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Seasonal cycles in and effects of starvation on egg development in *Mytilus edulis*

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ABSTRACT: Histological and quantitative cytochemical methods were used to investigate seasonal cycles in, and effects of starvation on, reproduction throughout the gametogenic cycle. Amount and composition of egg cytoplasm in ripe oocytes was found to be variable throughout the season. There was an overall decline in amount of egg cytoplasm as the gametogenic cycle progressed. Eggs developing early in the season had high glycogen levels but low lipid levels; protein levels remained constant from December to May but then declined. Results of experiments showed that during the first half of the gametogenic cycle starvation resulted in an increase in production of ripe oocytes. There was also an increase in volume of egg reserves on starvation but glycogen and protein levels declined. Timing of the gametogenic cycle was delayed by starvation. There was variability in the activities of hydrolytic enzymes throughout the gametogenic cycle; however, starvation did not significantly affect levels of activity.

INTRODUCTION

Inhibition of the reproductive potential of individuals by environmental factors is likely to have a significant ecological impact upon a population. The mussel *Mytilus edulis*, as a sedentary organism, is subjected to a wide range of environmental conditions, so the interactions between reproductive processes and environmental changes may assume great importance for the well-being of the population.

Observed effects of food scarcity on reproduction vary considerably from species to species. Generally, fecundity is reduced with a reduced ration (Calow & Woollhead 1977, Bayne et al. 1978, Townshend & Wootten 1984), although in some species it may be enhanced (McKillup & Butler 1979, Calow 1981). In other cases, although reduced fecundity has been demonstrated, reproductive effort (that is the proportion of acquired energy allocated for reproduction) has increased (Hirshfield 1980, Thompson 1982). Starvation has also been shown to affect egg size and composition in a number of species (Bayne et al. 1978, Thompson 1982, Townshend & Wootten 1984). Increased atresia and resorption of ripe oocytes have also been associated with a reduction in ration (Scott 1962, Bayne et al. 1978, Townshend & Wootten 1984).

The severity of environmental conditions and the point in the gametogenic cycle at which they are imposed are both likely to affect reproductive processes. Bayne et al. (1978), using a combination of thermal and nutritive stresses, showed increasing effects on reproduction in *Mytilus edulis* with increasing severity of the imposed conditions, and Gabbott & Bayne (1973) demonstrated varying effects of low food levels for different times of year.

The aims of the present study were to investigate seasonal cycles in, and effects of starvation on, egg production, size and cellular biochemical composition throughout the reproductive cycle of the common mussel, *Mytilus edulis* L.

MATERIALS AND METHODS

Samples of 20 mussels, 4.5 to 5.5 cm in length, were collected monthly from Sharrow Point, Whitsand Bay, Cornwall, from September 1981 to October 1982. Ten individuals were kept in the laboratory in filtered (2 μm), flowing seawater at seasonal ambient temperature; 10 others were processed immediately. The first 3 samples (Nov, Dec, Jan) were starved for 1 mo; for subsequent samples starvation was increased to 2 mo in an attempt to amplify the effects caused by lack of food.

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The following variables were measured, in individuals, for both starved and field samples: (1) shell length, (2) total flesh dry weight, (3) digestive gland dry weight, (4) mantle dry weight. Dry weights were determined by oven drying to constant weight at 80 °C. Means and standard errors for these variables were then calculated on a monthly basis. Histological and cytochemical analyses of mantle tissue were carried out as follows:

Histology. Slices of mantle tissue were fixed in Baker's formol calcium containing 2.5 % NaCl (w : v) prior to dehydration through an ascending alcohol series, processing through Histosol (Shandon Southern) and finally embedding in 56 °C Paraplast (Shandon Southern). Sections were cut (5 µm) on a Reichert rotary microtome and stained by the Papanicolaou method (Culling 1963). Stereological analysis was carried out, using the techniques described by Lowe et al. (1982), to assess the relative volumes of cell types present. Oocyte and germinal vesicle areas were also measured using a drawing tube attached to a Leitz Ortholux 2 microscope and a Kontron MOP3 image analyser interfaced to an SWTP 6800 microprocessor.

