mass (Table 3). Males were on average 23% heavier than females.

Residual correlations between dependent variables indicate that somatic mass was associated positively with shell growth and negatively with percent fat content (Table 3). In other words, when somatic mass decreased, shell growth decreased and the relative fat content increased (Table 3).

Brood production, reproductive output and reproductive investment of clams in the experiment

The probability of producing a brood during the experiment was affected more by transplant site (logit analysis, $\chi^2=17.27$, $df=4$, $P=0.002$) than by origin (logit analysis, $\chi^2=1.51$, $df=4$, $P=0.825$). However, the fit of the logit model in which the main effect of site was the only remaining term was only marginally adequate (Pearson $\chi^2=30.24$, $df=20$, $P=0.066$, likelihood ratio $\chi^2=37.28$, $df=20$, $P=0.011$; high P-value indicates good fit with the data). Only females that survived were included in the analysis.

Table 3 Multivariate analysis of covariance for differences in shell growth, somatic mass and percent fat content of female and male clams (SEX) from different populations (ORIGIN) transplant- ed to different sites (SITE). Length of clam (LENGTH) was used as covariate. Residual correlations indicate Pearson correlation co- efficients of the residuals of the dependent variables

Effect	Multivariate (Wilks Λ)			Univ.	Shell growth		Somatic mass		Percent body fat	
	df	F	P	df	F	P	F	P	F	P
ORIGIN	12, 447	2.69	0.002	4	2.84	0.026	4.26	0.003	1.40	0.233
SITE	12, 447	14.44	<0.001	4	29.87	<0.001	33.14	<0.001	4.00	0.004
SEX	3, 169	11.96	<0.001	1	0.71	0.401	23.01	<0.001	0.58	0.447
ORIGIN×SITE	48, 503	1.66	0.004	16	1.65	0.060	2.12	0.009	1.32	0.184
ORIGIN×SEX	12, 447	1.33	0.200	4	1.13	0.344	1.44	0.227	2.01	0.095
SITE×SEX	12, 447	1.35	0.188	4	0.28	0.885	2.03	0.091	1.37	0.246
ORIGIN×SITE ×SEX	48, 503	0.90	0.654	16	0.96	0.502	0.90	0.568	0.84	0.636
LENGTH	3, 169	186.98	<0.001	1	6.63	0.011	379.56	<0.001	6.17	0.014
Error MS				171	0.169		0.053		0.010	

|	Residual correlation	
	Shell growth	Somatic mass
Somatic mass	0.33***	
Percent body fat	−0.13ns	−0.35***

*** $P<0.001$, ns $P<0.05$
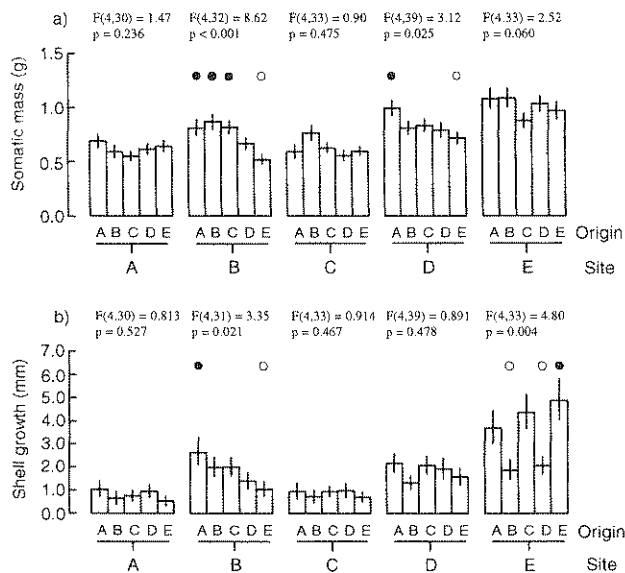
Fig. 6 Length-adjusted mean (±SE) of **a** somatic mass and **b** shell growth of clams 1 year after the start of the reciprocal transplant experiment. *Bars* indicate performance of clams of each origin (*first row of capital letters*) at each transplant site (*second row of capital letters*). *Error bars* are slightly asymmetrical due to back-transformation from logarithmic means. The effect of origin on so-matic mass and shell growth was tested within each site using a two-way ANOVA (origin, sex). If the effect was statistically sig-nificant (*F*-statistics for origin effect are given *above panels*), means were tested pairwise using post hoc Bonferroni tests. Means marked with *filled circles* are higher than means marked with *open circles* (5% error level)