Quantitative cytochemistry. Slices of mantle tissue were frozen on to aluminium cryostat chucks in liquid-nitrogen-cooled 2-methylbutane, wrapped with a double layer of Parafilm (American Can Company) and stored at −70 °C until completion of the 12 mo sampling period. Sections were cut (10 µm) in a Bright's motorised Cryostat (10 cutting strokes min^{-1}) with cabinet temperature below −25 °C and knife cooled with crushed 'dry ice'. Sections were collected on glass slides brought from room temperature into the cabinet which in effect flash-dries the section (Bitensky et al. 1973). All samples were coded prior to freezing and decoded only after all measurements had been made; each staining procedure was carried out simultaneously for all samples in order to eliminate variability in the cytochemical reactions for subsequent quantitative measurements.

Glycogen: sections were fixed for 15 min in dimethylsuberimidate (Hand & Hassel 1976) and then reacted with Schiff Reagent (Bancroft 1967).

Neutral Lipids: sections were fixed in Baker's formol calcium (+ 2.5 % NaCl) for 15 min and then stained with oil red 0 solution (Bancroft 1967).

Protein: sections were fixed in Baker's formol calcium (+ 2.5 % NaCl) for 15 min before being stained with the mercury-bromophenol blue method for protein (Pearse 1968).

Acid hydrolases: reaction for β-N-acetylhexosaminidase, β-glucuronidase, non-specific esterase, acid phosphatase and arylsulphatase activities was carried out on sections fixed in Baker's formol calcium (+ 2.5 % NaCl) for 15 min at 4 °C using the naphthol AS-BI methods (Pearse 1972) and postcoupling with the diazonium salt, fast violet B (Sigma Chemical Co), as described by Moore (1976).

Microdensitometric measurements (as relative absorption) were made (20 readings per section) on the ripe (i.e. post vitellogenic) oocytes, using a Vickers M85 scanning microdensitometer at wavelengths of 600 nm (protein), 560 nm (glycogen and acid hydrolases) and 530 nm (neutral lipids), with a measuring spot of 0.5 µm diameter, a mask size A3 and a ×40 objective.

RESULTS

The results compare *Mytilus edulis* that have undergone starvation for 1 or 2 mo with field specimens at that time. However, to interpret the data, condition of mussels when collected from the field is also considered for some parameters. For example, the graphed dry weight data following starvation reflect the weight of the non-starved animals on the date of collection. Thus, the elevated weights from starved mussels in March when compared with field specimens are a

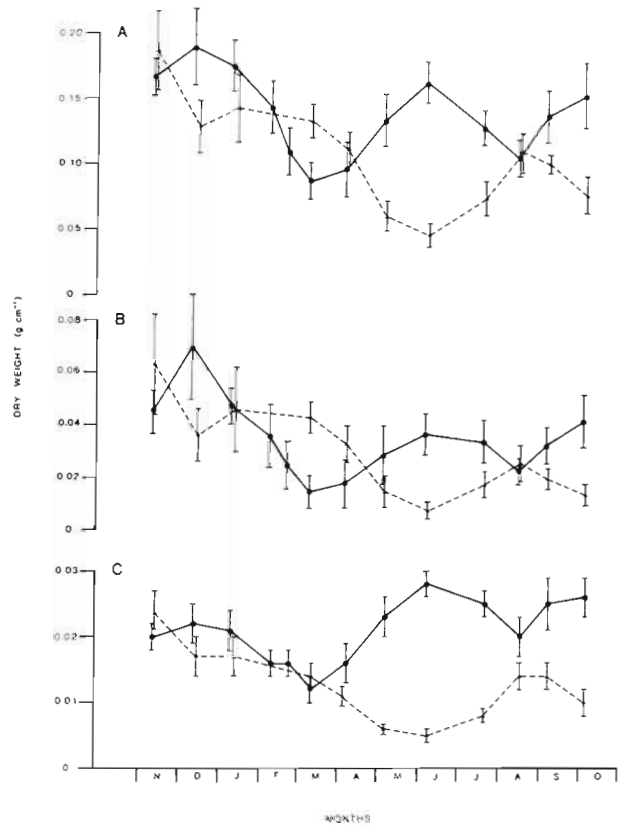

Fig. 1. *Mytilus edulis.* (A) Total dry flesh weights (g cm^{-1}); (B) dry mantle weights (g cm^{-1}); (C) dry digestive gland weights (g cm^{-1}). All values are means ± 2 SE. Solid line: field sample; broken line: Sample starved for 1 or 2 mo

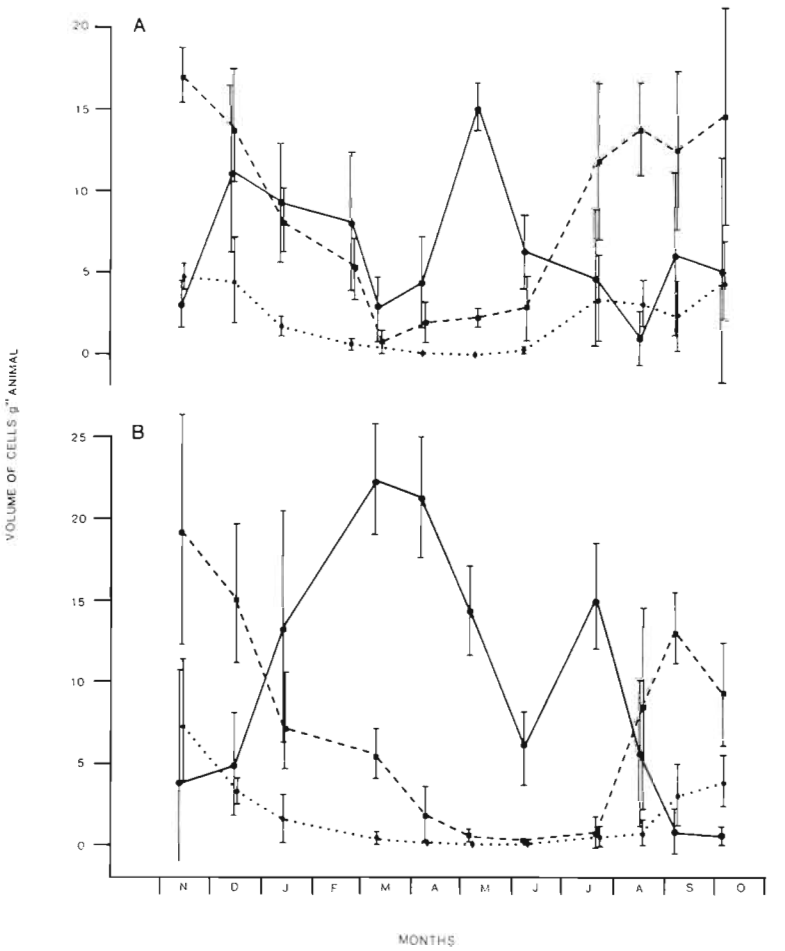

Fig. 2. *Mytilus edulis*. (A) Mantle tissue volume fraction g^{-1} animal, for field samples; (B) mantle tissue volume fraction g^{-1} animal, for samples starved for 1 or 2 mo. All values are means ± 2 SE. Solid line: ripe gamete; broken line: vesicular connective tissue cells; dotted line: adipogranular cells

reflection of the relatively high weights when the mussels were transferred from the field and demonstrates that the natural population has sustained an increased weight loss due to spawning (see Fig. 2).

Total flesh dry weight for non-starved mussels showed 2 peaks (Fig. 1A); the first, in December, can be attributed largely to mantle tissue (Fig. 1B) and the second, in May, is due to an overall tissue weight increase.

Effects of starvation on total dry weight and digestive gland dry weight are considerably increased in summer when food levels for the natural population would be very much higher than in winter. The results for mantle tissue show that the loss in weight throughout the season is not as great as for the digestive gland or total mussel, indicating that reproduction is being maintained, possibly at the expense of other tissues.

Results from stereological analysis show, for non-starved mussels (Fig. 2A), 2 peaks in the volume fraction of ripe gametes, the first from December to February and the second in May. The 2 storage cell types, the vesicular connective tissue (VCT) cells containing mainly glycogen and the adipogranular (ADG) cells containing predominantly lipid and protein, both declined from November, reaching minimal levels during April and May before rising again from June/July onwards.