If transplant groups (origin-site combinations) are treated as independent populations (25 groups), it is pos-sible to analyse the association of mortality and proba-bility of brood production. Correlation between mortality and probability of brood production was strong and neg-ative ($r= -0.50$, $df=25$, $P=0.011$) indicating that at
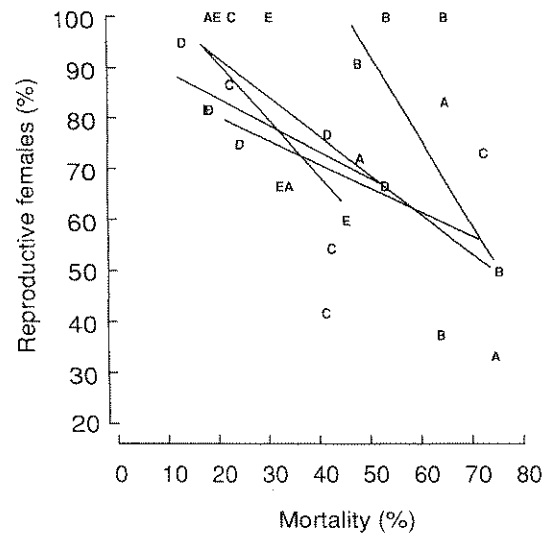
Fig. 7 Association of mortality and probability of brood produc-tion (among survivors) in the experiment (Pearson $r= -0.50$, $df=25$, $P=0.011$). *Points* represent transplant groups ($n=25$). Groups of same origin are indicated by *capital letters*. *Lines* indi-cate the trend for each origin

groups facing high maintenance allocation lower propor-tion of females reproduced (Fig. 7).

Transplant site affected the reproductive output of fe-males, measured as the dry weight of the gill blades (ANCOVA, for SITE, $F_{4, 82}=7.99$, $P<0.001$), whereas or-igin did not (ORIGIN, $F_{4, 82}=1.82$, $P=0.130$). The inter-action between site and origin was not statistically sig-nificant ($F_{16,82}=1.34$, $P=0.196$; error MS=0.17), whereas length as a covariate was ($F_{1, 82}=85.90$, $P<0.001$). At site E, where the reproductive output was highest, the clams produced on average 1.9 times more glochidia, than at site A, where it was lowest (Bonferroni test, $P<0.001$) (Fig. 8a). The average reproductive output at site E was also higher than that at site B ($P=0.011$), and at site D higher than at site A ($P=0.001$) (Fig. 8a).

We repeated the analysis described above using so-matic mass, percent fat content, shell growth and repro-ductive output as dependent variables in a MANCOVA
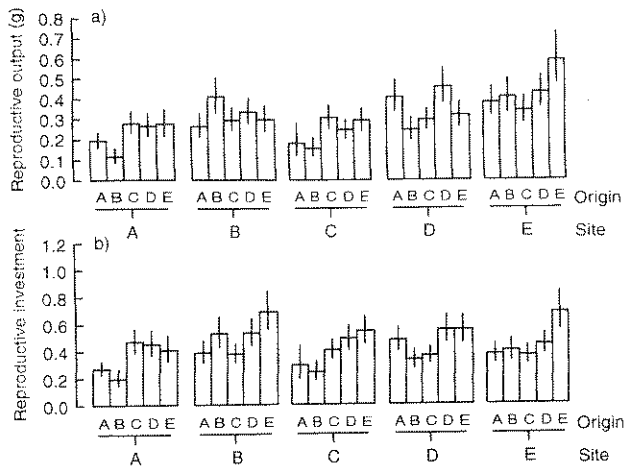
Fig. 8 Length-adjusted mean (±SE) of **a** reproductive output, measured as dry weight of gill blades, and **b** reproductive investment, measured as reproductive output/body mass-ratio of female clams 1 year after the start of the reciprocal transplant experiment. *Bars* indicate performance of clams of each origin (*first row of capital letters*) at each transplant site (*second row of capital letters*). *Error bars* are slightly asymmetrical due to transformation from logarithmic means

model. We do not present the full results of the model because they do not significantly add to results presented in Table 3. However, we present the results of the residual correlation analysis. Like the analysis including both sexes (Table 3), shell growth of females increased together with somatic mass ($r=0.42$, $P<0.001$) and as percent fat content increased, somatic mass decreased ($r=-0.38$, $P<0.001$). Additionally, shell growth and percent fat content were negatively correlated ($r=-0.27$, $P=0.006$). This indicates that females that grew most had higher somatic mass but relatively lower fat content. Reproductive output was positively correlated with somatic mass ($r=0.29$, $P=0.003$), but was not correlated with either percent fat content ($r=-0.12$, $P=0.219$) or shell growth ($r=0.17$, $P=0.091$).