In the starvation experiments (Fig. 2B) a marked increase in volume fraction of the ripe gametes occurred between January and March with a further peak in July. The VCT and ADG cells followed a similar pattern to the natural population between November and June but levels did not start to increase until July/August.

Relative measures of egg cytoplasm, calculated as total oocyte area minus germinal vesicle area, are shown in Fig. 3. A one way analysis of variance was carried out on the results and demonstrated that oocytes in starved mussels had significantly greater area of cytoplasm from November through to April than those from the field. The analysis of variance also showed, for oocytes from non-starved mussels, that for November to March cytoplasmic areas were significantly larger than for April to July. Similarly for the starved condition, cytoplasmic areas were significantly larger for November to April than for May to August.

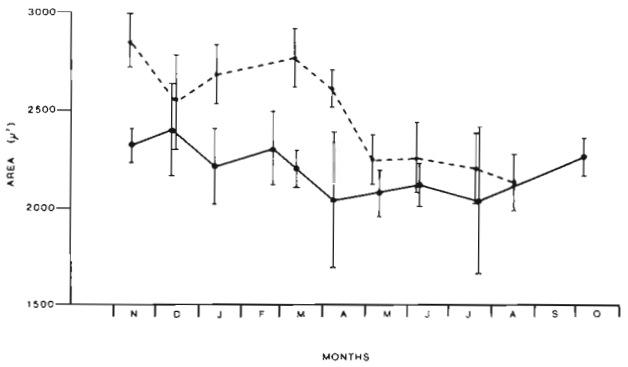

Fig. 3. *Mytilus edulis*. Oocyte cytoplasmic area, calculated as mean ± 2 SE. Solid line: field sample; broken line: sample starved for 1 or 2 mo

Cytochemistry of the Ripe Ooocytes

The results are summarised in Table 1.

Glycogen levels for ripe oocytes in mussels from the field showed a steady decline in levels from November to June before increasing again (Fig. 4A), indicating that eggs spawned earlier in the season had higher levels of glycogen than those spawned later. Starved mussels showed a similar pattern of oocyte glycogen throughout the season. However, following starvation levels fell considerably, particularly from November to January.

Oocyte lipid content in field mussels increased from November to a maximum in May (Fig. 4B), corresponding to the peak in volume fraction of ripe oocytes (Fig. 2A). There was also a much smaller peak in lipid content in January, again relating to the spawning period. Similarly, in oocytes from starved mussels the main peak in lipid levels corresponded to the second peak in ripe oocytes. It would appear, therefore, that eggs spawned later in the season have a greater lipid content than those which are spawned earlier. On starvation the effects appear variable when compared with field specimens. However the data can be interpreted more clearly if comparison is with levels on the date of collection, i.e. displace the starved data back 2 mo; then it can be seen that lipid levels increased slightly but consistently throughout the season.

Oocyte protein levels showed a plateau from December to May, during the spawning period (Fig. 4C). There was no overall indication of increasing or decreasing levels throughout the season as there was with lipid and glycogen levels. Starved mussels showed significantly reduced levels in oocyte protein compared with baseline specimens. Maximum levels, however, still correlated with the spawning period.

Of the 5 acid hydrolases tested, only acid phosphatase showed no detectable activity in the oocytes; the

Table 1. *Mytilus edulis*. Cytochemistry of ripe oocytes

Glycogen	Neutral lipids	Protein	β-glucuronidase	Non-specific esterase	Arylsulphatase	N-acetyl-β-hexosaminidase
			Non-starved			
Decline in levels Nov to Jun before increasing again	Increase in levels from Nov reaching a maximum in May. Smaller peak in January	Increase in levels Nov to Dec, then constant until May, then rapid decline	Declining trend in levels of activity Nov to Jun	2 peaks in activity, first Jan to Feb, second May to Jun	Rapid decline in activity Nov to Dec, increasing in Jan, constant until Apr, declining in May, then increasing again	No significant differences in activity throughout the season
			Starved			
Decline in levels Nov to May before increasing again. Levels considerably lower than in non-starved individuals	Increase in levels from Nov reaching a maximum in Jul. Smaller peak in Mar. Levels slightly, but consistently, higher than in non-starved individuals (see text)	Increase in levels Nov to Apr followed by rapid decline. Levels considerably lower than in non-starved individuals	Declining trend in levels of activity Nov to Apr before increasing. Levels of activity similar to non-starved individuals	2 peaks in activity, first in Apr, second in Jul. Levels of activity similar to those in non-starved individuals although some months did show significant decline	Decline in activity to May before increasing again. Levels of activity very similar to those in non-starved individuals	No significant differences in activity throughout the season. Levels similar to those in the non-starved individuals

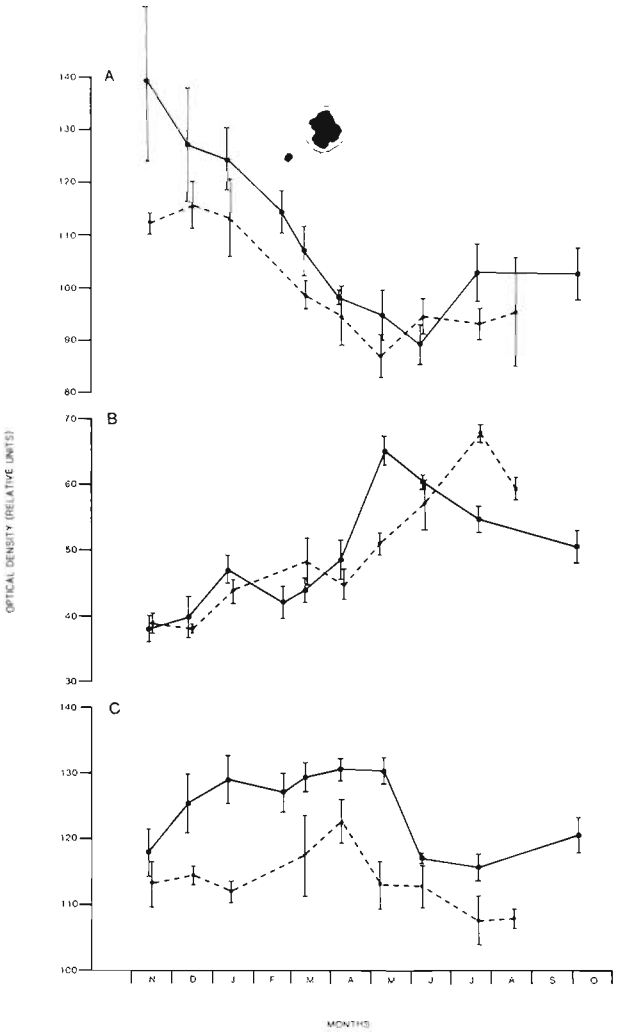

Fig. 4. *Mytilus edulis*. Volumes (measured as optical density) of cytochemical contents in ripe oocytes. (A) Glycogen; (B) neutral lipids; (C) protein. Values are means ± 2 SE. Solid line: field sample; broken line: sample starved for 1 or 2 mo