Differences in the reproductive investment of female clams were statistically significant among populations (ORIGIN, $F_{4,82}=4.93$, $P=0.001$), but not among transplant sites (ANOVA, for SITE, $F_{4,82}=2.23$, $P=0.072$) (Fig. 8b). Length as a covariate was statistically significant ($F_{1,82}=7.53$, $P=0.007$), whereas the interaction term was not ($F_{16,82}=1.07$, $P=0.394$; error MS=0.18). On average, clams from population E invested more in reproduction than clams from populations A, B or C (Bonferroni tests, $P=0.023$, $P=0.002$, $P=0.038$, respectively). In addition, clams from population D invested more than clams from population B ($P=0.002$) (Fig. 8b).

Discussion

Phenotypic response to environmental change

Our results show that *Anodonta* clams do respond flexibly to environmental change. When clams from six pop-

ulations were transplanted to new sites, their shell growth, somatic mass, percent body fat, and, in females, the proportion of individuals reproducing and the reproductive output, were affected more by transplant site than by origin. Since there was no significant interaction between origin and transplant site for most of the traits studied (except for somatic mass and survival of old clams) our results do not support hypotheses of local physiological adaptation.

Origin, however, clearly affected somatic mass, shell growth and reproductive investment during the experiment (Table 3). On average, clams from the more productive habitats (origins D and E) invested more in reproduction (Fig. 8b) than the clams from the poorer habitats, irrespective of the quality of the transplant site, as indicated by the significant origin terms and the nonsignificant interaction terms in the statistical analyses. These results are in accordance with those of Haukioja and Hakala (1978a). They found that reproductive investment was negatively correlated with the reproductive life span, which was shorter for clams living in productive environments. Our results suggest that, in addition to phenotypic modulation, reproductive investment, somatic mass and shell growth are also affected by canalized genetic factors, or by the early phenotypic fixation of these traits (developmental conversion).

Priority rules for energy allocation

Can the priority rules for energy allocation be detected using this kind of field experiment? Although we did not try to measure the energetics and physiological traits involved in the energy allocation (it would have been impossible), two lines of indirect evidence may be followed. Key questions are:
1. Is allocation to maintenance the top priority?
2. Which is more important, allocation to reproduction or allocation to growth?

This experiment does not give direct evidence of the priority of allocation to maintenance over allocation to reproduction or to somatic growth. However, when we treated experimental transplant groups as independent populations, we found a negative correlation between mortality and the probability of survivors producing a brood (Fig. 7). The trend remained the same if origin was taken into account (Fig. 7). This negative correlation suggests that high relative allocation to maintenance was associated with a higher proportion of females not reproducing. This interpretation is based on the assumption that survivors in a high-mortality group were forced to allocate relatively more to maintenance than survivors in a low-mortality group. This is a plausible assumption given that we designed the experiment to reduce the amount of resources available and further manipulated the environment by transplanting groups of clams among sites. Thus, we interpret our results as supporting the hypothesis that allocation to maintenance overrides reproductive allocation in these clams if resources decrease

substantially (Fig. 1b-d). Laboratory experiments using Cladocera have come to the same conclusion (Glazier and Calow 1992).

The comparison between control females in the experiment and undisturbed females of the same population answers the question of the comparative importance of reproduction and growth. Female clams in the experimental control groups grew more slowly and had lower somatic mass than those in the unstressed samples. In addition, there were no differences in reproductive output between the control groups, and the fat content of experimental females was higher than that of the undisturbed population. Together these results suggest that the effect of resource limitation on shell growth and body mass is higher than on reproduction and energy content of clams (Fig. 5). In other words, when short of resources, reproductive allocation overrides allocation to shell growth and somatic mass (corresponding to Fig. 1b or d).

Analysis of allocation trade-offs at the individual level is an alternative way of viewing the data to the among-group comparisons presented above. In this experiment "the individual level" refers to within-individual correlations of the measured traits, removing the effects of length, origin and transplant site. In other words, residual correlations among dependent variables describe how the measured traits covary at the individual level. Analysis including both sexes indicated that shell growth and somatic mass were positively correlated (Table 3). This suggest that shell growth did not take place at the expense of somatic condition, i.e. if allocation to somatic mass decreased, the allocation to growth also decreased. Similar results were obtained from the analysis of females. Additionally, in females, reproductive output was positively correlated with somatic mass, suggesting that reproduction did not take place at the cost of lower somatic condition. In an extensive field experiment with a marine clam, *Protothaca*, Peterson (1982) observed similar patterns in energy allocation: growth rate decreased at the same rate as reproductive effort in response to increased density. Furthermore, it has been shown that female freshwater clams provide resources for the developing brood during the winter (Arey 1932; Castilho et al. 1989). Hence, by producing fewer offspring, individuals in poor condition also reduce the need to allocate resources for the maintenance of the brood during the following winter.