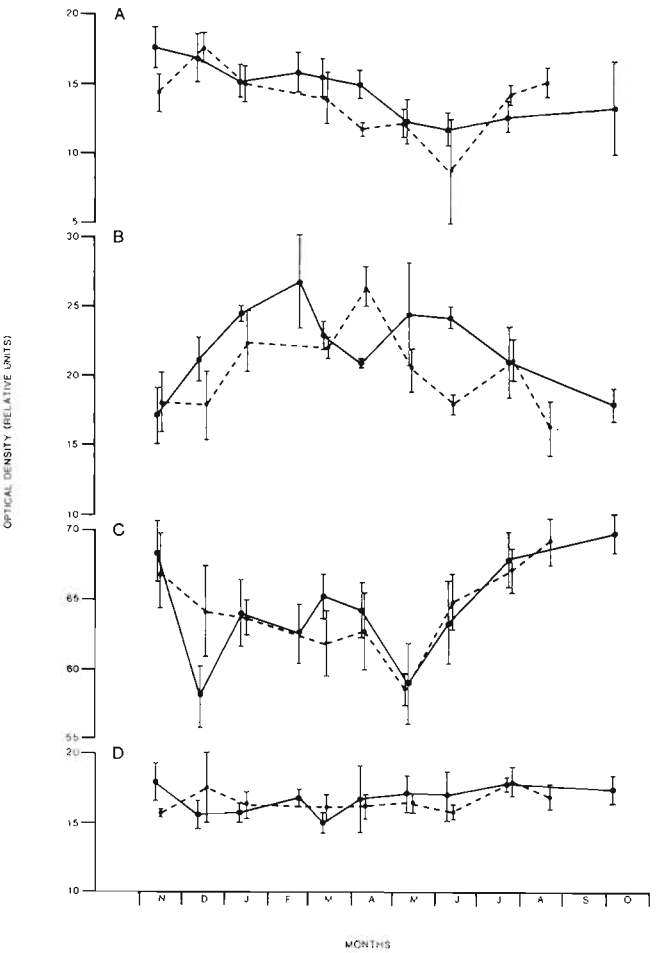

Fig. 5. *Mytilus edulis*. Relative activities (measured as optical density) of lysosomal acid hydrolases in ripe oocytes. (A) β-glucuronidase; (B) non-specific esterase; (C) arylsulphatase; (D) N-acetyl-β-hexosaminidase. Values are means ± 2 SE. Solid line: field sample; broken line: sample starved for 1 or 2 mo

others demonstrated variable levels of activity according to time of year (Fig. 5: Table 1). Oocytes from field specimens showed a declining trend in levels of activity of β-glucuronidase from November to June (Fig. 5A). A similar pattern was shown with oocytes from starved mussels, declining from November to April before increasing. Overall levels of activity were similar for starved and non-starved mussels although for occasional months were significantly lower in the starved condition. Non-specific esterase activity in oocytes from the field specimens showed 2 peaks, the first from January to February and the second from May to June (Fig. 5B). Both of these peaks in activity related to the 2 peaks in ripe oocytes (Fig. 5B cf. Fig. 2A). Similarly with starved mussels, the oocytes showed 2 peaks in activity both corresponding with levels of ripe oocytes. On starvation, levels of activity in the oocytes tended to remain similar although in some months they declined significantly. Levels of arylsulphatase activity in oocytes from the Whitsand field specimens appeared inversely proportional to percentage of ripe oocytes present, i.e. with lowest activity in December and May corresponding to peak numbers of oocytes (Fig. 5C cf. Fig. 2A). Activities in oocytes from starved mussels were not as variable as those from field specimens, declining from November to May before increasing again. The overall levels of activity in the oocytes were very similar for starved and non-starved mussels. There appeared to be no significant difference in activity for N-acetyl-β-hexosaminidase throughout the season for either baseline or starved mussels (Fig. 5D).

DISCUSSION

The effects of environmental change on reproduction must inevitably vary according to the condition of the animal when the change is applied; responses will therefore differ according to time of year and gametogenic state of the animal. In the present experiments, effects of starvation were investigated at monthly intervals throughout the gametogenic cycle of *Mytilus edulis*.

Dry weight and stereological results show that starvation appears to prevent spawning of ripe gametes, thus after starvation mussels were still at the same stage of gametogenic development as when they were brought from the field. An increased proportion of ripe gametes was produced as a result of starvation, particularly for the first spawning. However these values were calculated on a 'per g animal' basis and so actual fecundity may not have been different. The relative proportion of storage cells did not appear to change on starvation indicating that the enhanced ripe fraction was principally caused by developing gametes reaching maturation without subsequent spawning.

Ripe oocytes from the starvation experiment also had a greater cytoplasmic area, particularly during the first half of the gametogenic cycle when the ripe fraction was very large, suggesting that the delay in spawning causes an increase in egg size. It has been shown for the marine gastropod *Nassarius pauperatus* that when trophic conditions deteriorate, egg and egg capsule production increase (McKillup & Butler 1979). Increased reproductive effort at the expense of somatic growth has also been shown for sea urchins (Thompson 1982) and fish (Hirshfield 1980). From the present experiments one cannot say with certainty whether there was a positive increase in reproductive effort at the expense of other tissues or whether the lack of food prevented spawning thus causing a build up of larger oocytes. However the increased loss in weight, on starvation, of digestive gland compared with mantle tissue does indicate a positive increase in reproductive effort. Bayne et al. (1978) showed not only a decline in egg production for *Mytilus edulis* from an estuarine population, but also that the eggs were smaller and contained less organic matter when the mussels were subjected to extremes of temperature combined with nutritional stress. This indicates that mussels react differently either according to the degree of severity of environmental change to which they are subjected, or, possibly, according to their natural habitat.