In both sexes, however, somatic mass was negatively correlated with percent fat content, suggesting that when somatic mass decreased the relative fat content of the clam increased. Additionally in females, the percent fat content increased when shell growth decreased. The functional explanation of this result may be that the decrease in somatic mass takes place in organs that are low in fat, e.g. the muscular foot (Jokela et al. 1993), and thus the fat content, measured as the percentage of fat per unit weight, actually increases. From an energy allocation point of view, this indicates that the total amount of fat reserves in the individual does not change as much as expected when somatic condition decreases. Since the

somatic mass and shell growth covary strongly, a negative correlation between fat content and shell growth in females may be a side-effect.

The analysis of the residual correlations did not reveal a trade-off between shell growth and reproduction, as did the comparison of averages of experimental controls and undisturbed population. This is not surprising taking into account the results of the recent theoretical studies dealing with the nature of life-history trade-offs (van Noordwijk and de Jong 1986; de Jong and van Noordwijk 1992). The conclusion from these studies is that the larger the among-individual differences in the covarying traits, the more unlikely it is that trade-offs will be observed using correlative data. In this experiment, the variation in performance among individuals was large, and we observed positive correlations between somatic mass and reproduction and somatic mass and shell growth. This suggests that the better the somatic condition of the clam, the more energy is left over for growth after the prior energy demands are satisfied.

Survival and reproductive activity

The observed survival pattern indicates that clams of different origins had different stress tolerances, and that young clams were more sensitive to stress than the old ones (Table 1). Although the differences among origins were substantial, the large variation in survival rates among the transplant sites indicates that survival was largely mediated by the environment (Table 1). The origin effect may have a genetic component, but it may also follow from differences among origins in the amount of biochemical storage energy collected before the experiment began. Unfortunately a 1-year experiment is too short to distinguish between these alternatives.

Mortality at site F was exceptionally high. We believe that this was caused by the high rate of sedimentation due to the dredging of the upstream lake. Judged from the rate of sedimentation observed and the colour of the water, the food particle-clay particle ratio at site F was very low.

During the experiment, the highest mortality occurred in early spring (Fig. 4) when the clams undergo reproductive stress; the females release glochidia larvae and the males produce and release sperm (Haukioja and Hakala 1978a; Jokela et al. 1991). At least for the females, the release of glochidia involves increased activity (Jokela and Palokangas 1993), which may increase the total metabolic energy demand of females. If resources are scarce in spring, these increased demands may not be met, and if the resources stored in the previous autumn are not sufficient, reproductive activities may increase mortality. Other possible important factors are stress due to temperature, changes in food availability, physical disturbance, and activity of the trematode parasites. Most of these factors may interact with reproductive stress.

The stress treatment may have revealed the costs of the reproductive activity. The glochidia, brooded by the

females throughout the winter, were produced the previous autumn, before the experiment started. Thus, the amount of glochidia to be brooded was a consequence of an allocation decision based on a normal situation, without any additional stress. It is not clear if these clams are capable of aborting offspring and redirecting the energy allocated to developing brood to the maintenance of the female (Kozlowski and Stearns 1989). Hence, brooding and reproduction may have been relatively more costly during the experiment than usual; this is also supported by the high proportion of mortality occurring before late May in clams of origins A and C (Fig. 4), where the overall tolerance of stress was lowest.

Conclusions

Our results support the view that allocation to maintenance is of primary importance, reproduction is of secondary importance and somatic growth of the least importance for *Anodonta* clams when resource availability changes (Fig. 1b). Thus, we suggest that rules for energy allocation exist in *Anodonta*. A large potential for phenotypic modulation allows flexible responses to environment and allows modification of the allocation pattern. However, some of the traits studied, especially reproductive investment, were less plastic than others, showing that genetic canalization or developmental conversion also plays a role in the expression of the traits. It is tempting to suggest that the documented properties of the allocation pattern are adaptive, and have evolved as a response to annual and seasonal variation in the resources. However, more information on the covariance of energy allocation patterns and the dynamics of the resources in the natural populations is needed before such a conclusion can be drawn with high confidence.

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