Egg cytoplasmic areas declined throughout the reproductive cycle for both starved and non-starved conditions, indicating that eggs spawned later in the season would have overall less reserves than those spawned earlier; composition was also shown to change. This could be due to the fact that eggs developing earlier in the season can draw on the large reserves in the ADG and VCT cells which were still present even in starved mussels, whereas those developing later must rely to a greater extent on direct conversion from the food supply.

When considering the cytochemical aspect of the study, the variability in cytoplasmic areas should be borne in mind as some alterations in cytochemical measurements could be a reflection of increased or decreased concentration due to changes in egg volume. However, generally cytoplasmic areas were not as variable as the measured oocyte constituents; also it would be expected, on the basis of electron microscope studies (Pipe unpubl.), that concentration of cytoplasmic reserves, in the form of lipid and protein yolk granules and glycogen, would vary independently of egg volumes.

Eggs spawned earlier in the gametogenic cycle had higher levels of glycogen but lower levels of lipid than those spawned later; protein levels, however, did not vary significantly during the spawning period. An explanation for this could be that egg development early in the season is largely at the expense of stored reserves, in particular glycogen from the VCT cells, but as gametogenesis proceeds there is a decline in VCT cells and so nutrient deposition in the egg may be as a result of a more direct conversion from the food supply. Zandee et al. (1980) suggested that during summer, lipids are the main source of energy production in growing mussels and Waldock & Holland (1979) suggest that the provision of egg lipid varies between exogenous (dietary) and endogenous (from mantle glycogen) sources depending upon food levels.

On starvation both glycogen and protein levels declined considerably. Lipids were variable but on comparison with levels on the date collected from the field showed an increase. This could be as a result of conversion of glycogen within the eggs or alternatively a build-up of lipid at the expense of other tissues. Gabbot (1983), in his review of reproduction in relation to biochemical cycles, cites Zandee et al. (1980) as evidence for suggesting that glycogen in the mantle may be converted to lipid within the developing eggs. Evidence from electron microscopy suggests that lipid yolk granules are in fact formed within oocytes in *Mytilus edulis* (Humphreys 1962, Reverberi 1971). However, further work is needed to clarify the situation, and incorporation of exogenous lipid in the eggs cannot be ruled out (see recent reviews by Jong-Brink et al. 1983, Dohmen 1983).

The lysosomal acid hydrolases showed considerable variability in levels of activity, both for the different enzymes and according to time of year. Of the 5 enzymes tested, 4 showed activity. Pasteels (1973) reported

activity for acid phosphatase in ovarian oocytes of *M. edulis* and so it is possible that this enzyme was also present but possibly at very low levels of activity. The role of acid hydrolases in ovarian oocyte yolk granules has not been established; in some species it appears that yolk utilisation is mediated by these enzymes in fertilised and atretic oocytes (Pasteels 1973, Masuda & Dan 1977, Decroly et al. 1979) and activity has been shown to increase significantly in degenerating oocytes (see Jong-Brink et al. 1983). In the present study the non-specific esterase and arylsulphatase appeared to be related to the volume fraction of ripe oocytes present. It has been shown for *Lymnaea stagnalis* and *Biomphalaria glabrata* that the number of yolk granules reacting positively for hydrolytic enzymes is variable according to the degree of maturation of the oocytes (Arni 1974, Jong-Brink et al. 1983). The variability in levels of enzyme activity throughout the year indicates an active role in ovarian oocyte development rather than a presence in a latent form for use during embryo development (see Pasteels 1973). The tendency for levels of activity to decline on starvation in the present study coincides with the marked decline in oocyte protein levels which occurred in the starved mussels and gives no indication of lysosomal enzyme breakdown of the oocyte contents. However, in electron microscope studies (Pipe & Moore 1985) we have shown lysosomal enzymes to be involved in the resorption of degenerating yolk granules.

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